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Biotechnological applications of purine and pyrimidine deaminases

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ABSTRACT

Deaminases, ubiquitous enzymes found in all living organisms from bacteria to humans, serve diverse and crucial functions. Notably, purine and pyrimidine deaminases, while biologically essential for regulating nucleotide pools, exhibit exceptional versatility in biotechnology. This review systematically consolidates current knowledge on deaminases, showcasing their potential uses and relevance in the field of biotechnology. Thus, their transformative impact on pharmaceutical manufacturing is highlighted as catalysts for the synthesis of nucleic acid derivatives. Additionally, the role of deaminases in food bioprocessing and production is also explored, particularly in purine content reduction and caffeine production, showcasing their versatility in this field. The review also delves into most promising biomedical applications including deaminase-based GDEPT and genome and transcriptome editing by deaminase-based systems. All in all, illustrated with practical examples, we underscore the role of purine and pyrimidine deaminases in advancing sustainable and efficient biotechnological practices. Finally, the review highlights future challenges and prospects in deaminase-based biotechnological processes, encompassing both industrial and medical perspectives.

1. Introduction

Enzyme-based processes have become crucial in various research fields, providing a compelling alternative to traditional, complex, and environmentally harmful chemical methods. Among these, salvage enzymes are a natural source of promising catalysts for synthesizing nucleic acid derivatives (NADs) through both mono- and multi-enzymatic approaches. During the last decades, various enzymes have been successfully utilized for NAD synthesis, including nucleoside phosphorylases (NPs) (Kamel et al., 2018; Rinaldi et al., 2020), 2′-deoxyribosyltransferases (NDTs) (Acosta et al., 2018; Del Arco et al., 2019a), phosphoribosyltransferases (Acosta et al., 2021; Cruz et al., 2022; Del Arco et al., 2019b), nucleoside kinases (NKs) (Frisch et al., 2021; Mikhailopulo and Miroshnikov, 2011), and deaminases (DAs) (Lewkowicz and Iribarren, 2017; Li et al., 2019; Slagman and Fessner, 2021), among others (Del Arco et al., 2021; Fernández-Lucas and Camarasa, 2019; Lapponi et al., 2016; Simić et al., 2021). In this context, deaminases (DAs) stand out as versatile biocatalysts, finding significant applications for the synthesis of high-value-added compounds for the pharmaceutical and food industries, and as precision tools for cancer treatment and genome and transcriptome editing.

Purine and pyrimidine DAs catalyze metal-assisted hydrolytic deamination of nucleobases, nucleosides, and nucleotides. These nitrogen catabolic enzymes catalyze essential steps in purine and pyrimidine salvage pathways, but also contribute to regulating the concentration of the purine and pyrimidine pool in living organisms (Del Arco and Fernández-Lucas, 2018; El Kouni, 2003; Gaded and Anand, 2018). Beyond this, these enzymes are integral participants in fundamental cellular processes, including dendritic ramification, as well as differentiation and maturation of the lymphoid system, among others. Depending on the nature of the target molecules, we can distinguish between nucleobase, nucleoside, nucleotide, or even, nucleic acid DAs. The present review aims to show the practical applications of DAs as catalysts for the synthesis of nucleic acid derivatives and food bioprocessing, while also functioning as valuable tools for the development of innovative therapeutic strategies. To this end, firstly a brief description of general concepts about reaction mechanisms and classification of DAs will be presented, to ensure a proper framework for better comprehension. Furthermore, we disclose the content in two separate sections related to the nature of the nitrogenated nucleobase, including practical examples of the diverse biotechnological uses of DAs.

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Abbreviations

Nucleic acid derivatives

Ade	adenine
(d)Ado	(2′-deoxy)adenosine
AFLDA	6-aminodeoxyfutalosine
AIMers	chemically modified oligonucleotides
ASOs	antisense oligonucleotides
ara A (vidarabine)	arabinosil adenine
Ara C (cytarabine)	arabinosil cytosine
Ara G	arabinosil guanine
Ara U	arabinosil uracil
arRNAs	ADAR recruiting gRNAs
BG	O6-benzylguanine
circ-arRNAs	closed circular arRNAs
(d)CMP	(2′-deoxy)cytidine-5′-monophosphate
CNAs	click nucleic acids
crRNAs	CRISPR RNAs
(d)CTP	(2′-deoxy)cytidine-5′-triphosphate
Cyt	cytosine
(d)Cyd	(2′-deoxy)cytidine
DAP	2,6-diaminopurine
DAPR	2,6-diaminopurine riboside
ddAdo	dideoxyadenosine
ddIno (didanosine)	2′,3′-dideoxyinosine
DiMetXan	dimethylxanthine
dsDNA	double-stranded DNA
dsRBD	double-stranded RNA binding domain
dsRNA	double-stranded RNA
DOX	doxorubicin
EONs	editing oligonucleotides
GCV	ganciclovir
gemcitabine	2,2-difluoro-2′-deoxycytidine
GMP	guanosine monophosphate
gRNA	guide RNA
Gua	guanine
(d)Guo	(2′-deoxy)guanosine
Hyp	hypoxanthine
IMP	inosine monophosphate
(d)Ino	(2′-deoxy)inosine
MetXan	methylxanthine
MetXao	methylxanthosine
MTA	5′-methylthioadenosine
NADs	nucleic acid derivatives
NAs	nucleoside analogues
(d)NTP	(2′-deoxy)nucleotide
pre-mRNA	precursor mRNA
pur	purine
pyr	pyrimidine
SAM	S-adenosyl-L-methionine
SAH	S-adenosyl-L-homocysteine
sgRNA	single guide RNA
ssDNA	single-stranded DNA
ssRNA	single-stranded RNA
TriMetXan	trimethylxanthine
(d)TTP	(2′-deoxy)thymidine-5′-triphosphate
(d)UMP	(2′-deoxy)uridine-5′-monophosphate
Ura	uracil
(d)Urd	(2′-deoxy)uridine
(d)UTP	(2′-deoxy)uridine-5′-triphosphate
tRNA	transfer RNA
Xan	xanthine
(d)Xao	(2′-deoxy)xanthosine
Xao	xanthosine
3TC (lamivudine)	(–) 2′,3′-dideoxy-3′-thiacytidine
5-azaCyd	5-aza-cytidine
5-FCyt	5-fluorocytosine
5-fdCyd	2′-deoxy-5-(formyl)cytidine
5-fdUrd	2′-deoxy-5-(formyl)uridine
5-FUMP	5-fluorouridine monophosphate
5-FUra	5-fluorouracil
5-hmCyt	5-hydroxymethylcytosine
5-hmdCyd	2′-deoxy-5-(hydroxymethyl)cytidine
5-MetCyt	5-methylcytosine
5-hmdUrd	2′-deoxy-5-(hydroxymethyl)uridine
5′-ClAdo	5′-chloroadenosine

Enzyme abbreviations

ABEs	adenosine base editor
ACBE	Ade-to-Cyt base editor
ADAR	duplexed RNA adenosine deaminase
$ADAR_{DD}$	ADAR deaminase domain
ADAT	eukaryotic tRNA-specific adenosine deaminase
AdeDA	Adenine deaminase
AdoDA	adenosine deaminase
AID	activation-induced deaminase
AMPDA	adenosine-5′-monophosphate deaminase
APOBEC	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like
ATPDA	adenosine-5′-triphosphate deaminase
cAMPDA	cyclic adenosine-5′-monophosphate deaminase
CydDA	cytidine deaminase
CytDA	cytosine deaminase
DAs	deaminases
dCMPDA	2′-deoxycytidine-5′-monophosphate deaminase
dCTPDA	deoxycytidine triphosphate deaminase
dUTPase	2′-deoxyuridine-5′-triphosphate nucleotidohydrolase
GuaDA	guanine deaminase
GuoDA	guanosine deaminase
IMPDH	inosine-5′-monophosphate dehydrogenase
6-MetAdeDA	6-methyladenine deaminase
MTADA	S-methyl-5’-thioadenosine deaminase
NDTs	2′-deoxyribosyltransferases
NKs	nucleoside kinases
NPs	nucleoside phosphorylases
8-oxoGuaDA	8-oxoguanine deaminase
PNP	purine nucleoside phosphorylase
SAHDA	S-adenosyl-L-homocysteine deaminase
SAM2	methionine adenosyltransferase (S-adenosylmethionine synthetase)
TadA	procaryotic tRNA-specific adenosine deaminase
TCS1	tea caffeine synthase
TK	thymidine kinase
UDG	uracil DNA glycosylase
UO	urate oxidase
UP	uridine phosphorylase
UPRT	uracil phosphoribosyl transferase
XanA	xanthine dioxygenase
XOD	xanthine oxidase

Other abbreviations

AAV	adeno associated virus
ACE-Seq	APOBEC-Coupled Epigenetic Sequencing
AHS	amidohydrolase superfamily
AMLV	amphotropic murine leukemia virus
BEs	base editors
BER	base-excision repair
CBEs	cytosine base editor
CDA	cytidine deaminase-like superfamily
CIRTS	CRISPR-Cas-Inspired RNA Targeting System

COGs	cluster of orthologous groups
DSBs	double-stranded DNA breaks
eGFP	enhanced-fluorescent protein
EPT	enzyme prodrug therapy
FKBP3	FK506-binding protein 3
FRB	FKBP-rapamycin binding
GALV	gibbon ape leukemia virus
GDEPT	gene directed enzyme prodrug therapy
HD	heterodimeric
HDR	homology-directed repair
LDL	low-density lipoprotein
LEAPER	Leveraging Endogenous ADAR for Programmable Editing of RNA
LPEI	polyethylenimine
MCP	MS2 bacteriophage coat protein
MNPHT	magnetic nanoparticle hyperthermia
MSCs	human mesenchymal stem/stromal cells
NES	nuclear export signal
NLS	Nuclear localization sequence
PAM	protospacer adjacent motif
PAMAM	polyamidoamine
PCI	photochemical internalization
PEG-PLGA	polyethyleneglycol-poly(lactic-co-glycolic acid)
REPAIR	RNA Editing for Programmable A to I Replacement
RESTORE	Recruiting Endogenous ADAR to Specific Transcripts for oligonucleotide-mediated RNA Editing
REWIRE	RNA editing with individual RNA-binding enzyme
RRVs	retroviral replicating vectors
RT	Room temperature
SNP	single nucleotide polymorphism
SV40	Simian virus 40
STAT1	signal transducer and activator of transcription 1
TaRGET	tiny nuclease RNA-based genome editing technology
UGI	DNA glycosylase inhibitor

2. Purine and pyrimidine DAs

2.1. General concepts, structure, and mechanism

From a functional perspective, purine and pyrimidine DAs (like other deaminases) belong to the amidohydrolase class (E.C. 3.5) which comprises a broad spectrum of enzymes that catalyze the hydrolysis of different substrates bearing amide or ester groups at carbon and phosphorus centers (Gaded and Anand, 2018; Iyer et al., 2011; Seibert and Raushel, 2005). More specifically, they catalyze the deamination of cyclic amidines (E.C. 3.5.4.).

Based on their amino acid similarity and protein fold, purine and pyrimidine DAs are unusual protein families included in cytidine deaminase-like superfamily (CDA, Pfam entry CL0109, 33 members), and in amidohydrolase superfamily (AHS, Pfam entry CL0034, 17 members). Despite the deamination reaction in both superfamilies occurring through a metal cation, profuse structural and sequence differences have been reported among CDA and AHS DAs (Gaded and Anand, 2018; Iyer et al., 2011; Seibert and Raushel, 2005) (Tables 1 and 2).

Up to date, all determined structures of DAs belonging to the AHS superfamily share a common distorted $(\beta/\alpha)_8$-barrel (triosephosphate isomerase, TIM barrel) fold (Gaded and Anand, 2018) (Fig. 1A). Residues responsible for metal recognition are also conserved among AHS DAs and generally located at the C-terminal ends of β1, β4–6, and β8 strands (Goble et al., 2011). In this respect, the metal ion is coordinated by an aspartate residue, three histidine residues, and a water molecule. As a point of interest, AHS DAs possess a mononuclear or binuclear metal center responsible for the activation of the nucleophilic water molecule (Gaded and Anand, 2018) (Fig. 1B). Regarding the active site, protons from the metal-bound water molecule are transferred to the reaction products throughout three conserved residues: a glutamate (HxxE motif), a histidine and an aspartate residue (Gaded and Anand, 2018; Kamat et al., 2011a) (Fig. 1C) (Fig. 2). In this sense, generally speaking, the reaction mechanism for deaminases of the AHS superfamily follows a highly conserved sequence. Activation of the water molecule occurs when the zinc ion at the active site coordinates with a water molecule. Through interactions with conserved residues, particularly the glutamate residue, the water molecule is deprotonated, generating a hydroxide ion. Nucleophilic attack follows, wherein the metal-bound hydroxide acts as a nucleophile and attacks the substrate's carbon, typically at a nitrogen-carbon bond, forming a tetrahedral intermediate. Protonation of the leaving group is facilitated by the conserved aspartate residue, which helps in the proton transfer to the departing amino group, leading to the cleavage of the carbon-nitrogen bond. Finally, this reaction results in the formation of the deaminated product and the release of ammonia (Manta et al., 2014).

According to the NCBI database of Clusters of Orthologous Groups (COGs), which arranges ortholog proteins coded by whole genomes within the same cluster (Tatusov et al., 2001), AHS DAs could be phylogenetically classified in three distinct clusters: cog0402, cog1001, and cog1816 (Goble et al., 2011). DAs acting on cytosine, guanine, S-adenosylhomocysteine, N-formimino-L-glutamate, *S*-methyl-5′-thioadenosine, and 8-oxoguanine belong to cog0402 (Hall et al., 2011). The binuclear metal center deaminase (AdeDA) and N-6-methyladenine deaminase (6-MetAdeDA) are clustered within cog1001 (Kamat et al., 2011a). Finally, cog1816 groups together all bacterial enzymes that catalyze adenosine deamination (Goble et al., 2011).

On the other hand, according to the COGs database, CDA superfamily members fall into cog0590. All these CDA DAs share a compact core displaying a conserved αβα layered fold (Gaded and Anand, 2018) (Fig. 3A). This deaminase fold is formed by a four-stranded β-sheet, arranged in β2, β1, β3, and β4 order, with β1 running antiparallel to the rest of the strands. The α1 helix is positioned before the hairpin formed by β1and β2 strands. The second α2 helix is located right after α1, and the remaining β3 and β4 strands are separated by the α3 helix. In some cases, an additional fifth β strand (β5) could be present running either parallel or antiparallel to β4 (Iyer et al., 2011). Depending on the presence of an extra α helix (α4) in their overall fold, two main subgroups within the CDA superfamily could be distinguished. When α4 helix is present β4 and β5 strands stack parallel to each other, while α4 absence leads to the antiparallel stacking (Iyer et al., 2011). Additionally, CDA superfamily comprises both mononucleotide and NA DAs (Gaded and Anand, 2018). Mononucleotide DAs include blasticidin, cytidine, cytosine, deoxycytidylate (dCMP), guanine, and riboflavin deaminase, which play a key role in nucleotide metabolism. NA DAs, however, catalyze the *in situ* deamination of the nitrogenous bases present in DNA and RNA, so they are responsible for gene diversification and antiviral defense (Iyer et al., 2011). The deamination reaction catalyzed by CDA DAs can only occur assisted by a single zinc cation. The metal binding site consists of conserved residues represented in signatures HXE and PCXXC (a hallmark of this superfamily) (Mistry et al., 2021; Reizer et al., 1994). Among them, the zinc cation directly interacts with cysteine and histidine residues, whereas the glutamate residue acts as a proton shuttle during reaction (Ko et al., 2003; Navaratnam and Sarwar, 2006) (Fig. 3B) (Fig. 4). During the catalytic cycle, the zinc-bound water molecule is deprotonated to form a hydroxide ion, which acts as a nucleophile and attacks the substrate, forming a tetrahedral intermediate. In the reaction, the proton is transferred from the metal-bound hydroxide to the substrate, often through hydrogen bonding with the mentioned conserved active site residues (cysteine, histidine and glutamate residues). Key residues, such as the glutamate

residue, help stabilize the transition state and facilitate proton transfer, while a conserved aspartate residue assists in the cleavage of the nitrogen-carbon bond, leading to the formation of the deaminated product and the release of ammonia. The protonation state of the pyrimidine ring nitrogen also influences the nucleophilic attack, enhancing the reaction's efficiency (Xu and Guo, 2004). CDA DAs have been reported very specific towards their respective substrates (Bitra et al., 2013a, 2013b; Gaded and Anand, 2018). However, some DAs have acquired a certain degree of promiscuity through the modification of some essential structural features close to their active sites (Iyer et al., 2011; Bitra et al., 2013a, 2013b).

2.2. Purine DAs

As mentioned earlier, purine DAs have the ability to act on diverse purine substrates. Despite all targeted substrates containing a purine ring, we can distinguish between, nucleobase, nucleoside, nucleotide of nucleic acid DAs based on the structure of the molecules they target. Even though all purine DAs display a remarkable interest, we will focus our interest on those that can be applied as practical biocatalysts in the food and pharma industry.

2.2.1. Purine nucleobase DAs

Depending on the substituents of purine ring, nucleobase DAs can be classified as 6-aminopurine and 6-oxopurine DAs (Table 1). Adenine DA (AdeDA) and guanine DA (GuaDA) are the representative members of these subfamilies (Fig. 5). However, several examples of other unusual 6-aminopurine and 6-oxopurine DAs have been recently reported, namely 8-oxoguanine DA (8-oxoGuaDA) (Hall et al., 2010) or 6-methyladenine DA (6-MetAdeDA) (Kamat et al., 2011b).

2.2.1.1. Adenine DAs

2.2.1.1.1. General comments. AdeDA (EC 3.5.4.2), also known as adenine amidohydrolase, is responsible for the hydrolytic deamination of adenine (Ade) **(1)** to hypoxanthine (Hyp) **(2)**, releasing ammonia (Goble et al., 2011; Kamat et al., 2011a) (Fig. 5), in presence of a divalent metal cation (M^{2+}) AdeDA belongs to the AHS superfamily and is conserved in prokaryotes and lower eukaryotes (Pospísilová et al., 2008). One of the main roles of this enzyme is the deamination of Ade to Hyp in the first step of the purine salvage pathway (Pospísilová et al., 2008). AdeDA is also involved in the purine degradation pathway, in which after adenine deamination by AdeDA, hypoxanthine is transformed to uric acid by xanthine oxidase (XOD) through a xanthine

Table 1
Main characteristics of the purine deaminases discussed in the manuscript, including a representative structure from the PDB for each enzyme and their most relevant biotechnological applications.

Enzyme	EC number	Superfamily	COG number	Target substrate	PDB id	Biotechnological applications
Adenine deaminase (AdeDA)	EC 3.5.4.2	AHS	cog1001 (binuclear metal center) cog1816 (mononuclear metal center)	Adenine	3NQB (binuclear metal center) 3PAO (mononuclear metal center)	• Lowering the purine content in food
Guanine deaminase (GuaDA)	EC 3.5.4.3	AHS (mammalian, insect, fungal, and some bacterial) CDA (plant, archaeal, and some bacterial)	cog0402 (AHS) cog0590 (CDA)	Guanine Ammeline	6OHA (AHS) 4LC5 (CDA)	• Lowering the purine content in food • Increasing caffeine production
Adenosine deaminase (AdoDA)	E.C. 3.5.4.4	AHS	cog1816	Adenosine N6-methyladenosine	1A4L	• Production of nucleoside derivatives • Therapeutic target for inhibitor design • Markers for the diagnosis of different illnesses
Guanosine deaminase (GuoDA)	EC 3.5.4.15	CDA	cog0590	Guanosine	7DBF	• Markers for the diagnosis of different illnesses
S-adenosylhomocysteine deaminase (SAHDA)	EC 3.5.4.28	AHS	cog0402	S-adenosylhomocysteine	2PLM	• Potential for the regulation of gene expression
S-methyl-5′-thioadenosine deaminase (MTADA)	EC 3.5.4.31	AHS	cog0402	*S*-methyl-5′-thioadenosine	4F0R	• Potential therapeutic target for bacterial infections
Adenosine-5′-monophosphate deaminase (AMPDA)	EC 3.5.4.6	AHS	cog1816	Adenosine-5′-monophosphate	2A3L	• Production of nucleoside derivatives • Enhancement of umami flavor in food production • Improved plant growth and stress resistance • Enhancement of biomass and lipid production
Adenosine deaminases that act on dsRNA (ADAR)	EC:3.5.4.37	CDA	cog0590	dsRNA	7ZLQ (ADAR1) 8E0F (ADAR2)	• Base editors for RNA editing
Adenosine deaminases that act on tRNA (ADAT, in higher eukaryotes) (Tad, in yeast) (TadA in prokaryotes)	EC:3.5.4.34 (ADAT1; Tad1) EC:3.5.4.33 (ADAT2; Tad2; TadA)	CDA	cog0590	A37 in $tRNA^{Ala}$ (ADAT1; Tad1) A34 in tRNA (ADAT2/ADAT3; Tad2/Tad3) A34 in $tRNA^{Arg2}$ (TadA, prokaryotes)	3DH1 (ADAT2) 7BV5 (Tad2/Tad3) 1Z3A (TadA)	• Base editors for genomic editing

intermediate (Del Arco and Fernández-Lucas, 2018; El Kouni, 2003; Gaded and Anand, 2018; Oestreicher, 2003; Oestricher et al., 2008).

Similar to other AHS, two distinct types of AdeDAs (mono or binuclear metal center AdeDAs) can be identified based on the presence of either a single or two M^{2+} in the active site. The binuclear metal center AdeDAs, unlike their mononuclear counterparts in the AHS superfamily, exhibit a unique requirement for two divalent cations to achieve optimal catalytic activity (Seibert and Raushel, 2005; Kamat et al., 2011b). Another difference between both types of AdeDAs lies in the molecular weight, since binuclear AdeDA has a molecular weight of around 65 kDa, whereas the molecular weight of mononuclear AdeDA is approximately 35 kDa (Kamat and Raushel, 2011). It is also worth noting that the binuclear metal center is especially sensitive to iron, significantly affecting the catalytic activity (Goble et al., 2011). Consequently, before protein expression, the sequestration of iron by a metal chelator and supplementation of the growth medium with Mn^{2+} are essential steps to enhance the production of a more active enzyme (Kamat et al., 2011b). The reason behind the presence of mononuclear AdeDA in certain bacterial species, while others from the same class possess the binuclear form, remains unclear to date.

2.2.1.1.2. Biotechnological applications. Among the different biotechnological applications of AdeDA, its use in food bioprocessing has garnered significant attention. Although it is not as widely used as other enzymes, AdeDA offers a more efficient and environmentally friendly alternative to conventional food processing methods. Currently, deaminase-based bioprocessing methods are mostly focused on lowering the purine content in food.

Purines derived from exogenous sources and cell turnover are degraded to uric acid, which is subsequently excreted through the kidneys. However, in certain individuals with impaired renal function and a purine-rich diet, uric acid levels can rise, leading to hyperuricemia (Kutzing and Firestein, 2008; Saag and Choi, 2006; Tausche et al., 2010). This condition affects approximately 1–2 % of the population in developed countries and is a risk factor for hypertension, different cardiovascular and metabolic disorders (Richette and Bardin, 2010) and gout (Terkeltaub, 2010). In conventional clinical practice, gout treatment typically involves non-steroidal anti-inflammatory drugs during attacks and medications to lower urate levels during symptom-free intervals (Burns and Wortmann, 2011; Eggebeen, 2007; Schlesinger et al., 2009). Additionally, adhering to a purine-restricted diet is essential (Choi and Curhan, 2004; Choi et al., 2004), though many patients encounter difficulties in implementing these dietary modifications due to the exclusion of a wide array of purine-rich foods and beverages.

To address these challenges, the application of deaminases, such as AdeDA, offers a promising solution for the production of low-purine foods. AdeDA functions as part of a broader enzymatic systems that combine deaminases with other purine-degrading enzymes, including phosphorylases and oxidoreductases. The deamination process catalyzed by AdeDA modifies purines, making them more susceptible to further degradation by these enzymes. As a result, these systems effectively reduce the overall purine content in food products by breaking down purines into less harmful compounds. This enzymatic approach supports the development of methods for producing low-purine foods, thereby expanding dietary options and improving adherence to purine-

Table 2
Main characteristics of the pyrimidine deaminases discussed in the manuscript, including a representative structure from the PDB for each enzyme and their most relevant biotechnological applications.

Enzyme	EC number	Superfamily	COG number	Target substrate	PDB	Biotechnological applications
Cytosine deaminase (CytDA)	EC 3.5.4.1	AHS (bacterial) CDA (fungal)	cog0402 (AHS) cog0590 (CDA)	Cytosine Isocytosine (AHS) Isoguanine (AHS) 5-Methylcytosine (CDA)	3O7U (AHS) 1UAQ (CDA)	• Potential therapeutic target for microbial infections • Negative selection marker in transgenic plants • Prodrug-mediated cancer therapy
Cytidine deaminase (CydDA)	EC 3.5.4.5	CDA	cog0590	Cytidine	1MQ0	• Therapeutic target for cancer treatment • Therapeutic target for the treatment of tropical diseases • Gene therapy for the protection of healthy tissue from the toxicity of anticancer chemotherapy • Prodrug-mediated cancer therapy • Production of nucleoside derivatives • Optimization of pyrimidine biosynthesis in bacteria
Deoxycytidine monophosphate deaminase (dCMPDA)	EC 3.5.4.12	CDA	cog0590	2′-deoxycytidine-5′-monophosphate	2W4L	• Marker for diagnosing of various disease
Deoxycytidine triphosphate deaminase (dCTPDA)	EC 3.5.4.13	CDA	cog0590	2′-deoxycytidine-5′-triphosphate	1XS1	• No specific technological or biomedical applications
Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC)	EC 3.5.4.36 (APOBEC1; APOBEC2) EC 3.5.4.38 (AID; APOBEC3A-H)	CDA	cog0590	ssRNA (APOBEC1) ssDNA (AID; APOBEC2; APOBEC3A-H)	5JJ4 (AID) 6X91 (APOBEC1) 2NYT (APOBEC2) 5KEG (APOBEC3A) 5CQH (APOBEC3B) 3VOW (APOBEC3C) 5HX4 (APOBEC3F) 4ROW (APOBEC3G) 5W45 (APOBEC3H)	• Epigenetic sequencing • Base editors for genomic editing • Base editors for multi-nucleotide deletions • Base editors for random base editing • Base editors for RNA editing

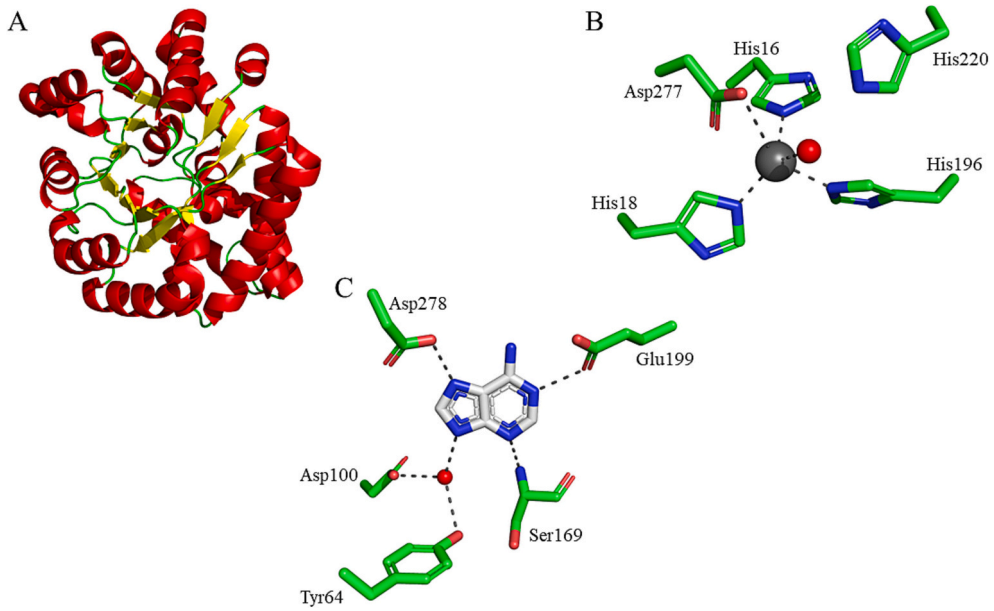


Fig. 1. Overall structure and active site residues of mononuclear adenine deaminase from *P. aeruginosa* (*Pa*AdeDA) (Goble et al., 2011). A) Overall structure of the monomer (apo form, PDB id: 3OU8) is represented as a cartoon showing the distorted $(\alpha/\beta)_8$ barrel core. B) Metal coordination center showing the residues and the water molecule responsible for the binding of the zinc cation. C) Active site center showing the residues and the water molecule responsible for the hydrogen bond network with adenine (adenine-bound *Pa*AdeDA, PDB id: 3PAO). Residues are represented in sticks (colored by atom type, carbon atoms in green), adenine is represented in sticks (colored by atom type, carbon atoms in light gray), the zinc cation is represented as a gray sphere, and water molecules are represented as red spheres. Interactions between residues and the zinc cation are represented as gray dotted lines. Hydrogen bonds among residues and adenine are represented as gray dotted lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

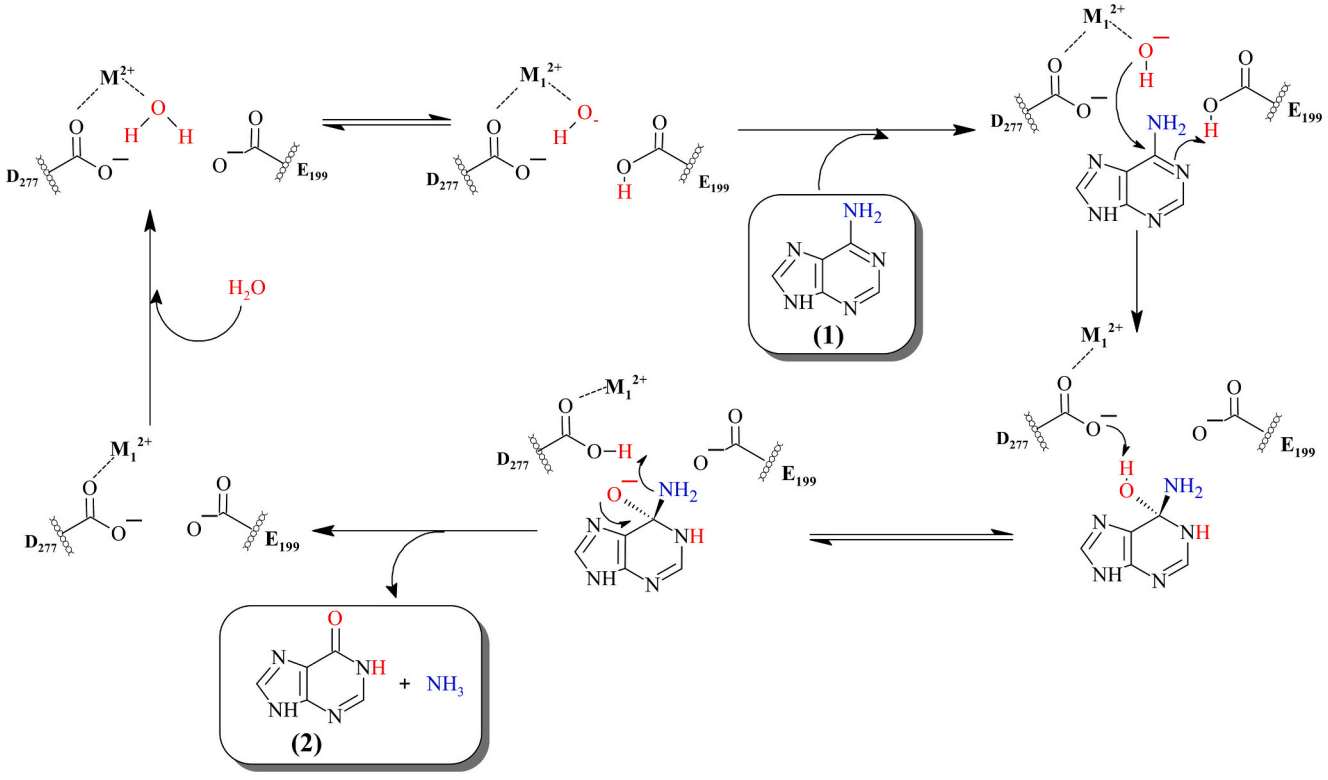


Fig. 2. Reaction mechanism of hydrolytic deamination of adenine **(1)** to hypoxanthine **(2)** catalyzed by *Pa*AdeDA (Goble et al., 2011).

restricted diets for patients with gout.

In a practical example, a transgenic yeast *Arxula adeninivorans* strain was engineered to overexpress AdeDA (together with GuaDA), as a component of an enzymatic system comprising other purine-degrading enzymes, with the goal of lowering purine content in food (Jankowska et al., 2015; Trautwein-Schult et al., 2014). In a subsequent step of the experimental procedure, those recombinant DAs (either as single enzymes or enzyme blends) were tested as catalysts for purine reduction in

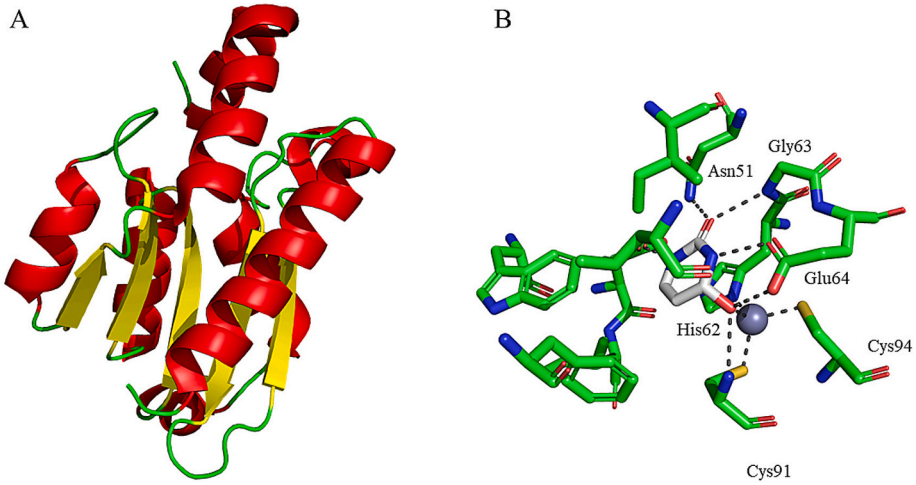


Fig. 3. Overall structure and active site residues of mononuclear yeast cytosine deaminase (CytDA) (Ko et al., 2003). A) Overall structure of the monomer (PDB id: 1UAQ) is represented as a cartoon. B) Active site center showing the residues responsible for hydrogen bond network with dihydropyrimidine-2,4(1H,3H)-dione, including metal coordination center showing the residues responsible for the binding of the zinc cation (PDB id: 1UAQ). Residues are represented in sticks (colored by atom type, carbon atoms in green), dihydropyrimidine-2,4(1H,3H)-dione is represented in sticks (colored by atom type, carbon atoms in light gray), and the zinc cation is represented as a gray sphere. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

food matrices. In a first approach by Jankowska and coworkers, recombinant AdeDA was successfully employed as a catalyst for purine content reduction in the beef extract (Jankowska et al., 2013). Incubation with AdeDA led to a substantial drop in the Ade content in beef broth samples, while the remaining purine levels stayed constant. Later on, the same authors developed a four-enzyme system -AdeDA, GuaDA, XOD, and urate oxidase (UO)- for the conversion of purines in food to a water-soluble 5-hydroxyisourate (Jankowska et al., 2015). After confirming reduction of the purine concentration in beef broth and yeast extract, a rolled fillet of ham was incubated with the enzyme mixture, resulting in a substantial reduction in levels of Ade **(1)**, Gua **(5)**, urate, and Xan **(6)**.

In another recent example, the potential of AdeDA (together with GuaDA) from *K. lactis* (*Kl*AdeDA and *Kl*GuaDA, respectively) was evaluated in combination with *K. lactis* purine nucleoside phosphorylase (*Kl*PNP), xanthine dioxygenase (*Kl*XanA), and commercial *Candida utilis* UO to produce low-purine-content beverages (Mahor and Prasad, 2018). Since different purine sources are present in beer samples, both enzymes were combined with *K. lactis* purine nucleoside phosphorylase *Kl*PNP), xanthine dioxygenase (*Kl*XanA), and commercial *Candida utilis* UO, to collectively reduce the overall purine content in beer. As expected, the experimental findings demonstrated a decrease in the overall purine

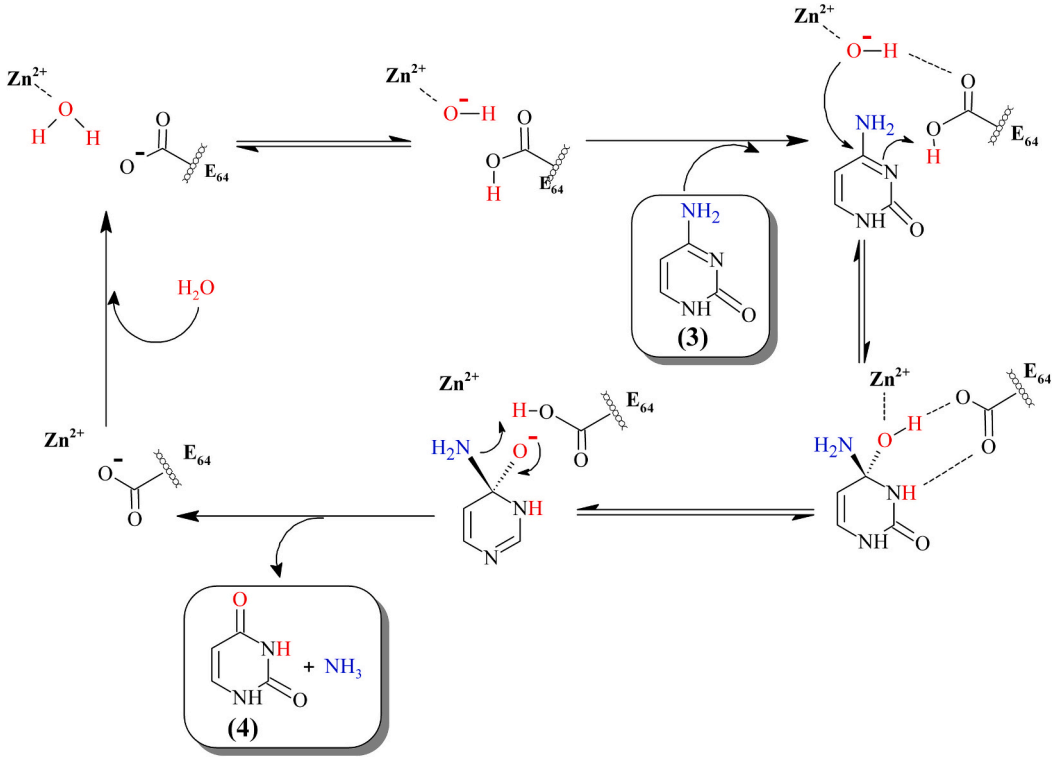


Fig. 4. Reaction mechanism of hydrolytic deamination of cytosine **(3)** to uracil **(4)** catalyzed by yeast CytDA (Ko et al., 2003).

content of beer (Mahor and Prasad, 2018).

2.2.1.2. Guanine DAs

2.2.1.2.1. General concepts. GuaDA (EC 3.5.4.3), also known as guanase or guanine aminohydrolase, catalyzes the hydrolytic deamination of Gua **(5)** into Xan **(6)**, releasing ammonia (Fig. 5) (El Kouni, 2003; Gaded and Anand, 2018). GuaDA is a zinc-dependent enzyme found in all lower and higher organisms. It plays a key role in the purine salvage pathway, regulating the guanine levels within the intracellular guanylic pool (Shek et al., 2019). In contrast to prokaryotes and lower eukaryotes, GuaDA expression in higher eukaryotes is tissue-specific and varies during different developmental stages of the organism (Paletzki, 2002). Many studies have demonstrated that GuaDA regulates dendritic ramification in mammals, being a key factor in the development of neuronal morphology (Charych et al., 2006; Chen and Firestein, 2007). In addition, Seffernick and coworkers established that GuaDA is also responsible for the deamination of ammeline (an intermediate in the melamine pathway) to ammelide (Seffernick et al., 2010). Notably, GuaDA is the only endogenous enzyme in eukaryotes capable of ammeline deamination.

Two types of GuaDA enzymes have evolved separately, and thus they are classified within the AHS or CDA superfamily (Holm and Sander, 1997; Liaw et al., 2004). According to COGs database, plant, archaeal, and some bacterial GuaDAs belong to the CDA superfamily (Fernández et al., 2010; Ko et al., 2003) and these are clustered within cog0590 (Gaded and Anand, 2018). Meanwhile, mammalian, insect, fungal, and other bacterial GuaDAs are clustered within AHS superfamily (Ko et al., 2003; Liaw et al., 2004), falling within cog0402 (Shek et al., 2019). Despite lacking structural similarities, GuaDAs from both superfamilies require the presence of a M^{2+} for the deamination reaction (Shek et al., 2019).

2.2.1.2.2. Biotechnological applications. Regarding biotechnological applications, similar to AdeDA, GuaDA has been utilized as a component of enzymatic systems for lowering the purine content in food and beverages. As mentioned above, a four-enzyme cocktail containing GuaDA, AdeDA, XOD, and UO was successfully employed to achieve complete reduction of the purine content in beef samples (Jankowska et al., 2015; Trautwein-Schult et al., 2014). As also mentioned for AdeDA, further practical examples include the use of GuaDA from *K. lactis* together with other purine degrading enzymes (*Kl*PNP, *Kl*XanA and commercial *Candida utilis* UO) for decreasing purine levels in beer (Mahor and Prasad, 2018). Apart from lowering the purine content in food and beverages, GuaDA can be also utilized for the production of caffeine **(15)** (Li et al., 2017a; Pan et al., 2019), providing a more environmentally friendly alternative to traditional chemical manufacturing procedures (Wang et al., 2008).

The primary caffeine synthetic pathway in tea plants involves several steps. First, Xao **(13)** undergoes methylation to form 7-MetXao **(16)**, which is then hydrolyzed to 7-MetXan **(17)**. Subsequently, it is methylated to reach theobromine **(18)** and caffeine **(15)** (Denoeud et al., 2014; Pan et al., 2019). However, different pathways for caffeine biosynthesis have been described for some microbes, wherein a methylation order differs from that observed in caffeine biosynthesis in plants (Pan et al., 2019) (Fig. 6).

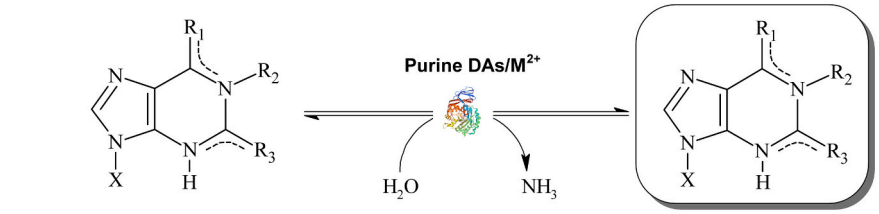
Nowadays, genetic and metabolic engineering techniques have significantly improved microbial platforms for the industrial production of plant-derived bioactive compounds (Sun et al., 2015). For instance, an increase in the caffeine level in pu-erh tea was reported when *Saccharomyces cerevisiae* GuaDA overexpression was induced in recombinant *E. coli* and *Corynebacterium glutamicum* during pile-fermentation (Pan et al., 2019).

Another interesting example involves the metabolic engineering of *E. coli* BL21 for *de novo* caffeine production from low-cost food sources (Li et al., 2017a). Since *E. coli* does not naturally synthesize this methylxanthine compound, a novel guanine-to-caffeine biosynthetic pathway was developed within the organism (Fig. 6). To this end, the codon-optimized *Camellia sinensis* TCS1 gene was introduced into *E. coli*. The recombinant *Ec*TCS1 enzyme catalyzes the methylation of Xan **(6)** to produce 3-MetXan **(19)**, followed by the conversion of 3-MetXan **(19)** to 1,3-DiMetXan **(20)**. Finally, *Ec*TCS1 converts 1,3-DiMetXan **(20)** to caffeine **(15)**.

Furthermore, in consideration of future industrial implementation, a series of genetic modifications were conducted to optimize the bioproduction of Xan **(6)** in *E. coli*. Initially, the *cs1* gene was cloned into T7 promoter containing pRSF-Duet-1 vector (pRSF-eCS1) to get a higher-level expression of the target enzyme. Subsequently, to further enhance S-adenosyl-L-methionine (SAM) **(21)** production, the coding sequences (*sma2* and *vgb* genes) for *S. cerevisiae* SAM2 and *Vitreoscilla* hemoglobin (*V*Hb), were also cloned in the vector pRSF-eCS1 (namely pRSF-eCS1-SMA2-vgb, according to the authors' nomenclature). This strategy aimed to increase SAM levels by leveraging the synergistic effects of these genes. Finally, a codon-optimized version of the *S. cerevisiae* GuaDA gene (*Sc*GuaDA) was further cloned into pRSF-eCS1-SMA2-vgb plasmid (pRSF-eCS1-SAM2-vgb-eGUD1), and *E. coli* competent cells were transformed with the final construct (Fig. 6). Through this metabolic engineering approach, the authors enabled TCS1 expression and enhanced Xan **(6)** and SAM **(21)** biosynthesis in *E. coli*, resulting in a significant increase in caffeine **(15)** production (Li et al., 2017a).

2.2.2. Purine nucleoside DAs

Purine nucleoside DAs, like purine nucleobase DAs, are primarily categorized based on their specificity towards 6-aminopurine and 6-oxopurine nucleosides (Table 1). In this regard, two distinct purine nucleoside DAs, adenosine DA (AdoDA) and guanosine DA (GuoDA), can be identified (Fig. 5). Although purine nucleoside DAs typically act on ribo- and 2′-deoxyribonucleosides, several instances of DA activity on other purine nucleosides have been documented. For instance, the deamination of 5′-deoxyadenosine (5′-dAdo) has been reported (Miller et al., 2013), as well as the deamination of *S*-methyl-5′-thioadenosine (MTA) (Hermann et al., 2007; Hitchcock et al., 2013), S-adenosylhomocysteine



(1) R_1= NH_2, R_2= H, R_3= H, R_4= H, X= H — AdeDA — **(2)** R_1= OH_2, R_2= H, R_3= H, R_4= H, X= H
(5) R_1= OH, R_2= H, R_3= NH_2, R_4= H. X= H — GuaDA — **(6)** R_1= OH, R_2= H, R_3= OH, R_4= H, X= H
(7-8) R_1= NH_2, R_2= H, R_3= H, R_4= H. X= (d)rib — AdoDA — **(9-10)** R_1= OH_2, R_2= H, R_3= H, R_4= H, X= (d)rib
(11-12) R_1= OH, R_2= H, R_3= NH_2, R_4= H. X= (d)rib — GuoDA — **(13-14)** R_1= OH, R_2= H, R_3= OH, R_4= H, X= (d)rib

Fig. 5. Enzymatic deamination of purine nucleobases and nucleosides catalyzed by purine DAs.

(SAH) (Zulty and Speedie, 1989; Hermann et al., 2007; Hitchcock et al., 2013; Miller and Maier, 2013), 6-aminodeoxyfutalosine (AFLDA) (Goble et al., 2013), and, more recently, N6-methyladenosine (Jiang et al., 2021).

2.2.2.1. Adenosine DAs

2.2.2.1.1. General concepts. AdoDA (E.C. 3.5.4.4), also called adenosine aminohydrolase, catalyzes the water-mediated deamination of Ado **(7)** and dAdo **(8)** to Ino **(9)** and dIno **(10)**, respectively (Gracia et al., 2012) (Fig. 5). AdoDAs are essential enzymes in the purine salvage network across living organisms, from mammals to bacteria. Both bacterial and mammalian AdoDAs are members of the AHS superfamily. Collectively, AdoDA are grouped within cog*1816*, a feature indicative of their common orthology (Miller and Maier, 2013). They also share a mononuclear Zn^{2+} center and display significant sequence similarity to one another (Seibert and Raushel, 2005; Miller et al., 2013). However, Jiang et al. (2021) recently described an AdoDA from *Bacillus subtilis*, which exhibits a novel activity against N6-methyladenosine and possesses a binuclear metal center.

Interestingly, two AdoDA isoforms have been identified: AdoDA1 and AdoDA2. The primary differences between the two AdoDA isoforms lie in the lower expression level (Atta et al., 2015) and substrate activity (Zhou et al., 2014) of AdoDA2 compared to AdoDA1. Additionally, AdoDA1 is expressed as an intracellular monomer enzyme with a molecular weight of 41 KDa, while AdoDA2 is specifically secreted as a homodimer with a molecular weight of 110 KDa (Skaldin et al., 2018; Gupta and Nair, 2006). AdoDA1 is considered ancient and plays a key role in maintaining cellular adenosine homeostasis (Skaldin et al., 2018). In addition, mammalian AdoDA1 is involved in the differentiation and maturation of the lymphoid system (Cristalli et al., 2001), therefore a disequilibrium in its activity can lead to severe combined immunodeficiency disease (Aldrich et al., 2000), or Diamond-Blackfan anemia (Hubert and Sutton, 2017). Conversely, AdoDA2 is considered a growth factor for the development and differentiation of endothelium cells and leukocytes in model organisms. Again, a disequilibrium in AdoDA2 activity can result in vasculopathy and immunological dysfunction (Dolezal et al., 2005; Zhou et al., 2014). Recently, Skaldin et al. (2018) linked AdoDA2 to cell signaling, particularly in organisms that rely on cell-cell communication, as well as quorum sensing in prokaryotic cells. Additionally, 2′-dAdo is a cytotoxic metabolite that promotes apoptosis, which also reinforces the essential role of AdoDAs for the survival of surrounding cells (Garcia-Gil et al., 2015a, 2015b; Giannecchini et al., 2003).

2.2.2.1.2. Biotechnological applications. Due to its broad substrate specificity, AdoDAs have been widely employed as catalysts for the synthesis of a wide range of nucleoside and nucleoside derivatives of pharmaceutical interest (Gupta and Nair, 2006; Trelles et al., 2018) (Fig. 7). It is well known that AdoDAs can deaminate 6-substituted and 2,6-substituted purine ribosides (Chassy and Suhadolnik, 1967). In this context, Nair et al. (2003) reported the dichlorination activity of AdoDAs over different substituted 6-chloropurine nucleosides with functionalized substitution at the 2-position. Interestingly, the broad-spectrum RNA antiviral 2-vinylinosine-5′-monophosphate **(21)** was synthesized by a chemo-enzymatic approach including AdoDAs, resulting in higher yield than the chemical approach (Pal et al., 2002; Nair et al., 1987). Similarly, Gupta and coworkers were able to increase up to 3.5-fold the production yield of 2-acetonylinosine **(22)** by employing AdoDA, while also reducing the reaction times compared to chemical synthesis. Another notable example of an AdoDA-catalyzed reaction is the synthesis of 2-aza nucleosides, such as 2-aza-2′-dIno **(23)** derived from 2-aza-2′-dAdo (Sugiyama et al., 2000).

Oxanosine and 2′-deoxyoxanosine are products of nucleoside nitrosation activity, which is involved in genotoxicity events (Lonkar and Dedon, 2011), and consequently serve as toxicological markers. Majumdar et al. (2005) demonstrated that AdoDA can transform oxanosine and 2′-deoxyoxanosine into 1-β-(D-ribo-furanosyl)-5-ureido-1H-imidazole-4-carboxylic acid **(24)** and 1-β-(D-2′-deoxyribofuranosyl)-5-ureido-1H-imidazole-4-carboxylic acid, respectively **(25)**. The lactone hydrolysis of oxanosine and 2′-deoxyoxanosine can be readily measured spectrophotometrically at 300 nm within the canonical nucleoside pool, making it suitable as a biological sensor.

Furthermore, due to the biological implications of AdoDA in metabolic drug inactivation (Rabie, 2022), inflammation (Bagheri et al., 2019), and infectious disease processes, researchers have been focusing their efforts on the development of AdoDA inhibitors. For this purpose, Sinkeldam et al. (2013) developed a high-throughput screening methodology based on fluorophore production. In this regard, AdoDA catalyzed the conversion of 1-(4-Aminothieno[3,4-*d*]pyrimidin-7-yl)-1,4-anhydropentitol to 1,4-Anhydro-1-(4-oxo-1,4-dihydrothieno[3,4-*d*]pyrimidin-7-yl)pentitol **(26)** which absorption/emission patrons are 339 nm and 410 nm, and 315 nm and 391 nm respectively. Regarding C-nucleoside fluorophore, Rovira et al. (2017) demonstrated the ability of AdoDA to convert different isomorphic and isofunctional adenosine analogs to other emissive purine-based nucleoside analogs with high rate and selectivity.

On the other hand, AdoDAs can also act on ribose-modified adenosine analogs. Early works demonstrated the significance of 5′-OH group in deamination reaction catalyzed by AdoDA (Maury et al., 1991). However, there are some exceptions to this general rule (Ciuffreda et al., 2002a). For instance, 5′-deoxy-5′-amino-2′,3′-O-isopropylidene adenosine was selectivity converted into the corresponding 6-oxopurine nucleoside, 5′-deoxy-5′-amino-2′,3′-O-isopropylidene inosine **(27)** by AdoDA from calf intestinal mucosa (calfAdoDA) at 98 % yield in just 360 min (1/24 time less than adenosine conversion) (Ciuffreda et al., 2002b). Also, calfAdoDA catalyzed the deamination of 5′-dAdo (26 % yield of isolated product) 5′-ClAdo (25 % yield of isolated product), and 5′-*O*-acetyladenosine (25 % yield of isolated product) into the corresponding 6-oxo derivatives **(28–30)** after 24 h (Ciuffreda et al., 2003). Recently, it has been reported several AdoDAs with unusually high activity on different modified 5′-nucleosides. Interestingly, these AdoDAs exhibit higher catalytic efficiencies than that shown for Ado. For instance, AdoDA from *Thermotoga maritima* showed greater catalytic efficiency for SAH ($5.8{\cdot}10^{4}$ M^{-1} s^{-1}) and MTA ($1.4{\cdot}10^{5}$ M^{-1} s^{-1}) compared to Ado ($9.2{\cdot}10^{3}$ M^{-1} s^{-1}) (Hermann et al., 2007). Likewise, AdoDA from *Methanocaldococcus jannaschii* catalyzes 5′-dAdo ($9.1{\cdot}10^{9}$ M^{-1} s^{-1}), SAH ($4.4{\cdot}10^{6}$ M^{-1} s^{-1}), and MTA ($1.1{\cdot}10^{6}$) with more efficiency than Ado ($7.5{\cdot}10^{5}$ M^{-1} s^{-1}) (Miller et al., 2013). Finally, a similar tendency is observed for AdoDA from *Pseudomonas aeruginosa* and *Plasmodium falciparum*. They catalyzed the deamination of MTA ($1.6{\cdot}10^{7}$ M^{-1} s^{-1} and $9.0{\cdot}10^{4}$ M^{-1} s^{-1}, respectively) more efficiently than Ado ($3.7{\cdot}10^{5}$ and $6.2{\cdot}10^{4}$ M^{-1} s^{-1}, respectively) (Guan et al., 2012).

Furthermore, AdoDA catalyzed the deamination process across a wide range of purine nucleosides bearing substitutions at positions 2′, 3′, and/or 4′, unfortunately, neither wild-type nor engineered AdoDA have shown activity on 1′-modified nucleosides. Cappellacci et al. (2002) determined that 1′-methyladenosine is not a substrate or inhibitor for AdoDA (Cappellacci et al., 2002; Vistoli et al., 2009). Regarding deamination on 2′-modified nucleosides, Taj et al. (2008), developed a chemo-enzymatic approach for the production of 2′-O-methoxyethylguanosine **(31)**, an important building block for the synthesis of chemotherapeutic oligonucleotides, starting from DAPR. In this approach, AdoDA catalyzed the enzymatic deamination of a mixture of 2′ and 3′ alkylated DAPR products to selectively afford the desired 2′-O-methoxyethylguanosine without resorting to chromatography for purification. The deamination reaction is completed after 70 h with less than 1 % of the 3′ alkylated byproduct, which could be easily removed during the next isobutyrylation step. This methodology was successfully applied to the kilogram scale, demonstrating its applicability for large-scale production.

In another practical example, Tritsch et al. (2000) tested different 3′-C-ethynyl-, 3′-C-ethenyl-, 3′-C-ethyl-(deoxy)nucleosides to study the

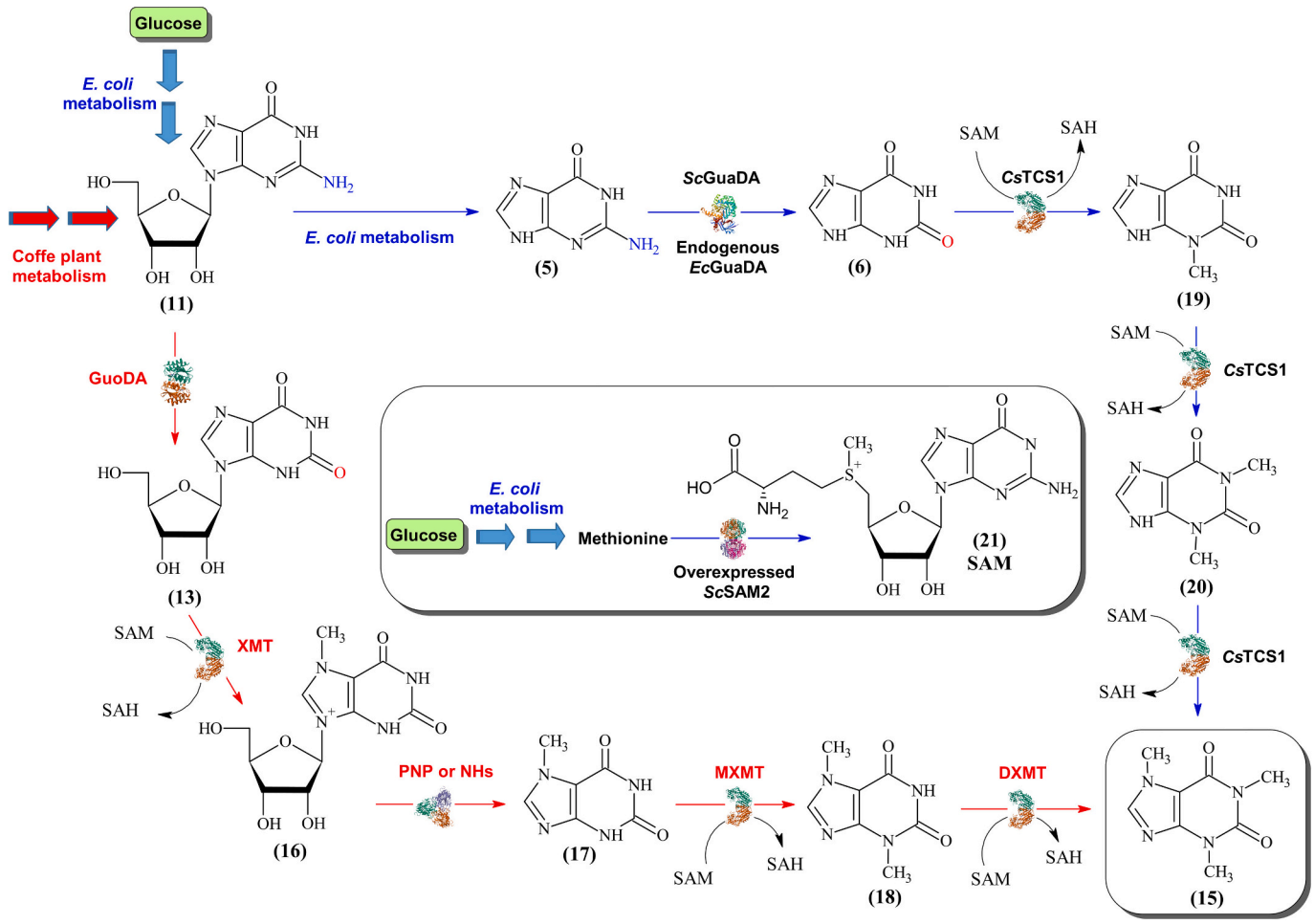


Fig. 6. Different metabolic networks for caffeine synthesis. (→) Plant *de novo* caffeine biosynthetic pathway in tea plants. (→) Engineered metabolic network for caffeine production from glucose in *E. coli*. GuoDA: guanosine deaminase; PNP: nucleoside phosphorylase; NH: nucleoside hydrolase; XMT: xanthosine methyltransferase; MXMT: methylxanthine *N*-methyltransferase; DXMT: 3,7-dimethylxanthine *N*-methyltransferase; SAM2: S-Adenosylmethionine synthetase; tea caffeine synthase, TCS1;

effect of the carbon hybridization (C_{sp}, C_{sp2} and C_{sp3}) on AdoDA activity. As a result, different 3′-β-branched (deoxy)inosine derivatives were synthesized for the first time **(32–35)**.

More recently, Díaz-Rodríguez et al. (2009), reported the chemo-enzymatic synthesis of different conformationally restricted bicyclic hexahydroisobenzofuran nucleoside analogs with potential anti-HIV activity from D-mannitol. Interestingly, AdoDa catalyzed the final step of this synthetic process (50 % yield; 1 % ADA, phosphate buffer pH = 7, 3 % DMSO, 35 °C, 72 h), leading to the synthesis of the bicyclic inosine analog **(36)**.

Didanosine (ddIno) **(37)** is a highly potent antiviral agent widely used for the treatment of HIV and Hepatitis B Virus infections. Consequently, different strategies have been developed for the synthesis of ddIno starting from ddAdo, using either whole cells carrying the AdoDA enzyme (73 % yield, 2 h) (Médici et al., 2008) or isolated AdoDA (87 % yield, 1.75 h) (Beach et al., 1991). It is worth noting that the use of whole-cells containing AdoDA enzyme as catalysts for the synthesis of nucleoside analogs offers several advantages over purified AdoDA enzyme. Notably, there is no need to purify the enzyme in whole-cell systems, and they also maintain activity at higher temperatures (60 °C) compared to the pure enzyme (50 °C). However, purified enzymes generally display higher overall activity than whole cells (Li et al., 2010). In efforts to get more cost-effective processes, both systems have been optimized to utilize less expensive starting nucleosides. On the one hand, *A. oxydans* was used in combination with other microorganisms in two-step processes (involving transglycosylation and deamination activity) to synthesize various 6-oxopurine nucleosides (Médici et al., 2008). On the other hand, a chemo-enzymatic synthesis of ddIno was developed, involving enzyme-mediated deamination of dAdo, followed by specific 5′ acylation catalyzed by *Candida antarctica* lipase, and subsequent chemical deoxygenation and 5′-deprotection step (Ciuffreda et al., 1999). More recently, calf AdoDA was used to transform ddA into ddI (100 mM phosphate buffer pH 7, 3 % DMSO, 95 %, 3 h) (Martín-Nieves et al., 2022).

AdoDA was also shown to be active against 8-substituted 2′,3′-dideoxy-6-aminopurine nucleosides, which show more antiviral activity and stability than 8-unsubstituted derivatives. For example, 8-amino, 8-hydroxy, and allopurinol 2′,3′-dideoxynucleoside analogs can be converted into corresponding oxo 2′,3′-dideoxynucleosides **(38)** (74 %, 4 h), **(39)** (79 %, 12 h), **(40)** (84 %, 2 h) (Buenger and Nair, 1990; Seela and Kaiser, 1988).

In another interesting example, Marquez et al. (1990) studied the conversion of 2′,3′-dideoxy-2′-ara-fluoroadenosine to the corresponding inosine derivative **(41)** by AdoDA, resulting in a 77 % yield after overnight incubation at room temperature (RT). However, in this case, a chemical synthesis with sodium nitrite in acetic acid allowed a higher yield (95 %, 20 h). Furthermore, to achieve more lipophilic anti-HIV-prodrugs (for the targeted delivery in central nervous system), a series of 6-substituted 2′,3′-dideoxy-2′-ara-fluoropurine nucleosides were tested as alternative substrates for human AdoDA. Experimental findings revealed that 6-fluoro nucleosides were shown to be the most active substrates (233 %, normalized from F-ddA; $IC_{50} < 5$ μM) followed, by

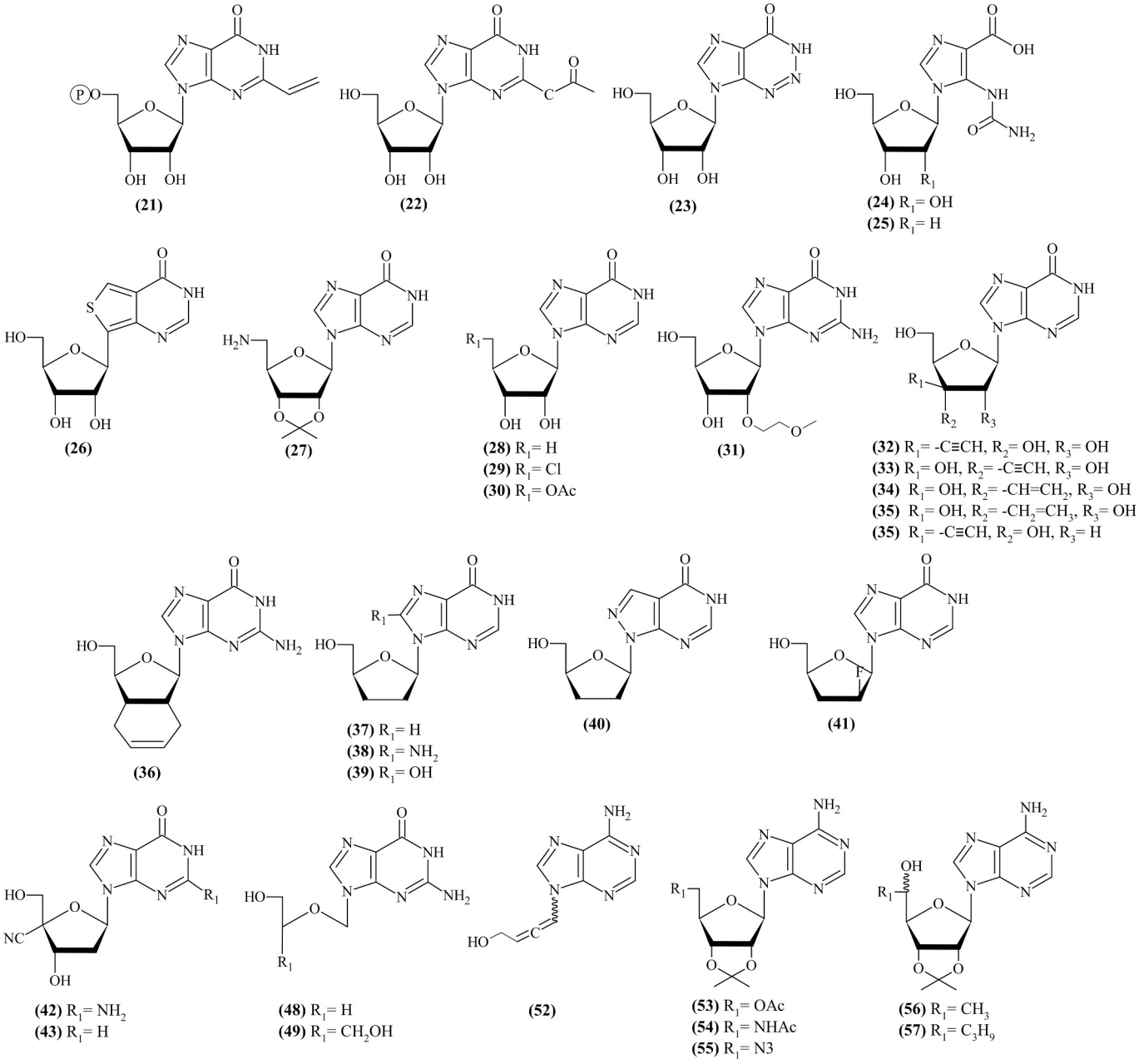


Fig. 7. Chemical structure different 6-oxo and 6-amino purine nucleoside derivatives.

far, by 6-chloro and 6-iodo derivatives (1.6 %; IC_{50} = 5.9 μM and 1.5 %; IC_{50} = 6.5 μM, respectively) (Driscoll et al., 1996; Ford et al., 1995).

Kohgo et al. (2004) developed an efficient chemoenzymatic synthesis of 4′-C-ethynyl-2′-deoxy purine nucleosides **(42–43)** with anti-HIV activity, using dAdo and DAP 2′-deoxyriboside as the starting materials. Compared to the conventional chemical methodologies of condensation of sugars with nucleobases, this methodology enhanced up to 3.4–17-fold the total yields. More recently, calf spleen AdoDA was found to be capable of transforming another set of different 4′-substituted nucleoside substrates into their corresponding guanosine analogs at high yields (exceeding 70 %, Tris-HCl buffer pH 7.5, 2–20 h, 40 °C) (Kohgo et al., 2018).

Delving into the endocyclic sugar moiety of Ado, Brakta et al. (2002) showed sugar moiety plays a crucial role in substrate binding to AdoDA. They demonstrated that appropriate substitution of a hydroxymethyl group and an adenine base on a benzene ring (ortho- or meta-) with an N9-C5' distance similar to that of adenosine, can mimic the structure of adenosine and, therefore, allows an efficient binding to AdoDA. These compounds have antiviral and antitumoral potential activity as aromatic neplanocin-A analogs (Kode and Phadtare, 2011).

Oxetanocin A (OXT-A), also known as 9-(2′-deoxy-2′-hydroxymethyl-P-D-erythro-oxetanosyl)adenine **(44)**, is a naturally occurring 4-member ring sugar moiety isolated from *Bacillus megaterium* with antibiotic activity. Additionally, related 6-oxopurine derivatives such as OXT-H (cyclobutyl hypoxanthine) **(45)**, OXT-X (cyclobutyl xanthine) **(46)**, and OXT-G (cyclobutyl guanine) **(47)** have antiviral activity. In addition, OXT-G, also called Lobucavir (BMS-180194), has been used for the treatment of herpes virus and hepatitis B infections. These all-related compounds were easily synthesized from OXT-A by enzyme-mediated deamination with AdoDA or *E. coli* 120,551 whole-cells (100 % yield) followed by OXT-H oxidation carried out by *Nocardia interforma* whole-cells (100 % yield) to reach OXT-X **(46)**. Then **46** was chemically converted (3 steps, 45 % yield) to 2-amino-OXT-A and finally deaminated again by AdoDA to produce OXT-G **(47)** (100 % yield) (Shimada et al.,

1987) (Fig. 8). Furthermore, Bristol-Myers Squibb prodrug Lobucavir l-Valine (BMS 233866) can be obtained through OXT-G transesterification using ChiroCLECTM BL (cross-linked enzyme crystals; 61 % yield). Alternatively, a more selective approach was achieved by using immobilized lipase from *Pseudomonas cepacia* (Amano PS-30), resulting in an 84 % yield) (Hanson et al., 2000; Simić et al., 2021).

Acyclonucleosides often show highly antiviral activity, such as acyclovir **(48)** or ganciclovir **(49)**. These 6-oxopurine analogs can be synthesized through a chemoenzymatic approach from different 2,6 substituted acyclonucleosides (including 2-amino-6-chloro- or 2,6-diamino-9-[(2-hydroxyethoxy)methyl]purines), by employing AdoDAs in phosphate buffer at 25 °C with a 100 % conversion rate (Ogilvie et al., 1984; Robins and Hatfield, 1982). Another complete hydrolytic defluorination for acyclovir **(48)** and ganciclovir **(49)** synthesis was achieved from 6-fluoropurine acyclonucleosides in presence of calf AdoDA excess and phosphate buffer solution at pH 7.5, carried out at RT overnight (Kim et al., 1994a).

In a similar way, the stereoselectivity of AdoDA allowed the resolution of a racemic mixture of synthetic carbocyclic nucleosides. Secrist et al. (1987) specifically deaminated (−)-aristeromycin **(50)** to obtain the corresponding hypoxanthine derivative (3 h, RT) from a mixture of (±)-racemates, facilitating the pharmaceutical active (+)-isomer purification (Fig. 9). Likewise, AdoDA was employed to transform the adenine moiety to access the (−)-enantiomer of carbovir, which is twofold more potent and selective inhibitor of HIV (Patel, 2008) (Fig. 9). In this regard, GlaxoSmithKline synthesized this API through a chemoenzymatic route starting from (−)-aristeromycin **(50)**, which is readily available as a secondary metabolite of *Streptomyces citricolor*. After 8 reaction steps, different immobilized AdoDAs (calf AdoDA or *Aspergillus sp* AdoDA) onto Eupergit-C support were employed in the bioconversion of 2′,3′-didehydro-2′,3′-dideoxy-DAP nucleoside into (−)-carbovir **(51)** for up to 10 cycles, without significant loss of activity. This bioprocess was carried out in 70 L medium containing 20 g/L of substrate, demonstrating the potential of AdoDA as a biocatalyst for large-scale production of optically pure (−)-carbovir **(51)** (Carter et al., 1990; Patel, 2008). Katagiri et al. (1998) studied the effect of pressure on AdoDA activity over cyclaradine, an anti-HSV carbocyclic analog of ara-A. The stereospecificity for enzymatic resolution of (+)-cyclaradine was enhanced by increasing pressure. Additionally, it was observed that (−)-cyclaradine was not deaminated by AdoDA, even under high pressures.

Another interesting example of the catalytic application of AdoDA in the resolution of racemic mixtures was developed by Megati et al. (1992). The authors obtained optically pure (−)-adenallene from the racemic (±)-adenallene **(52)**. Interestingly, the deaminated product, (+)-hypoxallene, was further converted to optically pure (+)-adenallene using well-established methodologies.

Far from their applications in the synthesis of APIs, numerous examples highlighting the potential of AdoDAs as therapeutic targets in many different diseases and malignancies can be found in the literature (Bagheri et al., 2019; Huang et al., 2019a; Kutryb-Zajac et al., 2020; Leone and Emens, 2018). Due to the major objective of this review is to focus on their ability as practical catalysts rather than the study of potential inhibitors, we do not deep into this field.

Nonetheless, it is noteworthy to mention that adenosine deaminase deficiency (AdoDA deficiency) is a rare genetic disorder that leads to severe combined immunodeficiency, a condition characterized by a dysfunctional immune system. In this context, ADAs have commonly used in the diagnosis and treatment of immunodeficiency disorders. For instance, Hershfield et al. (1987) demonstrated AdoDA enzyme replacement therapy using polyethylene glycol-modified AdoDA (PEG-AdoDA) has shown promising results in restoring immune function in ADA-deficient patients. More interestingly, gene therapy has emerged as a promising approach for the treatment of AdoDA deficiency (Gaspar and Kinnon, 2020; Secord and Hartog, 2022).

2.2.2.2. Guanosine DAs

2.2.2.2.1. General concepts and biotechnological applications. Guanosine DAs (EC 3.5.4.15) specifically deaminate 6-oxopurine nucleosides, such as Guo **(11)** and dGuo **(12)** to produce Xao **(13)** or dXao **(14)**, which are essential molecules in purine metabolism and nitrogen recycling (Fig. 5). GuoDA belongs to the CDA superfamily and are clustered within cog0590. These enzymes, involved in nucleobase salvage but also in nucleotide degradation, are particularly important in plants where the products from the degradation of purine nucleotides serve as nutrient sources (Dahncke and Witte, 2013).

As shown above, GuaDAs are essential enzymes in the catabolism of purines in animals, invertebrates, and microorganisms, converting guanine to xanthine. However, in most of plants, there is an alternative catabolic pathway that involves the presence of GuoDA (Dahncke and Witte, 2013). This pathway is closely linked to the biosynthetic route of purine alkaloids (caffeine and theobromine) in some plants, like coffee and tea (Denoeud et al., 2014; Pan et al., 2019; Witte and Herde, 2020) (Fig. 6).

Similar to AdoDAs, GuoDAs have been studied as markers for the diagnosis of different illnesses. For example, Kalkan et al. (1999) studied the levels of AdoDA and GuoDA activities in patients with different types of viral hepatitis disease. Experimental results suggested that GuoDA levels might serve as an indicator of liver condition in hepatitis diseases. More recently, again, AdoDA and GuoDA activities have been used as markers for the diagnosis of rheumatoid arthritis (Suleyman, 2012).

2.2.2.3. Other purine nucleoside DAs

2.2.2.3.1. General concepts and biotechnological applications. Although AdoDA and GuoDA are the most representative members of purine nucleoside DAs, another type of purine nucleoside DAs can be found in nature, such as S-adenosylhomocysteine deaminase (SAHDA, EC 3.5.4.28) or *S*-methyl-5′-thioadenosine deaminase (MTADA, EC 3.5.4.31). Both SAHDA and MTADA enzymes are classified as members of the AHS superfamily and are clustered within cog0402.

On the one hand, SAHDA is an enzyme that plays a crucial role in the metabolism of adenosylhomocysteine (SAH), a byproduct of S-adenosylmethionine (SAM) methylation reactions. SAHDA catalyzes the conversion of SAH to S-inosyl-L-homocysteine and ammonia, thereby regulating the intracellular levels of SAH (Hermann et al., 2007; Hitchcock et al., 2013). SAH is a strong inhibitor of DNA methyltransferases (James et al., 2002). Consequently, fluctuations in cellular SAH levels can alter the extent of genomic DNA methylation, which in turn influences the expression of numerous genes. Specifically, an increase in SAH levels is associated with decreased DNA cytosine methylation (Rocha et al., 2005), whereas a reduction in SAH leads to enhanced DNA methylation (Gotarkar et al., 2021). Considering these characteristics, it is plausible that modulating SAHDA activity could be used to adjust SAH levels, thereby affecting DNA and histone methylation patterns. Such an approach could offer valuable insights into epigenetic reprogramming and the regulation of gene expression.

On the other hand, MTADA catalyzes the deamination of MTA (a byproduct of polyamine biosynthesis and methionine metabolism), resulting in the production of 5-methylthioinosine (Hermann et al., 2007; Hitchcock et al., 2013). In eukaryotes and archaea, the degradation of MTA is carried out by 5′-methylthioadenosine phosphorylase (MTAP) in the presence of inorganic phosphate, leading to the generation of adenine and 5-methylthio-α-D-ribose 1-phosphate. However, Guan et al. (2012) suggested a MTA → 5′-methylthioinosine (MTI) → hypoxanthine pathway with no significant MTAP or 5′-methylthioadenosine nucleosidase (MTAN) activity, which would implicate the existence of an MTADA to convert MTA to MTI. Based on this evidence, the authors hypothesized a possible role of MTADA in an alternative *quorum sensing* pathway in *Pseudomonas aeruginosa*. The disruption of MTADA activity in *P. aeruginosa* (and other organisms that utilize this unusual pathway for MTA degradation) can potentially hinder the

communication and virulence processes mediated by *QS*, providing a new approach for combating bacterial infections.

2.2.3. Purine nucleotide DAs

2.2.3.1. General concepts and biotechnological applications. Despite nucleoside-5′-mono-, −di, and triphosphates can be considered nucleoside derivatives, we have chosen to include purine DAs that act on nucleotides in a separate category, namely purine nucleotide DAs.

Adenosine-5′-monophosphate DA (adenylate DA, AMPDA, EC 3.5.4.6) catalyzes the conversion of adenosine monophosphate, AMP **(58)**, to inosine monophosphate, IMP **(59)** and ammonia (Fig. 10) (Table 1). AMPDA plays a vital role in nucleotide metabolism, maintaining the balance of nucleotide pools which are critical for cellular functions and overall metabolism.

Returning to the studies conducted by Ciuffreda et al. (2002a, 2002b, 2003), which primarily investigated the enzymatic deamination of 5′-substituted derivatives by AdoDA, the authors simultaneously examined the deaminase activity of AMPDA on a variety of different 5′-substituted derivatives. Remarkably, despite ADA's inability to convert some 5′-substituted-2′,3′-O-isopropylidene adenosine derivatives such as acetate, acetamido, and azido **(53–55)**, the authors demonstrated that AMPDA converts all of these compounds into their corresponding 5′-substituted inosine derivatives during their biotransformation process (Ciuffreda et al., 2002b). Furthermore, the same authors also demonstrated both AMPDA and AdoDA enzymes exhibit the ability to catalyze the stereoselective hydrolytic deamination of (5′*R,S*)-methyl-2′,3′-isopropylidene adenosine **(56)**. However, it was observed that the 5′-butyl analog **(57)** was exclusively converted by AMPDA, indicating the substrate specificity of AMPDA for this particular compound (Ciuffreda et al., 2004).

Notably, a novel use of AMPDA as a potential enhancer of umami flavor has been recently proposed by Chew et al. (2017). The umami flavor arises from the existence of glutamate and is significantly intensified with the inclusion of nucleoside-5′-monophosphates, such as IMP **(59)** and GMP **(60)** (Del Arco et al., 2017). Therefore, substantial changes in glutamate, IMP and GMP levels could modify the umami taste in food. In this work, the authors generated transgenic tomato (*Solanum lycopersicum*) lines that overexpressed AMPDA. They found that the increased expression of AMPDA resulted in elevated levels of GMP in the nucleotide pool, while IMP did not accumulate to significant levels (Fig. 10).

In a study by Kotchoni et al. (2016), a recombinant green alga *Chlamydomonas reinhardtii* was engineered through the downregulation of AMPDA expression. AMPDA is responsible for counteracting the accumulation of AMP by removing it from the adenylate pool in response to elevated ATP levels. This genetic manipulation resulted in significant increases in the steady-state levels of intracellular ATP. Notably, the observed ATP elevation led to remarkable enhancements in biomass production (3-fold higher growth rate), cold tolerance, and oil content (approximately 25 % higher lipid/oil). Based on these findings, the authors put forward the hypothesis that these results could be extrapolated to engineer plants with improved growth under cold stress conditions, thus contributing to sustainable agriculture and plant-based biofuel production. More recently, an experimental study conducted by Zhang et al. (2019) demonstrated that manipulating the copy numbers of three heterologous lycopene biosynthesis genes (*crtE*, *crtB*, and *crtI*), along with overexpressing the gene encoding AMPDA, resulted in the highest reported lycopene content (46–60 mg/g DCW) in *Yarrowia lipolytica*.

Like AMPDA, adenosine-5′-diphosphate DA (ADPDA, EC 3.5.4.7) and adenosine-5′-triphosphate DA (ATPDA, EC 3.5.4.18) catalyzes the hydrolytic deamination of ADP and ATP into corresponding 6-oxo derivatives. Unfortunately, there is no available information regarding the biotechnological application of these enzymes. A similar situation can be observed for cyclic adenylate deaminase (cAMPDA, EC 3.5.4.46), a key enzyme in cellular signaling by catalyzing the conversion of cyclic adenosine monophosphate (cAMP) to 5'-AMP.

2.2.4. Other purine deaminases

DAs acting on free nucleobases, nucleosides, and nucleotides have been the primary focus of this review. All these enzymes, whether from the AHS or CDA superfamily, play a pivotal role in nucleotide

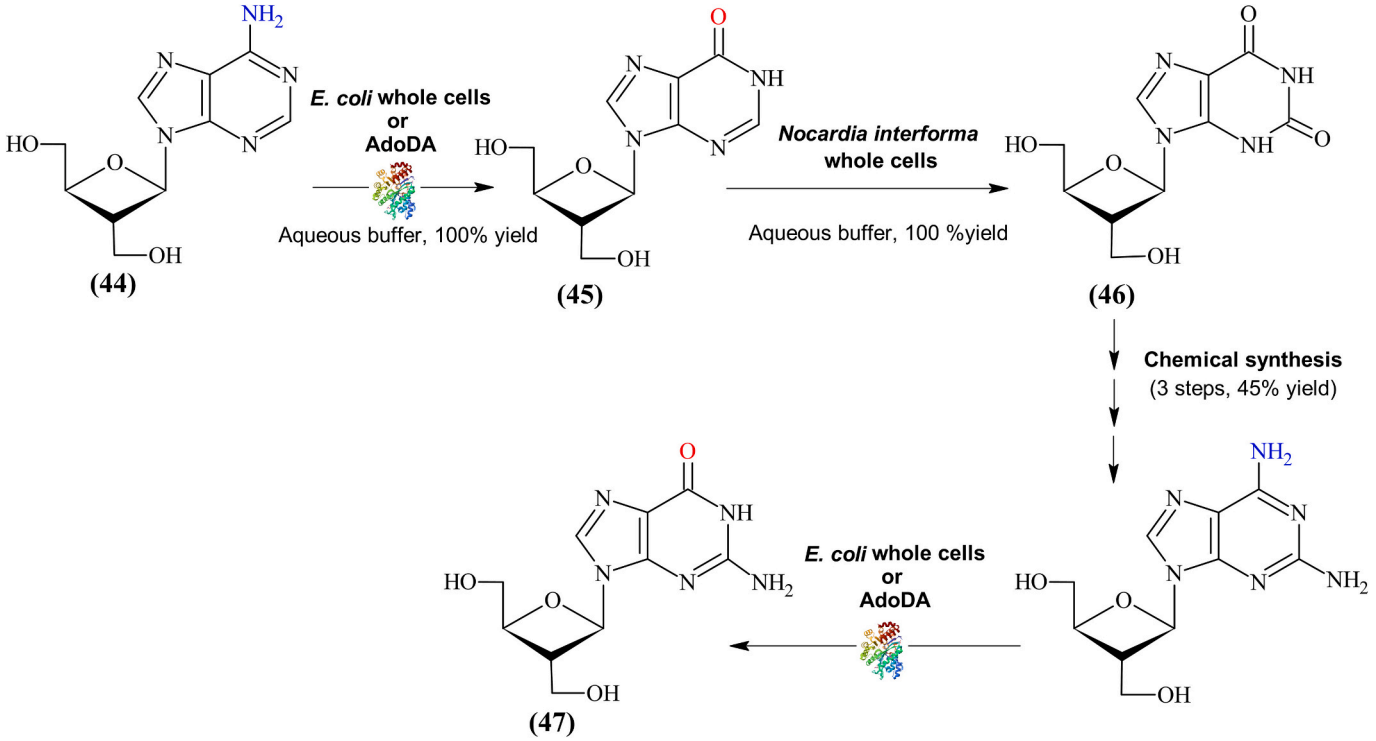


Fig. 8. Enzymatic and chemo-enzymatic synthesis of different oxetanocin analogues catalyzed by AdoDA.

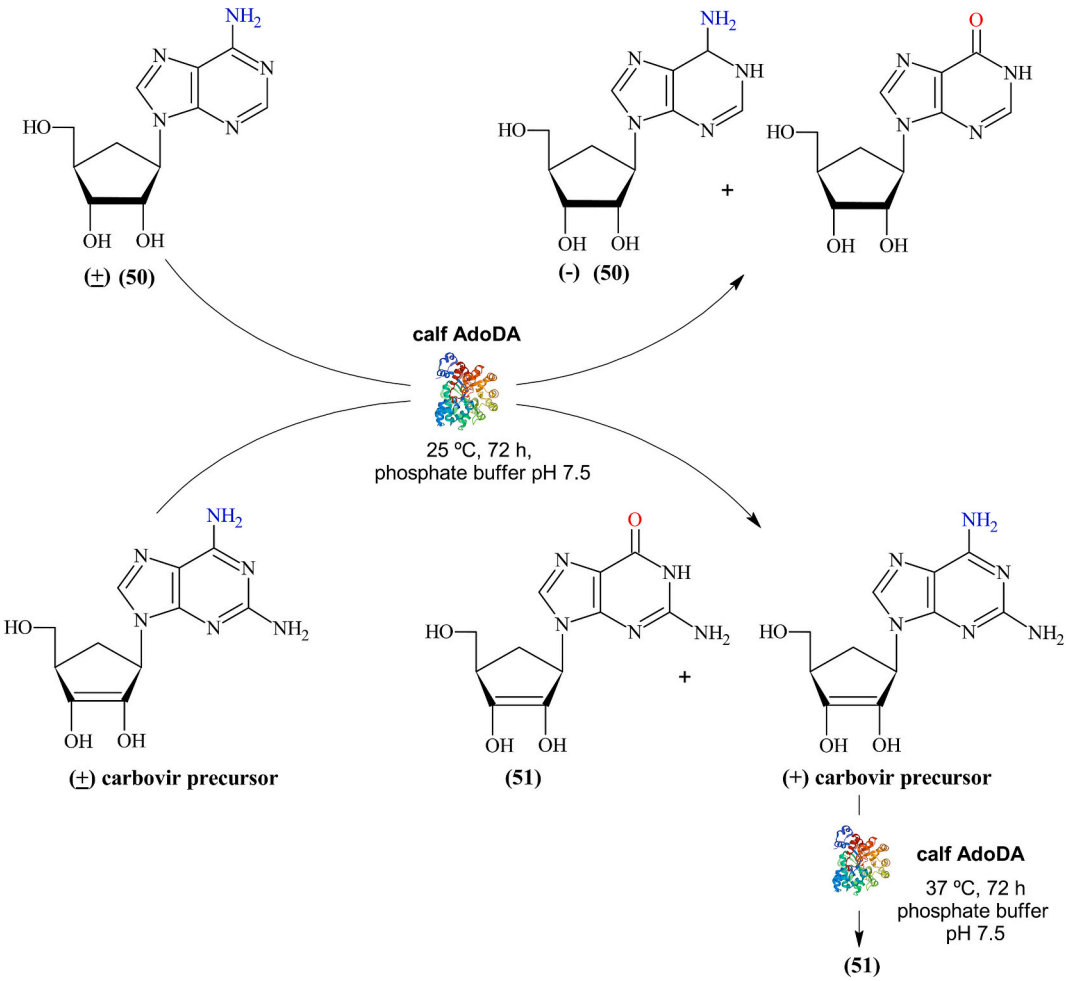


Fig. 9. Enzymatic resolution of racemic mixtures of synthetic carbocyclic nucleosides catalyzed by AdoDA.

metabolism (Iyer et al., 2011). In addition, as mentioned earlier, these enzymes have found extensive utility in various biotechnological applications. However, among the DAs catalyzing the hydrolytic deamination of nucleic acids, those acting on polynucleotide molecules also deserve special attention. These enzymes facilitate the *in situ* deamination of the nitrogenous bases present in RNA, thereby contributing to gene diversification and antiviral defense mechanisms (Gaded and Anand, 2018).

2.2.4.1. General comments. Among the post-transcriptional mechanisms, RNA editing reactions (insertion, deletion, or base modification) occur in many organisms and operate by different molecular mechanisms (Gerber and Keller, 2001; Savva et al., 2012; Schaub and Keller, 2002). In this regard, the hydrolytic deamination of Ado-to-Ino (referred to as A-to-I editing in the literature) at RNA level by RNA-dependent AdoDAs is an example of RNA editing. According to the literature, two types of AdoDAs acting on RNA have been reported, those acting on duplexed RNA structures (commonly named ADARs), and tRNA-specific AdoDAs (so-called ADATs) (Fig. 11) (Table 1).

2.2.4.1.1. AdoDAs acting on duplexed RNA structures. AdoDAs that act on RNA (ADARs) catalyze the hydrolytic deamination of Ado-to-Ino (referred to as "A" in Fig. 11) in double-stranded RNAs (dsRNA) (Bass, 2002) (Fig. 11A). Specifically, ADARs act on precursor mRNAs (pre-mRNAs) and noncoding RNAs (microRNA and endogenous siRNA) (Nishikura, 2010; Hundley and Bass, 2010; Warf et al., 2012). The conversion of Ado-to-Ino results in alterations to the information encoded in the primary sequence of the pre-mRNA, leading to changes in codons within open reading frames and modifications in RNA splicing patterns (Keegan et al., 2001). Moreover, since Ino is recognized as Guo during translation (base pairing with cytidine), the introduction of Ado-to-Ino changes by ADARs creates inosine-uridine mismatches, which in turn affect the structure of the RNA molecule (Bass, 2002). These alterations could potentially disrupt any biological function reliant on structure-specific interactions with RNA (Bass, 2002). Additionally, ADAR enzymes also target viral RNAs, thereby contributing to defense against viruses that possess double-stranded RNA stages in their life cycle (Kumar and Carmichael, 1997).

First discovered in *Xenopus laevis* (Bass and Weintraub, 1987), ADARs have evolved from the CDA superfamily and are present in most metazoans, including mammals. It is worth noting that multiple ADAR enzymes can coexist within the same organism, and such is the case for vertebrates that possess three distinct ADARs: ADAR1 (Kim et al., 1994b), ADAR2 (Melcher et al., 1996) and ADAR3 (Chen et al., 2000). Contrary to ADAR1 and ADAR2, ADAR3 does not exhibit the ability to deaminate any target site in RNA (Nishikura, 2016). Despite its lack of catalytic activity, ADAR3 can bind both double-stranded RNA (dsRNA) and single-stranded RNA (ssRNA), potentially exerting modulation on the editing activity of other ADARs (Chen et al., 2000). ADAR1 and ADAR2 in vertebrates display slightly different specificities regarding the target adenosine within the dsRNA (Bass, 2002). In addition, while ADAR1 is ubiquitously expressed in many tissues (Kim et al., 1994b), ADAR2 is mainly, though not exclusively, expressed in the brain (Melcher et al., 1996). Conversely, ADAR3 expression is limited to certain regions of the brain (Chen et al., 2000).

Regardless of the species, all ADARs display great similarities in their structures, with one to three dsRNA binding domains (dsRBDs) in the

vicinity of the N-terminal, and a highly conserved deaminase domain near the C-terminal (Bass, 2002; Maas et al., 2003). As of the present date, all reported ADARs exhibit notable structural similarities, featuring one to three dsRBDs located near the amino-terminal region, and a highly conserved deaminase domain situated close to the C-terminal (Bass, 2002; Maas et al., 2003). In all cases, the dsRBD comprises approximately 65 amino acids and adopts an α-β-β-β-α topology, while the C-terminal deaminase domain constitutes the catalytic center of ADARs (Nishikura, 2016).

The sequence analysis of this deaminase domain suggests a close sequence relation to the CDA superfamily (Wang et al., 2017). Moreover, the active site of ADAR displays a geometry like that of members within the CDA superfamily. In fact, much like CDA enzymes, the zinc ion present in the active site is coordinated by one histidine and two cysteine residues (Macbeth et al., 2005). Besides differing in the total number of dsRBDs, the distance between these domains also varies among different ADAR enzymes (Bass, 2002). Further structural differences have been observed among these enzymes, which are unique to the type of ADAR. For example, ADAR1 possesses two additional *Z*-DNA-binding domains (Zα and Zβ) located in a N-terminal extension (Herbert et al., 1997), while an arginine-rich single-stranded RNA (ssRNA)-binding domain (R domain) is present in ADAR3 (Chen et al., 2000). These domains might play a role in positioning the enzyme close to actively transcribed DNA (Herbert and Rich, 2001). However, a more comprehensive investigation is needed to gain a deeper understanding of the precise functions of these unique domains.

2.2.4.1.2. AdoDAs acting on tRNA. When talking about RNA editing, it is important to note that ADARs are not the sole DAs responsible for this function. Prokaryotic organisms, for instance, contain transfer RNA (tRNA) adenosine deaminase (TadA) capable of editing specific tRNAs (Wolf et al., 2002). In eukaryotes, orthologous enzymes of TadA are classified as AdoDAs acting on tRNAs. These include ADAT in higher eukaryotes and Tad in yeast (Keegan et al., 2004). ADAT and Tad enzymes share significant sequence similarity with ADARs, particularly in terms of their catalytic domain, although they lack the dsRBDs. The close resemblances between these enzymes suggest that ADARs may have evolved from ADATs through the acquisition of one or more dsRBDs (Savva et al., 2012). These similarities are also observed in certain members of the CDA superfamily, which strongly suggests that both ADAT, Tad and ADAR enzymes evolved from this superfamily (Bass, 2002).

Depending on the specific ADAT (in higher eukaryotes) or Tad (in yeast) Ado-to-Ino deamination occurs at distinct target sites within the tRNA (Fig. 11B). For example, ADAT1 and Tad1 target Ado at position 37 (A37 in the literature) in eukaryotic $tRNA^{Ala}$, located adjacent to the anticodon (Keegan et al., 2004). Meanwhile, in higher eukaryotes, the ADAT2-ADAT3 heterodimeric complex deaminates Ado at position 34 (A34) of tRNA, which is the wobble position of the anticodon. In this

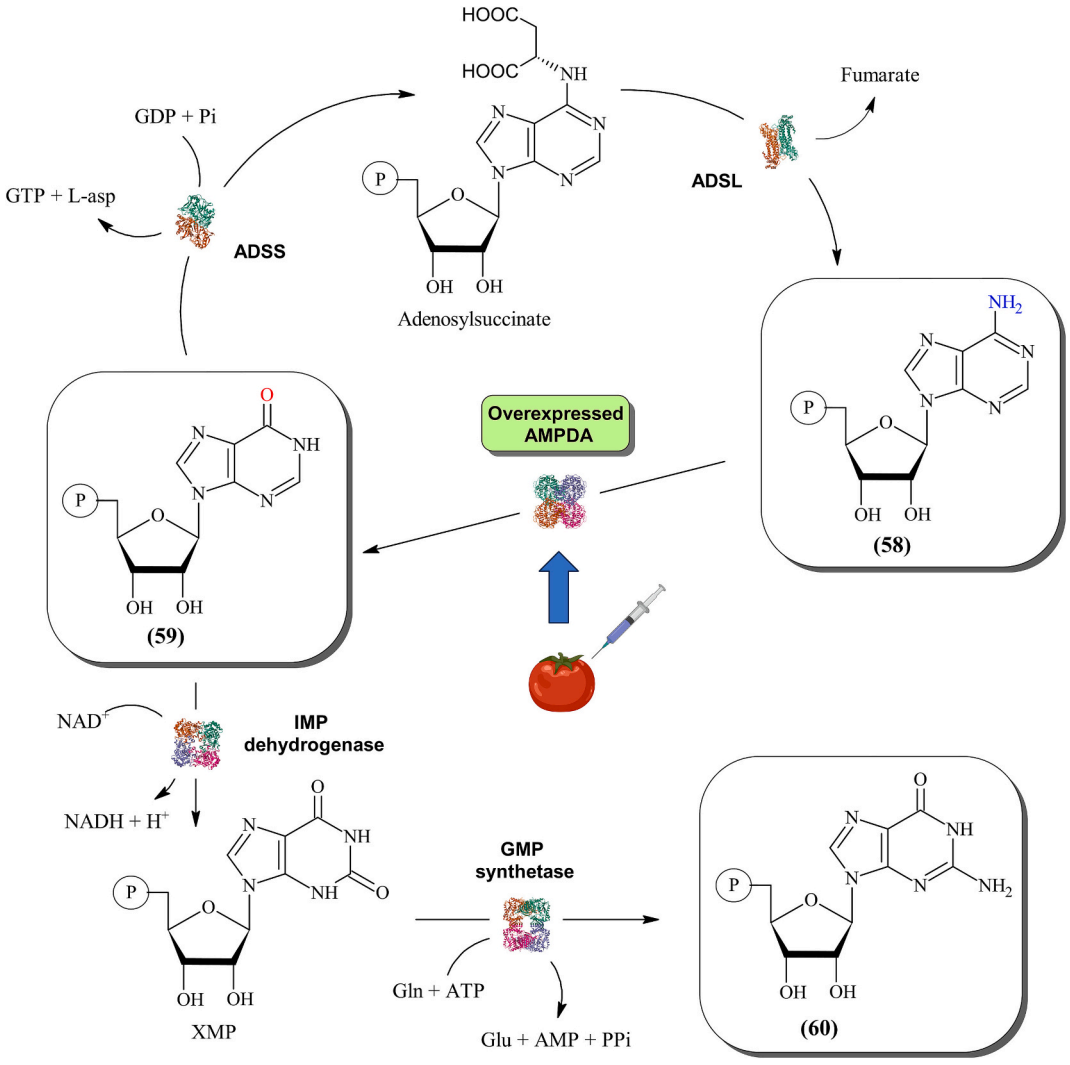


Fig. 10. Schematic representation depicting the biosynthetic pathway of AMP and GMP from IMP. ADSS: adenosylsuccinate synthetase; ADSL: adenosylsuccinate lyase; AMPDA: adenylate DA.

complex, ADAT3 is essential for tRNA binding but is catalytically inactive, leaving ADAT2 as the only active subunit of the heterodimer (Keegan et al., 2004). In yeast species like *Saccharomyces cerevisiae*, the homologous enzymes Tad2 and Tad3 perform a similar function, also acting as a heterodimer to deaminate A34 (Keegan et al., 2004). In contrast, in prokaryotes, the TadA enzyme, homologous to ADAT2, forms homodimers to catalyze A34 deamination specifically in the $tRNA^{Arg2}$ molecule, the only tRNA with this modification in prokaryotes (Wolf et al., 2002).

2.2.4.2. Biotechnological applications. Adenosine deaminase-mediated RNA editing represents an intrinsic mechanism of site-specific mutagenesis that is evident across all metazoans. As a result, the RNA editing capabilities of ADARs have proven their effectiveness in creating precise and reliable tools for RNA manipulation. Furthermore, despite the absence of reported instances of ADARs acting on single-stranded DNA (ssDNA) (Rees and Liu, 2018; Zheng et al., 2017), the direct evolution of a TadA enzyme has successfully led to deaminase activity at the DNA level (Gaudelli et al., 2017). As we will discuss later, this significant breakthrough has paved the way for the development of adenosine base editors for genomic engineering.

2.2.4.2.1. Adenosine base editors for RNA editing. RNA-editing has arisen as an attractive technology for the development of novel tools for *in vivo* research and therapeutic applications. As the name suggests, the alterations executed by RNA-editing systems take place at the RNA level, thus providing a clear safety advantage over the traditional DNA-editing tools. Since modifications are only introduced in transcripts carrying disease-causing point mutations, the genomic information remains unaltered (Aquino-Jarquin, 2020). In contrast, DNA editing requires a lower off-target rate (ideally below 0.1 %) to ensure safety, as even minor off-target effects can lead to permanent and potentially harmful genomic alterations (Tao et al., 2023). RNA editing, on the other hand, offers a more transient and reversible approach, reducing the risk of permanent damage from undesired off-target modifications (Casati et al., 2021). While RNA editing can tolerate a slightly higher off-target rate, efforts to minimize it are still crucial. The effects of RNA editing are dose-dependent, with their duration determined by the length of the administration period (Casati et al., 2021). However, while the exact threshold may vary depending on the specific disease, therapeutic approach, and target tissue, RNA editing *in vivo* should ideally achieve conversion efficiencies as high as possible (exceeding 50 %) for therapeutic relevance (Tao et al., 2023).

One of the main challenges when designing RNA-editing strategies for gene therapy applications is the capacity to target defined positions within distinct RNA transcripts. For achieving accurate base editing, either the full ADAR enzyme or solely the deaminase domain ($ADAR_{DD}$) is expressed as a fusion protein incorporating an RNA targeting domain (SNAP-tag, a λN peptide, an R/G motif, a Cas protein, or an MS2-tag) to form a fusion editase (Fig. 12A-D). Hereafter, this fusion-ADAR is introduced along with a guide RNA (gRNA), which is complementary to the target RNA sequence and capable of drawing the editase (Casati et al., 2021). Upon formation of the RNA-ADAR complex at the target site, Ado is deaminated into Ino, which is read as Guo during translation. Thus, Ado-to-Ino (referred to as A-to-I in the literature) is transformed into Guo, leading to the Ado-to-Guo (referred to as A-to-G in the literature) conversion. Consequently, when editing occurs within mRNAs, codons may change, leading to modifications that could impact protein functionality. These site-directed RNA editing approaches entail the administration of both the exogenous ADAR fusion editase and the gRNA, which could be challenging for therapeutic applications. In this regard, some groundbreaking procedures have been reported wherein site-directed base editing is carried out by the implementation of guides capable of luring endogenous ADARs (Merkle et al., 2019; Qu et al., 2019; Reautschnig et al., 2022; Monian et al., 2022; Katrekar et al., 2022a; Yi et al., 2022).

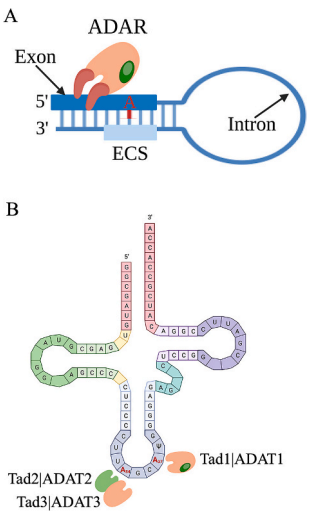


Fig. 11. Schematic representation of RNA-dependent ADAR and TadA deaminases, along with their respective substrate RNAs. The target adenosines are highlighted in red, the catalytic domains within the deaminases are depicted in green, and dsRBDs are shown in red. A) ADAR catalyzed Ado-to-Ino editing in pre-mRNAs. The target Ado (A) is located within an RNA duplex structure, formed through the pairing of exonic and intronic sequences, denoted as ECS (exon complementary sequence). B) TadA catalyzed Ado-to-Ino editing in yeast tRNA-Ala. The Ado at position 34 (A_{34}) is deaminated to Ino through the action of the Tad2p-Tad3p heterodimer. Additionally, the Ado at position 37 (A_{37}) is deaminated by Tad1p. Figure adapted from Gerber and Keller (2001). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The SNAP-ADAR was the first site-directed RNA editing approach that employed an exogenous ADAR fusion editase (Stafforst and Schneider, 2012). To this end, the authors employed the SNAP-tag protein, a self-labeling protein evolved from the DNA repair enzyme O6-alkylguanine-DNA alkyltransferase which covalently bonds to O6-benzylguanine (BG) (Keppler et al., 2003). The $ADAR_{DD}$ of human ADAR1 (lacking the dsRBDs) was combined with this SNAP-tag to create a SNAP-$ADAR1_{DD}$ fusion protein capable to target a short gRNA carrying BG (BG-gRNA). Thus, BG-gRNA determines the specificity of SNAP-$ADAR1_{DD}$. When paired with the target RNA sequence, BG-gRNA recruits the editing fusion protein, thereby initiating site-specific deamination (Fig. 12A). Initially, the developed system was employed for the *in vitro* repairing of the nonsense mutation $Stop^{66}$ (UAG) to Trp^{66} (UGG) within the ORF of the gene encoding the enhanced green fluorescent protein (eGFP), revealing a 60–90 % Ado-to-Guo conversion (Stafforst and Schneider, 2012).

Later on, seeking a future *in vivo* implementation, system components and the working procedure were modified (Vogel et al., 2014). First, the gRNA was subjected to both 2'-*O*-methylation and phosphorothioate modifications for improving its resistance against endogenous RNases. Additionally, in the pursuit of enhancing conversion rates, the $ADAR1_{DD}$ in the fusion protein was swapped for human $ADAR2_{DD}$. The clinical significance of this newly developed system was demonstrated *in vitro* by successfully repairing (70 % of editing efficiency) a disease-causing Factor 5 Leiden missense mutation (Vogel et al., 2014). In this context, the same authors conducted an extensive study on the potential modifications of gRNAs to optimize the selectivity and efficiency of the RNA editing system. The editing capacity of both $ADAR1_{DD}$ and $ADAR2_{DD}$ was tested on the four possible target codons (UAG, AAG, CAG, and GAG) by testing 64 gRNAs covering all possible match or mismatch combinations at the 5′-adjacent base of the target codon. In addition, these gRNAs carried Urd or Cyd as the complementary base to the targeted Ado to also cover a match or a mismatch, respectively, at this position. The experimental results indicated distinct preferences for

both the $ADAR_{DD}$ and the gRNA depending on the target codon, highlighting the need to optimize the SNAP-$ADAR2_{DD}$/gRNA system based on the specific target (Schneider et al., 2014). All these previous studies paved the way for the eventual development of a ready-to-use SNAP-ADAR system for site-directed editing of endogenous RNAs (Vogel et al., 2018). This time, a hyperactive version of $ADAR2_{DD}$ (E488Q mutant) (Kuttan and Bass, 2012) was utilized in the SNAP-ADAR system for the editing of two disease-related mRNAs coding KRAS and STAT1 signaling proteins. These transcripts were edited both individually and simultaneously, achieving a 46–76 % yield (Vogel et al., 2018). The E488Q variant was originally generated through random mutagenesis using error-prone PCR, which introduced a high frequency of mutations throughout the coding sequence of $ADAR2_{DD}$ (Kuttan and Bass, 2012). This approach led to the discovery of the E488Q mutant, which has since become an essential tool in RNA editing systems due to its significantly enhanced catalytic activity. The increased activity of the E488Q variant makes it particularly valuable for applications requiring efficient RNA editing. However, this heightened catalytic efficiency comes with a trade-off: the E488Q mutant exhibits reduced specificity, resulting in higher rates of off-target editing. This balance between enhanced activity and decreased specificity is a critical factor to consider in the development and application of RNA editing systems that incorporate the E488Q variant.

Apart from SNAP-ADAR, several other ADAR-mediated systems have been developed based on the use of protein components from bacteriophage organisms (Montiel-Gonzalez et al., 2013; Montiel-González et al., 2016; Vallecillo-Viejo et al., 2018; Sinnamon et al., 2017; Sinnamon et al., 2020; Katrekar et al., 2019; Tohama et al., 2020). One notable example is the λN-ADAR system, which is based on the ability the λ-phage N protein to non-covalently bind the boxB RNA hairpin structure (Legault et al., 1998). In this system, the RNA editing protein entails the fusion between $ADAR2_{DD}$ and the λ-phage N peptide (λN-$ADAR2_{DD}$), while the gRNA (complementary to the target RNA sequence) contains the boxB structure (boxB-gRNA) necessary for recruiting the λN-$ADAR2_{DD}$ fusion editase (Montiel-Gonzalez et al., 2013) (Fig. 12B). In a first approach, a λN-ADAR system was successfully employed *in vitro* to fix a premature stop codon in cystic fibrosis transmembrane conductance regulator (CFTR) encoding mRNAs (Montiel-Gonzalez et al., 2013). Thus, both λN-$ADAR2_{DD}$ fusion and boxB-gRNA were injected along with mutated CFTR transcripts in *Xenopus oocytes*, resulting in a 20 % editing efficiency and restoring the expression of the full-length protein. Finally, the RNA editing system was also tested in HEK293T cells, where it successfully corrected another premature stop codon to restore the activity of a nonfunctional enhanced eGFP, achieving a 20 % efficiency as confirmed by fluorescence readout (Montiel-Gonzalez et al., 2013).

Later on, in order to optimize λN-ADAR system (Montiel-González et al., 2016), a Cyd mismatch opposite to the targeted Ado (Ado-to-Cyd mismatch) and two boxB hairpins where introduced in the gRNA (Schneider et al., 2014). Similarly, the editing efficiency was further enhanced by utilizing a hyperactive $ADAR2_{DD}$ (E488Q mutant) and by the fusion of up to four λN peptides to this mutant $ADAR2_{DD}$ (4λN-$ADAR2_{DD}$). The optimized 4λN-ADAR system was employed in HEK293T cells for the editing of a premature termination codon in mRNAs encoding two fluorescent reporters (mCherry-eGFP). This led to an improvement in editing efficiency up to 80 %, however the hyperactive deaminase domain displayed high off-target editing (Montiel-González et al., 2016). Although off-target changes are less concerning in a transient system compared to DNA editing approaches, significant efforts have been made to completely eliminate unwanted off-target editing (Vallecillo-Viejo et al., 2018). In this respect, given that human ADAR2 is naturally located in the nucleus (Desterro et al., 2003), a nuclear localization sequence (NLS) was added to the N-terminal of the fusion construct (NLS-4λN-$ADAR2_{DD}$). Thus, the editing enzymes were redirected from the cytoplasm to the nucleus, reducing the off-target editing levels while maintaining the on-target editing efficiency. However, although the unspecific editing was reduced to a great extent, it was not entirely avoided, which indicates that the λN-ADAR system must be further optimized for therapeutic applications.

The recent advances in the development of the λN-ADAR system turn out in the implementation of this system for the editing of endogenous mRNAs. The initial effort in this endeavor involved correcting a Guo to Ado point mutation within the *MECP2* (methyl-CpG binding protein 2) gene, which causes the neurological disorder named Rett syndrome (RTT) (Sinnamon et al., 2017). To achieve this objective, the hyperactive λN-$ADAR2_{DD}$ (E488Q mutant) was fused to three copies of NLS from the large T-antigen of Simian virus 40 (SV40). Furthermore, the gRNA was optimized by adding two boxB structures and a Cyd opposite to the target Ado (Ado-to-Cyd mismatch). Once optimized, both the editase and the gRNA were endogenously expressed in cultured RTT neurons from mice. The authors reported a remarkable 72 % editing efficiency, and most importantly, the function of the MeCP2 protein was completely restored. However, despite a reduction in off-target editing events, several off-target sites were still detected. Fortunately, none of these off-target modifications resulted in pathological effects. These findings underscore the need for comprehensive global transcriptomic analysis to assess the extent of off-target editing and further optimization of the system to minimize unwanted edits, particularly for its therapeutic application. More recently, the same approach was applied *in vivo* in a RTT (*MECP2*$^{317G>A}$) mouse model (Sinnamon et al., 2020). Editing efficiency in mice was around 50 %, and localization of MECP2 protein to heterochromatin was observed at rates around 50 %, comparing to those values reported for the wild-type protein. Finally, around 30 % off-target editing was observed, with these undesired events increasing proportionally to the expression levels of the fusion editase (Sinnamon et al., 2020). Unfortunately, while the editing efficiency may already be sufficient for clinical application, the significant amount of off-target editing currently limits its therapeutic potential.

In recent years, several other site-directed RNA editing techniques have utilized similar strategies by employing protein components from bacteriophage organisms. In this context, the naturally occurring interaction between MS2 bacteriophage coat protein (MCP) and the RNA stem-loop from its genome can be exploited for the recruitment of ADAR enzymes (Katrekar et al., 2019; Tohama et al., 2020). In these techniques, RNA editing is driven by an editase comprising the fusion between a MCP protein and the $ADAR_{DD}$. So, the MCP-$ADAR_{DD}$ editase is recruited to bind the MS2 stem-loop present in the gRNA, which in turn is paired with the target mRNA (Fig. 12C). To assess the potential of the MS2-MCP-ADAR system, gRNAs carrying the MS2 stem-loop on either side were optimized to recruit MCP-$ADAR_{DD}$ (Katrekar et al., 2019). In this case, the fusion editase contained either the wild-type or hyperactive forms of $ADAR1_{DD}$ or $ADAR2_{DD}$. When tested in HEK293T cells, both $ADAR1_{DD}$ and $ADAR2_{DD}$ achieved substantial editing yields. However, editases containing a nuclear export signal (NES) or the hyperactive $ADAR1_{DD}$(E1008D) or $ADAR2_{DD}$(E488Q) displayed greater editing rates but also increased off-target editing. Conversely, in lined with previous work (Vallecillo-Viejo et al., 2018), the addition of a NLS to the editase resulted in lower off-target editing. Finally, AAV8 vectors encoding MCP-$ADAR1_{DD}$(E1008D)-NLS were administered to *mdx* mice, reporting almost a 3 % *in vivo* editing efficiency (Katrekar et al., 2019). Recently, significant efforts have been made for the implementation of the MS2-MCP-ADAR system for clinical applications (Tohama et al., 2020). First, all the components of the system (coding sequences for the gRNA, MCP-$ADAR1_{DD}$ fusion editase, and target genes) were packaged into a single construct. Additionally, concentrating primarily on the editase, the XTEN flexible linker was utilized for the MCP and $ADAR1_{DD}$ fusion instead of the conventional linker (Katrekar et al., 2019), contributing to protein stability (Chhabra et al., 2015). Moreover, the size of the MCP was reduced to create a smaller fusion editase more suitable for gene therapy applications. The constructed vector was introduced into HEK293T cells for editing of endogenous transcripts, displaying an editing efficiency of up to 40 % (Tohama et al., 2020).

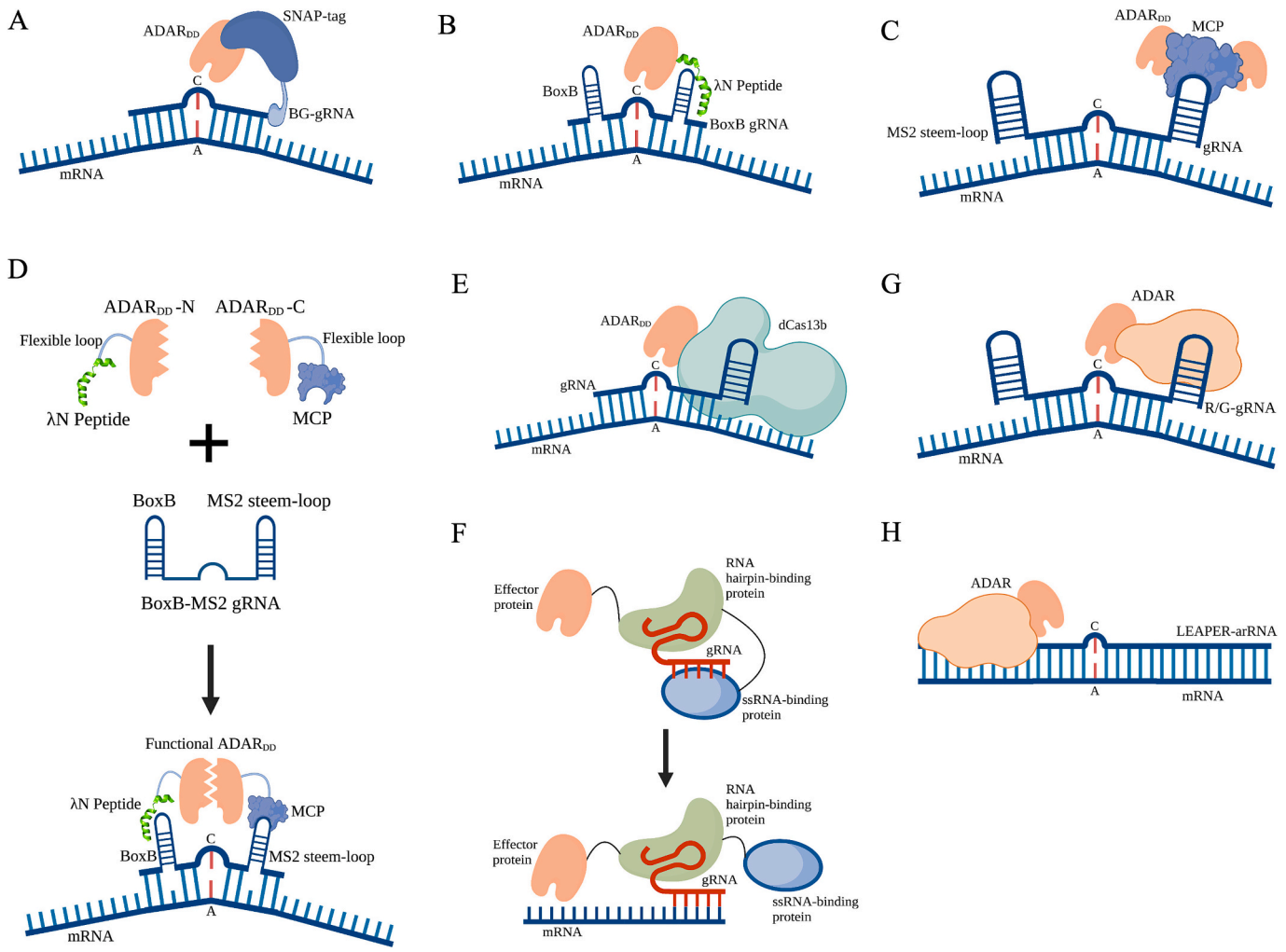


Fig. 12. ADAR-mediated RNA-editing procedures. A) Schematic representation of SNAP-ADAR system. B) Schematic representation of λN-ADAR system. C) Schematic representation of MS2-MCP-ADAR system. D) Schematic representation of Split-ADAR system. E) Schematic representation of REPAIR system F) Schematic representation of CIRTS system. G) Schematic representation of GluR2-ADAR system. H) Schematic representation of LEAPER system. $ADAR_{DD}$ stands for ADAR deaminase domain.

However, further studies are needed to evaluate whether this newly developed system can be successfully implemented *in vivo* for gene therapy applications.

Building upon previous studies that efficiently recruited ADARs by using MCP and λN systems (Montiel-Gonzalez et al., 2013; Katrekar et al., 2019), Katrekar et al. (2022b) recently developed a new methodology to reduce the off-target editing. This ingenious strategy involves the use of two inactive fragments of $ADAR2_{DD}$ that converge at the target mRNA to form a functional enzyme. The split-ADAR2 system (Fig. 12D), as its name suggests, involves N-terminal and C-terminal non-functional fragments of hyperactive $ADAR2_{DD}$ (E488Q), each bearing a MCP and λN peptide, respectively. The system utilizes chimeric gRNAs, complementary to the target mRNA, which incorporate both BoxB and MS2 stem loops to recruit the two split halves of $ADAR2_{DD}$. Surprisingly, the off-target editing events exhibit a remarkable reduction of 1000–1300-fold when compared to the intact $ADAR2_{DD}$ (E488Q), albeit with an editing efficiency approximately 40 % lower. To improve the efficiency of $ADAR2_{DD}$, the authors conducted deep mutational scanning to assess the impact of every possible single amino acid substitution at each position. This systematic approach led to the development of the split-ADAR2 system utilizing the $ADAR2_{DD}$ E488Q/N496F variant, which exhibited enhanced editing efficiency in 5′-GAN-3′ motifs. However, this variant displayed high promiscuity, as off-target editing events were comparable to those observed with the $ADAR2_{DD}$ E488Q mutant.

Since the first reports of Cas protein for site-specific RNA cleavage (Abudayyeh et al., 2016), many different CRISPR/Cas-based systems employing ADAR enzymes have been developed for site-directed RNA editing (Cox et al., 2017; Abudayyeh et al., 2019). These systems make use of the catalytically inactive dCas13b protein ("dead" Cas9) fused to the $ADAR_{DD}$. As early mentioned, a specific gRNA complementary to the target RNA is also required to recruit the fusion editase. Similar to the λN-ADAR system, this gRNA contains an Ado-to-Cyd mismatch opposite to the targeted Ado, as well as a direct repeat sequence in the 3′-terminal that forms a hairpin structure (DR domain), which is necessary for dCas13b recruitment (Abudayyeh et al., 2016).

REPAIRv1 (RNA Editing for Programmable Ado-to-Ino Replacement, version 1) was the first system based on this approach (Cox et al., 2017) (Fig. 12E). In this context, authors fused the hyperactive $ADAR2_{DD}$ (E488Q) to create a dCas13b-$ADAR2_{DD}$(E488Q) editase with enhanced editing efficiency. REPAIRv1 was first utilized in HEK293FT cells for the correction of defective transcripts from both nephrogenic diabetes insipidus (*AVPR2*, 878G > A) and Fanconi anemia (*FANCC*, 1517Guo > Ado) genes, resulting in an editing efficiency of 35 % and 23 %, respectively. Additionally, the editing capacity of REPAIRv1 was further tested in another 34 Guo-to-Ado pathogenic mutations, showing up to 28 % efficiency at 33 sites. Finally, to reduce off-target editing events, the authors focused on improving the RNA recognition by ADAR2DD in the REPAIRv1 system. A rational mutagenesis strategy was applied to

modify the ADAR2DD residues that interact with the RNA duplex. Through this approach, they identified the E488Q/T375G double mutant, which led to the development of the second version of REPAIR (REPAIRv2). Despite the marked reduction in off-target editing, REPAIRv2 showed significantly lower on-target editing efficiency when tested on endogenous mRNA targets (Cox et al., 2017). Unfortunately, in most cases, Cas13b becomes excessively large when fused with $ADAR2_{DD}$, making it unsuitable for widespread use for AAV *in vivo* delivery. Consequently, Xu et al. (2021) fused a truncated form of an already compact dCas13X.1 with $ADAR2_{DD}$ (E488Q/T375G) to create a reduced base editor (mxABE). When compared to the previously developed REPAIR system (Cox et al., 2017), mxABE exhibited higher RNA-editing efficiency in mammalian cells.

The major drawback of CRISPR/Cas-based techniques lies in the fact that general population possesses antibodies against CRISPR-Cas proteins due to their bacterial origin (Crudele and Chamberlain, 2018). In this regard, patients may manifest undesired adverse reactions because of the immune response after administration of these proteins. Moreover, the large size of Cas13 proteins (the smallest of the Cas13 family members, Cas13d, is around 100 kDa) (Yan et al., 2018a) hinders the introduction of the coding sequence into a viral vector for protein delivery. As an alternative to more traditional CRISPR/Cas-based systems employing ADAR enzymes, in recent years, the CRISPR-Cas-Inspired RNA Targeting System (CIRTS) has been developed (Fig. 12F) (Rauch et al., 2019). This interesting approach uses a ribonucleoprotein complex entirely derived from human protein parts, consisting of four components: i) an RNA hairpin-binding protein that selectively binds the gRNA; ii) a gRNA complementary to the target RNA sequence, capable of interacting with the RNA hairpin-binding protein; iii) a protein able to non-specifically bind the gRNA to stabilize and protect it before the interaction with the target RNA (ssRNA-binding protein); iv) an epitranscriptomic regulator acting on the target RNA (effector protein) (Fig. 12F). As mentioned earlier, the gRNA can be optimized to target different RNA sequences, which makes CIRTS an easily programmable technology. Additionally, the ribonucleoprotein complex can deliver distinct RNA-editing proteins, *e.g.* ADARs, which makes CIRTS a versatile tool for site-directed RNA editing (since multiple effector proteins can be combined). In fact, the delivery of $ADAR_{DD}$ from both wild-type and hyperactive human ADAR2 (E488Q mutant) for editing exogenous mRNAs encoding a luciferase reporter was reported (Rauch et al., 2019). All in all, the human origin of its components along with the reduced size of CIRTS represents a competitive advantage when comparing to other CRISPR/Cas-based systems.

Another example of site-directed RNA editing system is the GluR2-ADAR system, which is based on the ability of the dsRBDs of ADAR enzymes to recognize the R/G editing site of the GluR2 receptor (Fig. 12G) (Stefl et al., 2010). Similar to the previously described optimization strategies, the R/G motif containing gRNA (R/G-gRNA) carried the Ado-to-Cyd mismatch opposite to the target adenosine. Co-expression of R/G-gRNA and human ADAR2 in HeLa cells resulted in a 10 % editing efficiency of a premature stop codon in PINK1 kinase mRNAs, associated with Parkinson's disease (Wettengel et al., 2017). The implementation of the hyperactive ADAR2 (E488Q) increased the system's efficiency to 40 % in HEK293T cells (Katrekar et al., 2019). Then, ADAR2 (E488Q) and R/G-gRNA coding sequences were delivered *via* AAV8 vector in two mouse models: *mdx* for Duchenne muscular dystrophy and spf^{ash} for ornithine transcarbamylase (OTC) deficiency. In *mdx* mice, administration of AAV construct restored 1–2.5 % dystrophin protein to its full-length, while in spf^{ash} mice 4.6–33.8 % editing efficiency was achieved in OTC-expressing mRNAs, albeit observing off-target editing and toxicity (Katrekar et al., 2019).

The GluR2-ADAR offers a clear advantage over other systems since it uses a natural signal for the recruitment of ADAR enzymes. In this context, transfection of gRNA alone would be enough to leverage endogenous ADARs for triggering site-directed RNA editing. This simplifies the clinical application of these methodologies, as only one of the system components needs to be introduced, taking advantage of cellular tools for editing. The first steps in that direction were made *in vitro* by using HEK293 cells overexpressing human ADAR2 by Fukuda et al. (2017). They achieved the correction of a premature termination codon in mRNAs encoding reporter GFP (around 3 % efficiency) through gRNA expression only. A more recent method called RESTORE (Recruiting Endogenous ADAR to Specific Transcripts for oligonucleotide-mediated RNA Editing) relies upon the same molecular basis, recruiting native ADARs by the action of engineered antisense oligonucleotides (ASOs) (Merkle et al., 2019). The designed gRNAs carry an invariant domain comprising the ADAR-recruiting R/G motif and a programmable domain for target mRNA recognition. As an improvement of previous work (Wettengel et al., 2017), ASOs were optimized by utilizing a R/G motif variant with enhanced ADAR-recruiting capacity. Additional chemical modifications (2′-*O*-methylations and the presence of four phosphorothioate residues at the 3′ end) (Vogel et al., 2014) were also made in the programmable domain for higher editing efficiency. The therapeutic potential of RESTORE was shown by effective RNA editing in cancer cell lines and human primary cells. Specifically, the repair of the phosphotyrosine 701 site in endogenous STAT1 resulted in 3–30 % efficiency, and successful editing of the E342K mutation in SERPINA1 transcripts (causing α1-antitrypsin deficiency) achieved 10–20 % efficiency (Merkle et al., 2019).

Alternatively, the LEAPER (Leveraging Endogenous ADAR for Programmable Editing of RNA) system uses engineered linear gRNAs to recruit endogenous ADARs for Ado-to-Ino editing (Fig. 12H) (Qu et al., 2019). Based on previous work on the CRISPR/Cas-based system REPAIR (Cox et al., 2017), the authors observed that CRISPR gRNAs (crRNAs) lacking the Cas13a-recruiting structure could induce RNA editing in absence of the dCas13a-$ADAR_{DD}$ fusion. As a result, long (71–151 nt) ADAR recruiting gRNAs (arRNAs), with the Ado-to-Cyd mismatch opposite to the targeted Ado, were developed and engineered for high editing efficiency (Qu et al., 2019). In primary fibroblast cells from a patient with Hurler syndrome, both mature mRNA and pre-mRNA were edited by LEAPER system with efficiencies of 10 % and 30 %, respectively. Additionally, LEAPER successfully restored catalytic activity of α-l-iduronidase in fibroblasts from a patient with Scheie syndrome, with consistent 30 % editing efficiency, and without activating type-I interferon pathways or eliciting pro-inflammatory responses (Qu et al., 2019).

It's worth mentioning that the LEAPER system has recently been improved by incorporating covalently closed circular arRNAs (circ-arRNAs) (Yi et al., 2022). Consequently, the updated LEAPER 2.0 exhibited a 3.1-fold increase in editing efficiency and nearly eliminated bystander off-target editing. Interestingly, Katrekar and coworkers have also developed a range of circ-arRNAs with the aim of exploiting their longer half-life and extended target residence times to enhance ADAR enzyme recruitment for more efficient and precise RNA editing (Katrekar et al., 2022a). In this sense, AAV-mediated *in vivo* delivery of circ-arRNAs resulted in 53 % editing of the mPCSK9 transcript in mice liver and a 12 % correction of a nonsense mutation in IDUA transcripts in Hurler syndrome mouse models.

Despite the advantages demonstrated by circ-arRNAs, considerable efforts have been dedicated to optimizing linear gRNAs to achieve more efficient ADAR recruitment. An intriguing example involves the design of *in silico*-optimized CLUSTER guide RNAs capable of targeting transcript sequences that were previously inaccessible using traditional gRNAs (Reautschnig et al., 2022). When administered *in vivo*, RNA editing levels of up to 10 % were achieved in reporter transcripts in mice liver. In another recent study, chemically modified oligonucleotides (AIMers) were designed, incorporating chimeric backbones with phosphorothioate and nitrogen-containing linkages (Monian et al., 2022). These AIMers were able to effectively recruit endogenous ADARs for up to 50 % *in vivo* editing of the ACTB transcript in primate liver, with no observed off-target events, suggesting the potential of this system for clinical applications.

Thus far, all the RNA base editing systems require the assembly of a gRNA and a deaminase domain to create an RNA-protein complex, enabling the recognition and subsequent editing of the target RNAs. Despite the efforts to enhance gRNAs, inherent limitations persist within these systems. For instance, the formation of the RNA-protein complex remains a rate-limiting step, which hampers editing efficiency. Furthermore, the pairing of gRNA and target RNA may result in the undesired bystander effect. Recently, the REWIRE (RNA editing with individual RNA-binding enzyme) system has emerged as a solution to these limitations (Han et al., 2022a). This editase includes a programmable PUF domain of human origin, responsible for recognizing target RNAs, along with $ADAR_{DD}$ for the Ado-to-Ino editing process. Comparing to other gRNA-based RNA editing tools, like REPAIRv2 system (Cox et al., 2017), REWIRE has demonstrated improved editing efficiency, albeit with a slight increase in off-target effects. Furthermore, to evaluate its therapeutic potential, REWIRE was administered *in vivo* to mice *via* AAV, displaying 27–34 % editing efficiency in transcripts expressing eGFP, while also exhibiting minimal off-target editing.

2.2.4.2.2. Adenosine base editors for genomic editing. As mentioned earlier, one of the key advantages of RNA base-editing tools for therapeutic applications is their transient nature. By targeting RNA, these tools preserve the underlying DNA sequence, so any unintended modifications are temporary and can be reversed by discontinuing the treatment. This characteristic, while beneficial, necessitates the continuous administration of the system components, which may limit their applicability for some treatments (Casati et al., 2021). In contrast, DNA base-editing tools induce permanent changes at the genome level. While a single administration might be sufficient to correct a disease-causing mutation, achieving high conversion efficiencies (exceeding 50 %) is crucial for therapeutic success (Savić and Schwank, 2016). The exact threshold for effective DNA editing can vary depending on the specific disease, therapeutic approach, and target tissue. Given that DNA modifications are irreversible, it is essential to rigorously mitigate off-target effects (ideally less than 0.1 %) (Tao et al., 2023). Consequently, DNA editing requires more stringent off-target reduction compared to RNA editing. The challenges associated with off-target effects have notably impacted the broader application of DNA base-editing methods, underscoring the need for improved precision and safety in these approaches (Tang and Xu, 2020).

In this sense, interestingly, DA enzymes have been recently employed for genome editing through CRISPR/Cas-based techniques (Kim, 2018; Matsoukas, 2018; Pecori et al., 2022). These systems rely on chimeric proteins based on a DA catalytic domain (Ado or Cyd DA domain) fused with a DNA targeting unit, like dCas9 (Hess et al., 2017) or Cas9 nickase (Eid et al., 2018; Ran et al., 2013), both guided by a single guide RNA (sgRNA). The catalytically deficient Cas9 recognizes the target DNA through a protospacer adjacent motif (PAM), located ~15 nucleotides from the target base. This way, CRISPR RNA-guided DAs, so-called base editors (BEs), induce single nucleotide modifications within a small 4–5 nucleotide window in the protospacer. Thus, the CydDA domain deaminates Cyd to Urd (Cyd-to-Urd, referred to as C-to-U in the literature), which is then transformed into thymidine through DNA repair and replication, leading to the Cyd-to-Thd (referred to as C-to-T in the literature) conversion. In the case of the AdoDA domain, Ado is deaminated to Ino, which is interpreted as Guo at the DNA level. So, this progression ultimately results in the Ado-to-Guo (referred to as A-to-G in the literature) conversion. As a major advantage, neither donor DNA templates nor double-stranded DNA breaks (DSBs) are required, and therefore BEs do not rely on homology-directed repair (HDR) processes. Additionally, since base editors generally do not create DSBs, they minimize the risk of indels, translocations, or large DNA rearrangements.

The first DNA base editor leveraging the catalytic activity of DAs, known as Base Editor 1 (BE1), was developed in David R. Liu's group, through the fusion of APOBEC1 (Apolipoprotein B mRNA Editing Enzyme Catalytic Subunit 1) to dCas9 from *Streptococcus pyrogenes* (Komor et al., 2016). BE1 was further optimized leading to the development of following generations of Cyd Base Editors (CBEs): the second-generation Base Editors (BE2) and third-generation Base Editors (BE3) (Komor et al., 2016). Despite their importance, to fit the structure of the review, CBEs will be addressed in the section dedicated to pyrimidine DAs.

Conversely, ADARs act strictly on duplex RNA or DNA/RNA hybrids, so contrary to APOBEC enzymes, no ADARs have been reported to act on ssDNA (Rees and Liu, 2018; Zheng et al., 2017). It represents a severe limitation when designing Ado Base Editors (ABEs). Initial attempts in this direction involved substituting APOBEC1 in BE3 with different AdoDAs, such as TadA from *Escherichia coli* (*Ec*TadA), human ADAR2 and ADAT2, and mouse AdoDA2 (Gaudelli et al., 2017). In the absence of positive results, *Ec*TadA underwent extensive directed evolution and protein engineering to generate TadA variants (TadA*) displaying high activity on ssDNA and extended target sequence compatibility. The development of the TadA* variant was achieved through iterative rounds of mutation and selection, resulting in the accumulation of several key mutations. Notably, the mutations A106V and D108N were critical in enabling TadA to deaminate adenine in DNA rather than its natural RNA substrate. These mutations are located in the region of the enzyme that interacts with the nucleic acid substrate, specifically in or near the active site of TadA. By altering the enzyme's active site, these mutations effectively modified its substrate recognition, allowing TadA* to act on DNA with high efficiency instead of RNA. In addition, since native TadA acts as a homodimer, with one monomer responsible for the deamination reaction while the other contributes to tRNA substrate binding (Losey et al., 2006), the ABE system was ingeniously engineered to account for TadA* dimerization effects. A heterodimeric protein containing both, the catalytically inactive wild-type TadA monomer at the N-terminus and the TadA* monomer C-terminally linked to the Cas9 nickase (TadA-TadA*-Cas9 nickase), was constructed. All in all, this meticulous process of direct evolution and protein engineering culminated in the creation of the seventh-generation ABE, exemplified by ABE7.10. Thus, ABE7.10 system comprises three principal components: the evolved TadA-TadA* dimer, a Cas9 nickase, and a sgRNA (Gaudelli et al., 2017). ABE7.10 was then tested in human cells for the conversion of Ado-Thd base pairs to Guo-Cyd (Fig. 13), showing ~50 % conversion efficiency with almost negligible indel rates of indels.

Over the years, certain ABE versions (primarily ABE7.10) have been extensively used for genomic DNA editing in prokaryotes (Wang et al., 2019; Zhang et al., 2020; Wang et al., 2021a), plants (Kang et al., 2018; Li et al., 2018a; Yan et al., 2018b; Wang et al., 2022; Wang et al., 2023), human cell types (Kim et al., 2019; Sürün et al., 2020; Krishnamurthy et al., 2021), zebrafish (Qin et al., 2018) and mice (Liang et al., 2018; Ma et al., 2018; Ryu et al., 2018; Song et al., 2020). Despite the promising results, the ABE system developed by Liu's group (Gaudelli et al., 2017) exhibited certain limitations hindering its biotechnological application. Primarily, this group demonstrated that base-editor expression level within cells significantly influences editing efficiency (Koblan et al., 2018). Hence, an optimized ABE was engineered (ABEmax) by replacing the nuclear localization signal (NLS) and modifying the codon usage in the previous ABE7.10 system. ABEmax was then used in HEK293T cells to correct point mutations in promoters of the gamma globin genes (*HBG1* or *HBG2*) through two distinct sgRNAs (Fig. 13). The results were noteworthy, with ABEmax demonstrating approximately 2-fold higher editing efficiency compared to ABE7.10 for the first sgRNA, both in sorted and unsorted cells. For the second sgRNA, ABEmax achieved editing efficiencies that were 5.2- and 7.1-fold higher than those of ABE7.10 in unsorted and sorted cells, respectively (Koblan et al., 2018).

The efficacy and safety of ABEmax were also tested in the livers of long-tailed macaques (Rothgangl et al., 2021). As an alternative to other published works, ABEmax was delivered by lipid nanoparticles circumventing certain problems associated with AAV administration. ABEmax demostrated efficient editing of *PCSK9*, a negative regulator of low-density lipoprotein (LDL), achieving up to 34 % efficiency with no

off-target events. Despite higher editing efficiencies are desirable for clinical applications, the reported results underscore the potential of ABEmax for therapeutical use. As previously mentioned, DNA base editing using ABE systems occurs within a narrow nucleotide window adjacent to the PAM sequence recognized by the Cas9 domain. Thus, the targeting scope of ABEs is defined by the length of the base-editing window along with the PAM availability. For *Streptococcus pyogenes* Cas9 (*Sp*Cas9), the primary enzyme employed in genome editing, it selectively identifies an NGG sequence (with N representing any nucleobase) as the PAM (Sternberg et al., 2014). This strict recognition pattern limits the potential targetable regions within the genome. To minimize these limitations, the targeting scope was expanded by developing optimized ABEmax versions containing *Sp*Cas9 variants compatible with non-NGG PAMs. Furthermore, circularly permuted *Sp*Cas9 variants were also utilized to facilitate the access of the DA domains in ABEmax to the ssDNA loop. These circular permutants resulted in ABEmax variants with extended editing windows (from ~4–5 nucleotides to ~8–9 nucleotides) (Huang et al., 2019b). Interestingly, based on an analysis of disease-causing human SNPs in ClinVar (Ihry et al., 2018; Li et al., 2018b), authors claimed that the targetable SNPs increased from 31 % (in the case of the first version of ABEmax), to 51 % (for the latest variant of ABEmax) (Huang et al., 2019b).

Although the target scope of ABEmax was significantly expanded in subsequent versions, it is worth noting that minimal off-target DNA editing was detected (Huang et al., 2019b). Additionally, after a thorough study, off-target Ado-to-Ino editing in cellular RNA was also observed for the catalytically inactive wild-type TadA monomer and the evolved TadA* monomer (Rees et al., 2019). In this respect, to minimize the RNA editing activity (while preserving the ability to edit target DNA), both monomers were subjected to mutations following a structure-guided approach. The designed ABEmax variants were tested in three mammalian cell lines, displaying significantly reduced off-target RNA and DNA editing, as well as reduced indel by-product formation (Rees et al., 2019). In another work, ABEmax underwent direct evolution and directional screening in human cells, resulting in the development of three high-activity variants: NG-ABEmax-SGK (R101S/ D139G/ E140K), NG-ABEmax-R (Q154R) and NG-ABEmax-K (N127K) (Fu et al., 2021). Subsequently, the mutations present NG-ABEmax-R and NG-ABEmax-K were combined to generate the NG-ABEmax-KR variant, which contains the N127K mutation in TadA domain and the Q154R mutation in TadA* domain. NG-ABEmax-KR exhibited enhanced editing efficiency in HEK293 cells and a murine model. Additionally, when compared to the original ABEmax, NG-ABEmax-KR displayed up to 4-fold higher editing rates in gamma globin *HBG1* or *HBG2* promoters.

In a different approach, a truncated ABEmax (miniABEmax), lacking the wild-type TadA domain, was constructed to decrease the off-target RNA editing activity (Grünewald et al., 2019b) (Fig. 13). Moreover, to further decrease the RNA editing activity of miniABEmax, specific mutations were introduced into the remaining TadA* monomer using a protein-truncation strategy. The introduced mutations included K20A and R21A in one variant, resulting in miniABEmax(K20A/R21A), and V82G in another variant, creating miniABEmax(V82G). These mutations were designed to diminish the RNA-recognition capability of the enzyme, thereby reducing off-target RNA editing while preserving the efficiency of on-target DNA editing. Finally, Liu's group demonstrated the potential *in vivo* applicability of the optimized ABEmax variants (Levy et al., 2020). To this end, split-intein ABEs, together with split-intein CBEs, were introduced into optimized dual-AAV genomes. Thus, dual-AAV split-intein BEs were successfully utilized to correct a pathogenic mutation causing Niemann-Pick disease type C. *In vivo* treatment successfully repaired the point mutation in the central nervous system of mice, preserving target Purkinje neurons and prolonging mice lifespan (Levy et al., 2020). In a similar example, this very system was employed to correct a premature stop codon in the *Pcdh15* gene, aiming to treat Usher syndrome type 1F (Peters et al., 2023), characterized by congenital hearing impairment and gradual blindness. To this end, the split-intein ABE was administered in the inner ear of neonatal mice *via* dual-AAV vectors. Despite detecting base editing, hearing restoration was not achieved in *Pcdh15* constitutive knockout mice. However, when vectors were injected into the cochlea of late conditional knockout mice, hearing ability was indeed restored (Peters et al., 2023).

As mentioned earlier, the targeting scope of ABEs is limited to nucleobases located within a defined proximity to the PAM. Thus, Ado-to-Ino editing by ABEs depends to a great extent on the target site having a properly located PAM compatible with the Cas9 domain present on the ABE. While CBEs are compatible with many Cas homologs, ABEs have been reported to be compatible with a limited number of them: *Sa*Cas9 (Hua et al., 2019; Huang et al., 2019b), *Sa*Cas9-KKH (Huang et al., 2019b), evolved *Sp*Cas9 variants (Hu et al., 2018; Huang et al., 2019b; Hong and He, 2023) and SauriCas9 (Hu et al., 2020). Therefore, a logical step for improving the applicability of ABEs is to broaden their compatibility with a larger set of Cas homologs. To this effect, an engineered ABE variant (ABE8e) was developed, comprising an evolved TadA domain (TadA8e) compatible with greater number of Cas9 and Cas12a domains (Richter et al., 2020; Chen et al., 2022a). The development of ABE8e involved the introduction of eight additional mutations into the TadA domain through a combination of phage-assisted non-continuous and continuous evolution (PANCE and PACE) techniques (Richter et al., 2020). These specific modifications were aimed at enhancing the enzyme's compatibility with various Cas proteins and improving its overall performance. Notably, a V106W substitution was introduced to reduce off-target editing. These advanced methods allowed for the systematic evolution and selection of the TadA8e variant with improved base editing capabilities. Additionally, the TadA8e monomer proved to be compatible with SpRY-nCas9, bypassing PAM limitations and resulting in higher activity at NRN PAMs compared to NYN PAMs (R and Y representing purine and pyrimidine nucleotides, respectively) (Cao et al., 2022). Interestingly, SpRY-nCas9 was also employed for the development ABEs (as well as CBEs) for the metabolic engineering of *B. subtillis* (Xia et al., 2023). Overall, compared to previous ABE7.10, ABE8e displayed improved editing efficiency and expanded targeting scope. In a separate attempt to expand the targeting scope of ABE8e, the HNH domain of *Sp*Cas9 was replaced with a heterodimeric (HD) TadA domain ($TadA_{wt}$ linked to TadA8e) to create HNHx-ABE8eHD (Villiger et al., 2021). Apart from a higher editing efficiency, HNHx-ABE8eHD also displayed a broader editing window compared to classical ABEs (ABEmax and ABE8e), which enables the correction of additional pathogenic SNPs.

Recently, the cryo-electron microscopy structure of a DNA-bound ABE8e complex was determined (Lapinaite et al., 2020). Based on the kinetic and structural data presented, mutations in ABE8e led to the stabilization of the substrate DNA in a tRNA-like conformation, which could be the reason for the enhanced DNA-editing activity reported for ABE8e. Moreover, these mutations have been shown to elevate the positive charge density in the DNA-binding region, leading to an increased number of electrostatic interactions with DNA (Zhu et al., 2023). In pursuit of more active ABEs, especially when target Ado sites are located on the edges of the editing window (positions 3, 4, 7 and 8), TadA* present in ABE7.10 was further optimized by directed evolution to develop additional ABE8 systems (ABE8s). The capacity of ABE8s for multiplexed editing was demonstrated by simultaneous targeting of *B2M*, *CIITA*, and *TRAC* genes in primary human T cells. Among the tested ABE8s, ABE8.20-m edited each individual target with more than 98 % efficiency. Furthermore, almost negligible sgRNA-independent off-target DNA editing as well as very low mRNA editing levels were detected.

Apart from the promising applications for the treatment of several pathologies, the newly developed ABE8 systems have also been successfully applied for genomic engineering in mice (Badat et al., 2021), plants (Han et al., 2022b; Wang et al., 2021b) and microbial strains (Kozaeva et al., 2024). Unfortunately, when these high-activity ABE8e

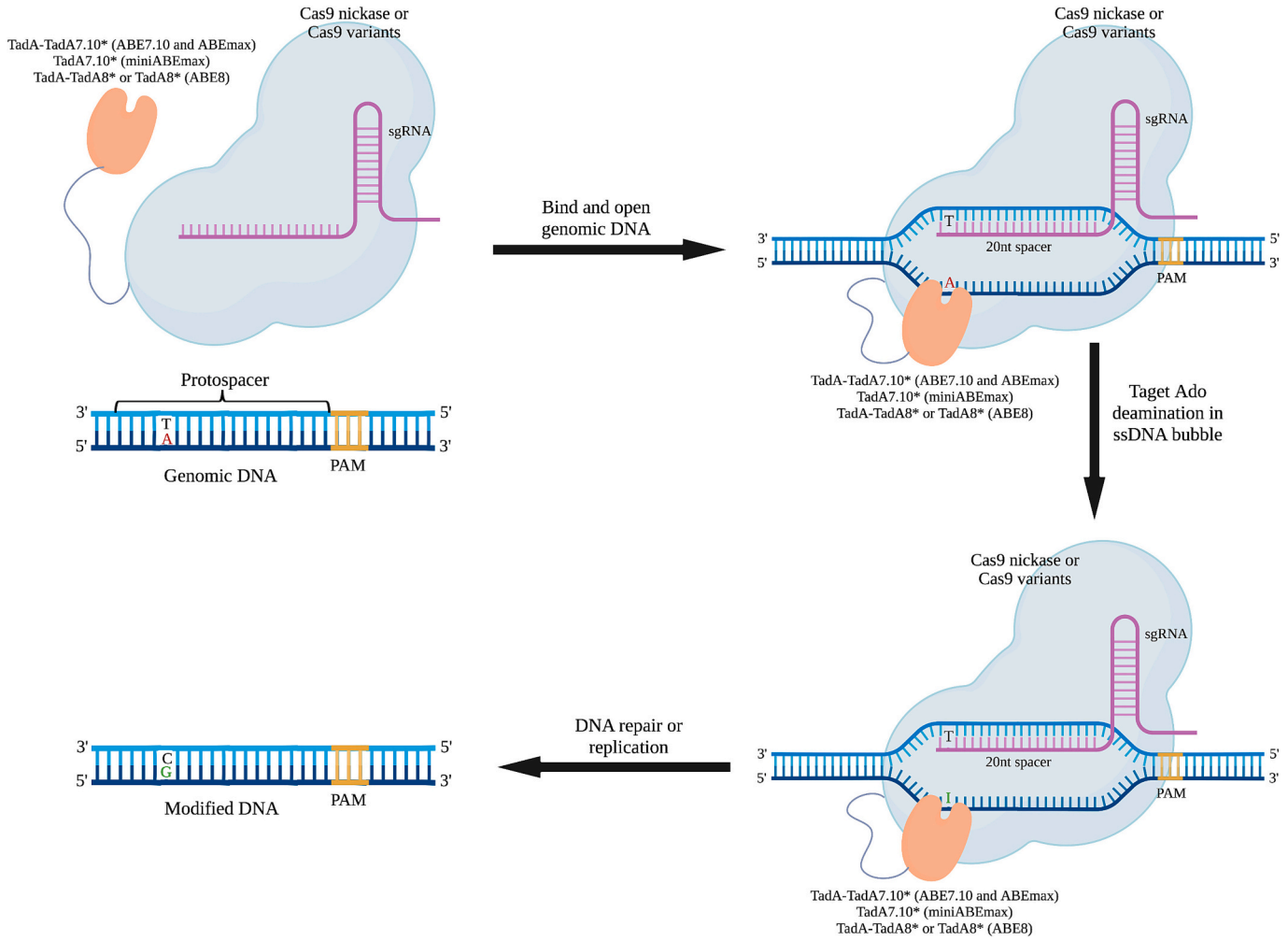


Fig. 13. Schematic representation of ABE-mediated base editing strategy for the Ado-to-Guo conversion.

(Richter et al., 2020) and ABE8s (Gaudelli et al., 2020) were tested in genomic sites containing both Ado and a Cyd target motifs, Cyd deamination activities were also detected (Jeong et al., 2021). Based on previous structural studies (Losey et al., 2006) and multiple sequence alignments of TadA orthologs, authors identified the critical amino acid residues involved in Ado and Cyd recognition. After extensive testing, TadA variants carrying D108Q or P48R mutations displayed improved selectivity for Ado or Cyd, respectively. So, D108Q mutation was introduced in ABE8e and ABE8s, thereby obtaining ABE8eQ and ABE8sQ variants with significantly lower Cyd editing activity. Interestingly, reduced RNA deamination was also observed with ABE8eQ and ABE8sQ, suggesting that the D108Q mutation effectively diminishes the RNA editing activity as well (Jeong et al., 2021). Finally, the ABE8r (TadA8r variant) has been recently developed with broaden editing window and greater efficiency than its predecessor ABE8e. This novel ABE8r system has proven successful in editing relevant sites that were previously difficult to access with existing editors. Notably, ABE8r was utilized for the editing of mutations such as ABCA4-c.5882G > A, the most common mutation associated with Stargardt disease (Xiao et al., 2024).

Lately, an additional mutation, L145T, has been incorporated to TadA8e through structure-guided engineering for the creation of the novel ABE9 system (Chen et al., 2023a). This mutation led to a narrowed editing window, effectively further reducing undesired RNA editing events and eliminating off-target DNA editing. All in all, ABE9 was up to 342-fold more precise than ABE8e when the editing occurs in homopolymeric Ado sites. The valuable knowledge obtained from this research led to the development of a novel technology called TaRGET (Tiny nuclease RNA-based Genome Editing Technology) (Kim et al., 2022). TaRGET-ABE was composed of a Tad-Tad mutant (V106W, D108Q) dimer attached to the C-terminal of a hypercompact type V dCas12f (D354A). Moreover, the system components underwent further engineering to expand the targeting scope. TaRGET-ABE could be successfully delivered into mammalian cells *via* AAV administration, ensuring high editing rates.

Finally, upon reviewing the most recent and intriguing publications on ABEs, we come across the split-ABE systems (Santos et al., 2023; Zeng et al., 2023), designed to minimize the bystander effect. Unlike the split-ADAR2 system developed by Prashant Mali's group (Katrekar et al., 2022b), the split-ABE system developed by Santos and colleagues comprises two Cas9 fragments, Cas9(C)-NLS and Cas9(N)-FRB (FKBP-rapamycin binding), which dimerize in the presence of rapamycin to form the functional editase. After optimization, authors electroporated BCi-NS1.1 cells with split-ABE for the correction of a premature stop codon (W1282X) that causes cystic fibrosis. The non-split editors exhibited a 14 % editing efficiency compared to the 2.1 % observed for split-ABE. Nonetheless, almost no undesired bystander events were detected ($\leq$ 0.5 %) with split-ABE. In a more recent approach the deaminase domain of TadA8e, present in ABE8e, was divided into two inactive segments: one fused with FRB protein and the other with FK506-binding protein 3 (FKBP3) (Zeng et al., 2023). Consequently, these two components could reassemble into an active editase by rapamycin-induced dimerization. Through extensive engineering, the latest iteration of split-ABE8e demonstrated a narrower activity window

and enhanced precision compared to ABE8e, showing reduced genomic and transcriptomic off-target events. When administered in mouse liver by dual-AAV vectors, the novel split-ABE8e could effectively edit the therapeutically significant *PCSK9* gene after induction (Zeng et al., 2023).

Within the state of the art of ABEs, one of the most cutting-edge technologies is the Ado transversion editors for Ado-to-Cyd and Ado-to-Thd editing (Chen et al., 2023b; Tong et al., 2023). These Ade-to-Cyt base editors (ACBEs), as reported to date, consist of an ABE fused with either an alkyladenine DNA glycosylase (Chen et al., 2023b) or an *N*-methylpurine DNA glycosylase (Tong et al., 2023). All in all, ACBEs have demonstrated successful correction of pathogenic mutations in mammalian cell lines, displaying high transversion editing activity. Very recently, transversion editors containing *N*-methylpurine DNA glycosylase (Tong et al., 2023) have been optimized for Ado-to-Thd editing in rice (Wu et al., 2023; Li et al., 2023, 2024) and tomato (Li et al., 2024) plants. Although A-to-T editing efficiencies were high (up to 82 %), A-to-C conversions were only detected in rice with very low editing efficiencies. The development of Ado-to-Thd/Cyd transversion systems in plants is crucial for enhancing crop genetics and generating novel germplasm resources.

2.3. Pyrimidine DAs

Pyrimidine DAs possess the capability to act on N-aminated pyrimidines. Due to the absence of deamination capability with uracil or thymine, pyrimidine DAs exclusively target cytosine nucleobases and nucleoside derivatives. Similar to the categorization observed for purine DAs, the information can be classified into three categories: nucleobase DAs, nucleoside DAs, and other pyrimidine Das (Table 2). Additionally, again, this revision will be focused on the use of pyrimidine DAs as practical catalysts in the food and pharmaceutical industries.

2.3.1. Pyrimidine nucleobase DAs

2.3.1.1. Cytosine DA

2.3.1.1.1. General concepts. CytDA (EC 3.5.4.1) is responsible for the hydrolytic deamination of Cyt **(3)** into Ura **(4)**, releasing ammonia (Ireton et al., 2002) (Fig. 14) (Table 2). However, various CytDAs have been shown to transform different Cyt derivatives **(61–62)** into their corresponding Ura derivatives **(63–64)** (Gaded and Anand, 2018; Hitchcock et al., 2014; Sakai et al., 1975). As reported for other DAs, the presence of a metal cation is crucial for optimal catalytic activity but also for protein expression in a standard medium (Porter and Austin, 1993). CytDA is an essential enzyme in the pyrimidine salvage pathway of prokaryotic and eukaryotic organisms but is utterly absent in mammalian species (Ireton et al., 2002). Consequently, the absence of CytDA in mammalian cells has been extensively exploited in antimicrobial drug design and gene therapy applications (Gaded and Anand, 2018).

While on the subject, the two different types of CytDA enzymes have evolved separately, providing a clear example of convergent evolution (Gaded and Anand, 2018). Fungal CytDA enzymes belong to the CDA superfamily and are classified within cog0590, while bacterial CytDA enzymes belong to the AHS superfamily and fall into cog0402 (Gaded and Anand, 2018; Hall et al., 2011). In addition to their differences in the structural fold, other distinctions have been observed between bacterial and fungal CytDAs, including their amino acid sequences, molecular mass, oligomeric states, and substrate specificities. Concerning the latter, in contrast to CytDAs from the AHS superfamily, CytDAs belonging to the CDA superfamily can catalyze the deamination of the modified base 5-methylcytosine **(61)** due to unique architectural features within their active site (Hitchcock et al., 2014). Among other notable characteristics, bacterial CytDA enzymes have been reported to exhibit higher thermal stability when compared to their yeast counterparts (Katsuragi et al., 1987). However, further research is required to fully explore this specific subject.

2.3.1.1.2. Biotechnological applications. CytDAs find extensive utility across various areas, including agriculture, the pharmaceutical industry, diagnostics, and medicine, among others.

Regarding their applications in agriculture, several examples can be found in the literature. Shao et al. (2015), demonstrated that a bacterial gene encoding a CytDA can serve as a negative selection marker in soybean (*Glycine max*). Like most plants, soybean does not naturally possess CytDA. In this context, transgenic *Glycine max* plants expressing CytDA and cultivated in the presence of 200 μ/mL or higher concentrations of 5-fluorocytosine (5-FCyt) **(62)** (a well-known antimetabolite a drug used in the treatment of a range of cancers, which triggers DNA and RNA damage) exhibit reductions in hypocotyl and tap-root lengths, as well as severe suppression of lateral root development. In similar studies, a bacterial CytDA gene was employed as a negative selection marker in transgenic tomato hairy root (Hashimoto et al., 1999) and in transgenic rice plants (Dai et al., 2001). More recently, Leonhardt et al. (2020) developed an inducible system for genetic toxicity at the tissular level by engineering a bi-functional CytDA/uracil phosphoribosyltransferase (UPRT) (Fig. 15). The utilization of negative selection in this context offers valuable prospects for the development of precise genomic engineering strategies for plant growing, but also allow researchers to investigate the functions and roles of targeted tissues, providing valuable insights into plant development, physiology, and molecular mechanisms.

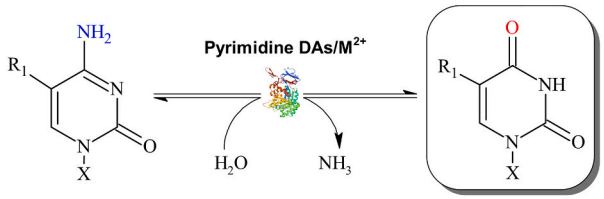
From a pharmaceutical and biomedical perspective, the capacity of CytDA to transform the prodrug 5-FCyt **(62)** into the antimetabolite 5-FUra **(64)**, together with the absence of this enzyme in mammalian cells, has been widely exploited for gene therapy applications (Gaded and Anand, 2018) (Fig. 16). 5-FCyt is not a toxic compound, but its deamination results in the highly mutagenic 5-FUra, which is known to induce cell apoptosis by RNA and DNA damage (Nishiyama et al., 1985). In this context, 5-FUra has been extensively utilized for the treatment of various cancers, mostly colorectal, breast, and gastric cancers (Longley et al., 2003). In the early 1990s, 5-FCyt conversion to the antimetabolite 5-FUra in mammalian cells was accomplished through CytDA-based gene directed enzyme prodrug therapy (GDEPT) (Mullen et al., 1992). Subsequent studies have confirmed the potential of CytDA as a successful suicide enzyme for prodrug-mediated cancer therapy approaches (Gaded and Anand, 2018; Lehouritis et al., 2013; Yata et al., 2012). In this regard, the CytDA coding transgene is introduced into tumor cells by viral or non-viral delivery systems. The expressed recombinant CytDA, in combination with local or systemic administration of 5-FCyt, is employed for tumor elimination (Fig. 16).

Regarding the feasibility of CytDA enzymes for GDEPT applications, yeast (*Saccharomyces cerevisiae*) CytDA (*Sc*CytDA) shows higher activity for 5-FCyt deamination, and thus it is preferred over bacterial (*E. coli*) CytDA (*Ec*CytDA) due to a higher therapeutic effectiveness (Yata et al., 2012). A recent study described the use retroviral replicating vectors (RRVs) for the development of a dual-vector gene therapy procedure (Kubo et al., 2019). Herpes simplex virus thymidine kinase (TK) and *Sc*CytDA coding genes were introduced in RRVs derived from gibbon ape leukemia virus (GALV) and amphotropic murine leukemia virus (AMLV), resulting in an AMLV/GALV-RRVs delivery system. Thereupon, human hepatocellular carcinoma (Hep3B) cells were transduced with GALV-TK, AMLV-TK, GALV-CD, and AMLV-CD (alone or in combination) and cultured in the presence of ganciclovir (GCV) and 5-FCyt. The combination of AMLV/GALV-RRVs mediated TK/GCV and CytDA/5FCyt gene therapy displayed a synergistic effect with improved *in vitro* cytotoxic efficacy. In another study, a viral vector derived from TK-deficient vaccinia virus Guang9 (VG9-TK–) was employed as a delivery system for the *Sc*CytDA transgene (Ding et al., 2020). The antitumor efficiency of VG9-CytDA in combination with 5-FCyt was evaluated in cellular (HCT116, CT26, and MC38 cell lines) and animal (BALB/c nu, BALB/c, and C57BL/6 mice models) colorectal cancer models. Excellent cytotoxicity was observed both *in vitro* and *in vivo*;

however intra-tumoral administration of 5-FCyt reduced the lifespan of mice. In response to the problem, 5-FCyt was orally administered through gavage, improving the antitumor effectiveness and the survival of mice.

Human mesenchymal stem/stromal cells (MSCs) possess the ability to target and access tumor cells (Cihova et al., 2011). Thus, MSCs-mediated GDEPT has emerged as an innovative alternative for cancer treatment (Kucerova et al., 2010) (Fig. 16). In recent years, exosomes obtained from human MSCs have been engineered to express the prodrug activating *Sc*CytDA/UPRT enzyme system (Altaner and Altanerova, 2019; Altanerova et al., 2017, 2019, 2021; Ho et al., 2020). Since the produced exosomes carry mRNA coding the suicide gene, when introduced within cancer cells, and in the presence of 5-FCyt, the growth of tumors is inhibited *in vitro* by conversion of 5-FCyt **(62)** to 5-FUra **(64)** and subsequently to 5-FUMP **(65)** (Cihova et al., 2011; Kucerova et al., 2010). Altanaerova and coworkers developed this system by retrovirus infection of MSCs obtained from different human tissues, such as adipose, bone marrow, dental pulp, umbilical cord, and menstrual blood-derived endometrial regenerative cells (Altanerova et al., 2019). Then, the efficiency of the *Sc*CytDA/UPRT-MSCs/5-FCyt system was evaluated in PC3 and HeLa1a cancer cell lines. All prepared *Sc*CytDA/UPRT-MSCs caused tumor cell death not simply by a bystander effect but primarily *via* exosomes endocytosed by the cancer cells. In a later study, this *Sc*CytDA/UPRT-MSCs system was further tested in a more extensive set of human and rat tumor cell lines (Altanerova et al., 2021). In this context, despite killing several types of tumor cells *in vitro*, suicide gene exosomes were unable to prevent the progression of human fibroblast cells. Finally, no signs of toxicity were detected when these exosomes along with the prodrug (5-FCyt) were intravenously and intraperitoneally injected into mice. In previous work, the same research group implemented an interesting approach where the commercially available Venofer (iron sucrose nanoparticles) was introduced into MSCs derived from different human tissues. This way, *Sc*CytDA/UPRT-MSCs/Fe exosomes were able to induce tumor cell apoptosis following two distinct strategies: 5-FCyt activation *via* the suicide fusion enzyme and alternating magnetic field mediated intracellular hyperthermia (Altanerova et al., 2017).

Despite the demonstrated efficacy of MSCs-mediated GDEPT therapies, the modification of MSCs to express prodrug-activating transgenes remains a great challenge. Most of the existing transfection methods rely on viral vectors, which entail safety concerns related to viral integration or transduction (Thomas et al., 2003). Furthermore, current non-viral transfection procedures for the preparation of prodrug activating MSCs have been reported to exhibit low efficiency (Halim et al., 2014). However, a promising solution has emerged through the introduction of a novel, cost-effective and highly efficient non-viral transfection technique (Ho et al., 2020). In this context, an easily available polyethylenimine (LPEI) based procedure was employed for the development of *Sc*CytDA/UPRT-MSCs from human adipose tissue. Interestingly, when administered to chemo-resistant mice in the presence of 5-FCyt, a single dose resulted in a significant 45 % reduction in glioma size.



(3) R_1= H, R_2= NH_2, X= H **CytDA** **(4)** R_1= H, R_2= OH, X= H
(61) R_1= CH_3, R_2= NH_2, X= H **CytDA** **(63)** R_1= CH_3, R_2= OH_2, X= H
(62) R_1= F, R_2= NH_2, X= H **CytDA** **(64)** R_1= F, R_2= OH, X= H
(66-67) R_1= H, R_2= NH_2, X= (d)rib **CydDA** **(68-69)** R_1= H, R_2= OH, X= (d)rib

Fig. 14. Enzymatic deamination of pyrimidine nucleobases and nucleosides catalyzed by pyrimidine DAs.

Even though *Sc*CytDA has been reported to exhibit greater activity in converting 5-FCyt to 5-FUra, the higher stability of *Ec*CytDA makes it an attractive choice when employing GDEPT procedures mediated by nanocomplexes (Chen et al., 2016). In this respect, Dore-Savard et al. (2017) developed and poly-L-lysine-based nanocarrier for the delivery of *Ec*CytDA and evaluated its cytotoxicity in immunodeficient mice bearing MDA-MB-435 cells. A triple-mutant (*Ec*CytDA$_{V152A, F316C, D317G}$) (Fuchita et al., 2009) with enhanced specificity for 5-FCyt was employed, thereby producing a *Ec*CytDA-PLL-PEI nanocomplex that displayed improved antitumor activity and bystander effect. Upon administration to mice and two consecutive doses of 5-FCyt, the growth of breast cancer cells in mouse lungs was significantly impeded. In another study, polyethyleneglycol-poly(lactic-co-glycolic acid) nanoparticles (PEG-PLGA) containing click nucleic acids (CNAs) were synthesized. The prepared PEG-PLGA-CNA-nanoparticles carried the *Ec*CytDA enzyme as well as the chemotherapeutic drug doxorubicin (DOX) (Harguindey et al., 2019). Consequently, this innovative nanocarrier facilitated the simultaneous delivery of both the prodrug-activating enzyme and the chemotherapeutic drug. *In vitro* antitumor efficacy was assessed for *Ec*CytDA/DOX containing nanocarriers, and for nanocarriers containing either *Ec*CytDA or DOX. Higher cytotoxicity in breast cancer cells was detected with the double-loaded PEG-CNA-PLGA delivery system in the presence of 5-FCyt. In another exciting example (Nemani et al., 2015), the authors explored triggering EPT with magnetic nanoparticle hyperthermia (MNPHT). To this end, they co-encapsulated engineered *E. coli* cells that overexpressed the *Ec*CytDA enzyme upon thermal induction with magnetic iron oxide nanoparticles in alginate microcapsules. The expression of *Ec*CytDA was then remotely triggered by alternating magnetic field-induced hyperthermia.

Beyond nanocarriers, additional non-viral systems have also been employed for the delivery of the *Ec*CytDA suicide enzyme. In a recent study, the VEGF-controlled expression of *Ec*CytDA within 4 T1 murine breast cancer cells, preceded by 5-FCyt administration, was reported (Emamian et al., 2021). To accomplish this, a *Ec*CytDA-pEGFP-N1 shuttle plasmid containing the VEGF promoter was prepared and transfected into tumor cells using non-viral polyamidoamine (G4-PAMAM) dendrimers. Successful *in vitro* results were reported, with a 90 % inhibition of tumor cell proliferation observed with the developed approach. In line with the non-viral delivery systems for GDEPT, the ability of macrophages to target cancerous cells makes them potential candidates for these types of procedures (Baek et al., 2011; Valable et al., 2007). In this regard, Romena et al. (2021) achieved more efficient non-viral transfection in rat alveolar NR8383 macrophages (Romena et al., 2021). The *Ec*CytDA coding gene was transfected *via* photochemical internalization (PCI), and the modified macrophages were used to induce glioma cell apoptosis in the presence of 5-FCyt. *Ec*CytDA-transfected macrophages exhibited significant cytotoxicity *in vitro*, and the growth of surrounding tumor cells was also inhibited due to a notable bystander effect.

2.3.2. Cytidine DA

2.3.2.1. General concepts. Cytidine DA (CydDA) (EC 3.5.4.5) catalyzes the hydrolytic deamination of Cyd **(66)** or dCyd **(67)** into Urd **(68)** or dUrd **(69)**, respectively, in presence of Zn^{2+} (Carter Jr., 1995) (Fig. 14) (Table 2). These enzymes are present a wide range of organisms, from bacteria to mammals, and play an essential role in pyrimidine scavenging and maintaining the intracellular pyrimidine pool (Costanzi et al., 2003). Moreover, this deamination process is also necessary for pyrimidine catabolism, which is linked to β-alanine production and, therefore, to the *de novo* pyrimidine synthetic pathway (Frances and Cordelier, 2020). As shown for other members of the CDA superfamily, CydDA displays a conserved signature motif, [HC]-[AV]-*E*-x(24–30)-P-C-x-x-C) (where "x" denotes any amino acid), including the Zn^{2+} binding

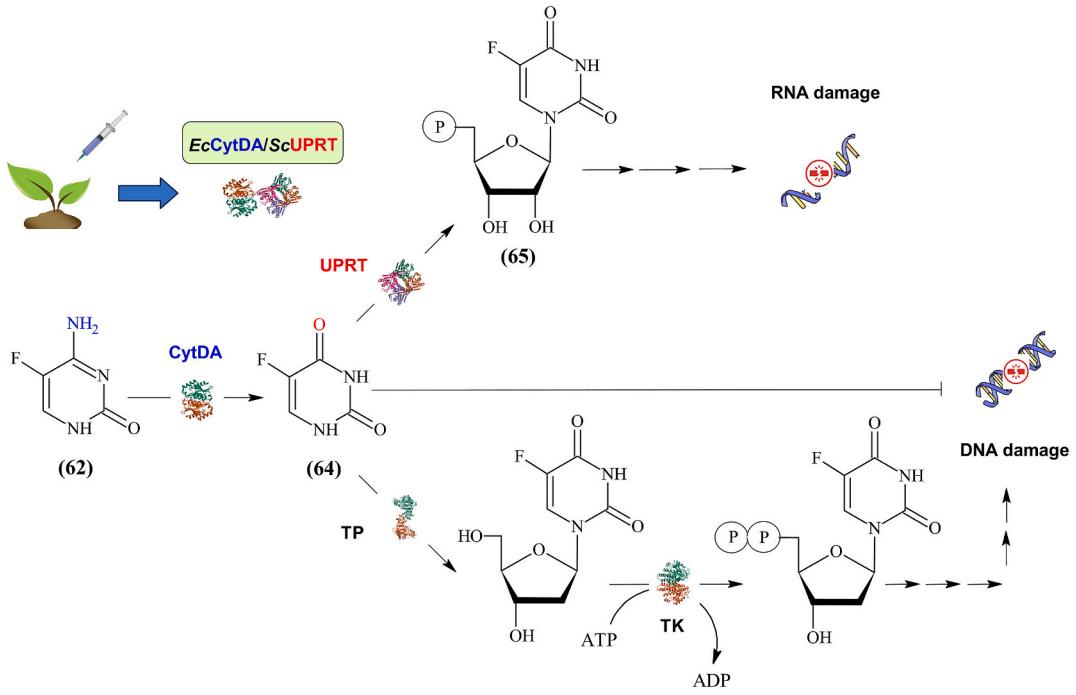


Fig. 15. Primary enzymes involved in the metabolism of 5-fluorocytosine in transgenic plant lines expressing the fusion enzyme *Sc*CytDA/*Ec*UPRT. *Sc*CytDA: *Saccharomyces cerevisiae* CytDA; *Ec*UPRT: *E. coli* uracil phosphoribosyltransferase; TP: thymidine phosphorylase; TK: thymidine kinase.

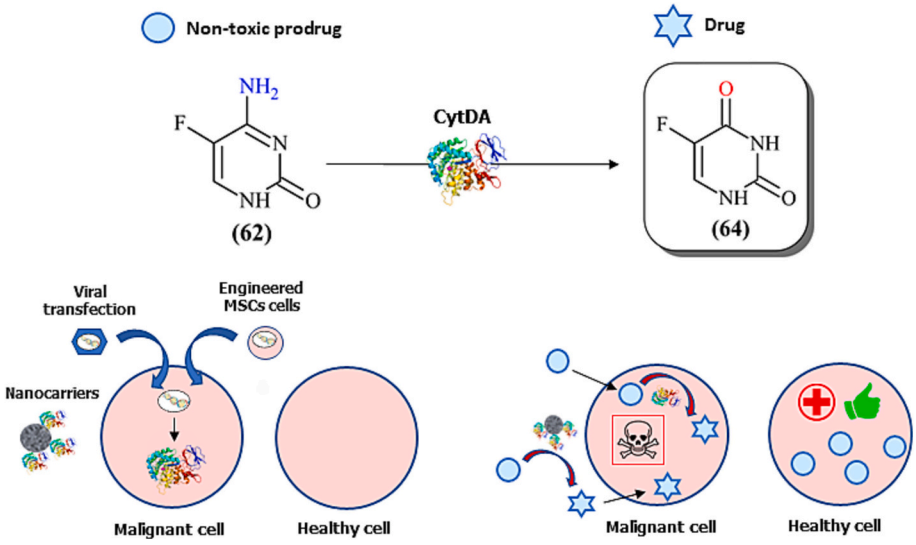


Fig. 16. Prodrugs cancer therapy.

residues (Hys and Cys) and the Glu residue responsible for the proton shuttling during deamination (Fig. 4) (Navaratnam and Sarwar, 2006).

2.3.2.2. Biotechnological applications. CydDA can be found in nature as dimers (*e.g. Escherichia coli* and *Arabidopsis thaliana* CydDAs) or tetramers (*e.g. Homo sapiens* and *Bacillus subtilis* CydDAs) (Carlow et al., 1999; Costanzi et al., 2003; Faivre-Nitschke et al., 1999). Interestingly, two different isoforms (*Hs*CydDA1 and *Hs*CydDA2) can be found in humans (Vincenzetti et al., 2004). This, together with the well-known CydDA-mediated inactivation of cytidine chemotherapeutic nucleoside analogs, such as Ara C **(70)**, 2,2-difluorodeoxycytidine (gemcitabine) **(71)**, and 5-aza-cytidine **(72)** (Fig. 17), has prompted research laboratories to invest significant efforts in developing potent inhibitors of human CydDA (*e.g.*, zebularine). These inhibitors are administered as coadjuvants to enhance the antineoplastic activity of these antimetabolites (Frances and Cordelier, 2020).

Similarly to CytDA, CydDA has found applications in different gene therapy strategies over the last few decades. Since low levels of CydDA are usually associated with severe toxicity in rapidly proliferating tissues, the overexpression of CydDA can protect these tissues from the side effects of chemotherapy. Moritz and coworkers successfully applied this strategy to protect the healthy marrow from the effects of the treatment of acute leukemia with Ara C (Lachmann et al., 2013a, 2013b). Additionally, there is an emerging role of CydDA in the development of novel therapeutic strategies based on CydDA overexpression in tumor or infected cells (Zauri et al., 2015). This strategy allows the deamination of synthetic 2′-deoxycytidine analogs, such as 5-hmdCyd **(73)** or 5-FdCyd **(74)** (Fig. 17), to get 5-hmdUrd and 5F-dU. These metabolites

are subsequently phosphorylated by NKs and inserted into DNA, triggering cell cycle arrest and cell death (Boorstein et al., 1992; Zauri et al., 2015). Finally, since some kinetoplastid pathogens, including *Trypanosoma* and *Leishmania* species, lack certain enzymes of the *de novo* pathway and depend entirely on the salvage pathway for nucleotide synthesis both, CydDA and AdoDA, can be considered valuable chemotherapy targets for the treatment of tropical diseases (sleeping sickness or leishmaniasis) (El Kouni, 2003; Leija et al., 2016; Naguib et al., 2018).

Stepping away from the exclusive realm of biomedical applications, CydDAs have found versatile utility as catalysts in the enzymatic and chemo-enzymatic synthesis of a wide range of nucleosides and nucleotides (Fernández-Lucas, 2015; Mikhailopulo, 2007) **(75–77)**. In this respect, the most common strategy involves the use of reaction schemes based on the sequential use of CydDAs and nucleoside phosphorylases (NPs), or *vice versa*. In the early 1990s, this tandem strategy was successfully employed for the synthesis of Ara G, an antimetabolite used in the treatment of T-cell acute lymphoblastic leukemia. The synthesis involved the deamination of Ara C **(70)** to get Ara U, followed by a transglycosylation using Ara U as a nucleoside donor, and Gua, Guo, or 3′-dGuo as sources of guanine. To this end, the authors employed glutaraldehyde-treated *E. coli* BM-11 cells (which contain highly active CydDAs and NPs), achieving moderate to high conversion yields (63 %–65 %) (Zinchenko et al., 1990).

In a similar way, Barai et al. (2002, 2003) conducted a multi-enzymatic synthesis to produce 3′-dGuo **(77)** from 3′-dCyd **(75)** and 2,6-diaminopurine (DAP) (Fig. 18). Initially, they executed a cascade synthesis of 2,6-diaminopurine-3′-deoxyriboside using *E. coli* BM-11 (expressing CydDA) and BMT-4D/1 A (expressing UP/PNP) whole cells as biocatalysts. The sequential action of CydDA (deamination) and UP/PNP (transglycosylation) led to the synthesis of 3′-dAdo (60 mM, pH 7.0, 26 h, 52 °C, 64 % yield). In the second step, the deamination of 3′-dAdo, catalyzed by *Ec*AdoDA, resulted in the formation of 3′-dGuo (85 % yield, RT, 16 h). The combined action of CydDA/UP/PNP/AdoDA in this two-step system afforded 68 % yield of the desired compound (Fig. 18) (Mikhailopulo and Miroshnikov, 2011).

In another practical application of CydDAs in the pharmaceutical industry, Mahmoudian et al. (1993) covalently immobilized *E. coli* CydDA onto epoxy-activated Eupergit C for the enzymatic resolution of a racemic mixture of (±) 2′,3′-dideoxy-3′-thiacytidine. The selective deamination of the (+) isomer led to the production of the optically pure (−) enantiomer lamivudine, 3TC **(76)** (an FDA-approved anti-HIV agent) with a remarkable 76 % overall yield and an optical purity exceeding 99.5 %. The one-pot, one-step enzymatic resolution catalyzed by *Ec*CydDA offered a more efficient, environmentally friendly, and cost-effective alternative to the most common three-step chemo-enzymatic process (Patel, 2008). As a proof of concept, 1.15 kg of highly optically pure lamivudine was produced from a 3 kg batch by reusing the immobilized *Ec*CydDA derivative up to 15 times, resulting in a 38.4 % overall yield and optical purity exceeding 99.5 %. Moreover, GlaxoSmithKline successfully implemented this biocatalytic approach in the industrial manufacturing of lamivudine on a tonne scale using these immobilized derivatives.

In another interesting example, Zhu et al. (2015) modified the production of pyrimidine nucleosides in *Bacillus subtilis* through various metabolic and genetic interventions. To achieve this, the researchers manipulated genes closely associated with pyrimidine nucleoside biosynthesis, such as *cdd* (encoding CydDA) and *hom* (homoserine dehydrogenase). This, together with the deregulation of the *pyr* operon (pyrimidine nucleotide biosynthetic operon), the overexpression of *prs* gen (phosphoribosyl pyrophosphate synthetase), and the deletion of the *nupC* (transporter responsible for nucleoside uptake) along with the *pdp* gene (PyNP) from the *dra-nupC-pdp* operon, collectively led to a significant increase in the production of pyrimidine nucleoside compounds. Interestingly, experimental results revealed that the activation or inactivation of the *cdd* gene determined the presence or absence of intracellular CydDA, consequently modifying the Urd/Cyd ratio. By using this methodology, Urd and Cyd were produced at high concentrations (1684.6 mg/L and 1423 mg/L, respectively).

Recently, Burke and colleagues pioneered a more environmentally friendly synthesis of Molnupiravir (MK-4482, 79), a promising anti-COVID-19 agent currently undergoing evaluation by global regulatory agencies. They achieved this by replacing a chemical hydroxyamination step with a straightforward enzyme-mediated transformation catalyzed by an evolved *E. coli* CydDA variant (*Ec*CydDA*) (Fig. 19) (Burke et al., 2022). The conventional chemo-enzymatic process involves a chemical hydroxyamination of Cyd **(66)**, followed by a lipase-mediated selective acylation of *N*-hydroxycytidine **(78)** catalyzed by Novozyme 435 lipase (immobilized *Candida Antarctica* Lipase B, EC 3.1.1.3). In this context, the authors aimed to develop a novel and environmentally friendly alternative to chemical hydroxyamination for the synthesis of **78**. To this end, $Ec\text{CydDA}_{wt}$ was subjected to three rounds of evolution, obtaining the $Ec\text{CydDA}_{T123G}$ variant which significantly improved the *N*-hydroxycytidine:uridine ratio by up to 48-fold (8:1). To assess the scalability of $Ec\text{CydDA}_{T123G}$ for manufacturing, they successfully synthesized **78** at high substrate concentrations ([substrates] = 100 g/L, 90 % conversion, 24 h) (Burke et al., 2022).

Finally, in a recent study, Urbelienė et al. (2023) unveil the remarkable catalytic capabilities of prokaryotic homo-tetrameric CydDAs to catalyze nucleophilic substitution reactions at the fourth position of diverse substrates, including N^4-acyl-cytidines, N^4-alkyl-cytidines, N^4-alkyloxycarbonyl-cytidines, S^4-alkylthio-uridines, and O^4-alkyl-uridines. Under their enzymatic influence, these substrates undergo transformation into Urd, yielding corresponding amides, amines, carbamates, thiols, or alcohols as leaving groups. This versatile catalytic activity expands the repertoire of chemical transformations mediated by CydDAs, holding great promise for applications in synthetic chemistry, biotechnology, and pharmaceutical research.

2.3.3. Pyrimidine nucleotide DAs

2.3.3.1. General concepts and biotechnological applications. Similar to purine nucleotide DAs, we have included pyrimidine DAs that act on nucleotides in a separate category namely pyrimidine nucleotide DAs (Table 2).

2′-deoxycytidine-5′-monophosphate DA, commonly referred to as dCMPDA (EC 3.5.4.12), serves as a catalyst for the irreversible hydrolytic deamination of dCMP, resulting in the formation of dUMP and ammonia. Within the enzymatic landscape, dCMPDA is categorized as a member of the CDA superfamily. Typically, dCMPDAs are encountered in nature as homohexamers, exemplified by dCMPs from *Homo sapiens* (Maley et al., 1993) *Gallus gallus* (Maley and Maley, 1990) and *Schistosoma mansoni* (Scortecci et al., 2017), however, it is noteworthy that a dimeric variant has been isolated from human spleen (Ellims et al., 1981). Unlike nucleobase and nucleoside DAs dCMPDA assumes a pivotal role in DNA synthesis and mitosis (Maley and Maley, 1990). The heightened levels of dCMPDA observed in actively dividing tissues prompted its consideration as a potential marker for diagnosing various disease states in which enzyme levels are elevated, particularly in the early 1990s.

In contrast to their nucleobase or nucleoside counterparts, dCMPDA has garnered relatively less attention in the realm of biotechnological and biomedical applications. However, recent discoveries have unveiled intriguing possibilities in this domain. One notable revelation comes from the identification of a bifunctional enzyme encoded in the genome of chlorovirus PBCV-1, possessing both dCMP and 2′-deoxycytidine-5′-triphosphate (dCTP) deaminase activities. This enzyme opens up the intriguing prospect of generating two distinct intermediates for dTTP synthesis (Zhang et al., 2007). More recently, a recombinant bifunctional dCMPDA/dUTPase from *Bacillus halodurans* strain C125 has been characterized (Oehlenschlæger et al., 2015). Furthermore,

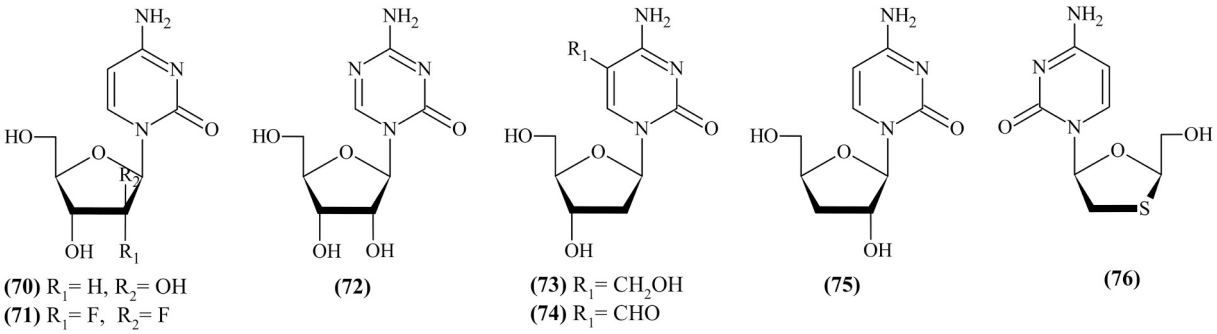


Fig. 17. Chemical structure of different pyrimidine nucleosides.

investigations have unveiled the presence of a dCMP deaminase in rice. This enzyme not only plays a role as a regulator in chloroplast development but also contributes to critical processes such as DNA damage repair, cell cycle progression, and plant development (Xu et al., 2014; Niu et al., 2017).

Another example of pyrimidine nucleotide DA is deoxycytidine triphosphate deaminase, dCTPDA (EC 3.5.4.13), which is involved in DNA metabolism. Its primary function is to catalyze the conversion of dCTP into 2′-deoxyuridine-5′-triphosphate (dUTP) (Huffman et al., 2003). Similar to other DAs, dCTPDA plays a crucial step in maintaining the integrity of the DNA nucleotide pool. Unfortunately, despite several studies deepen into the physiological role and implications in DNA metabolism, structural basis, or substrate specificity, no specific technological or biomedical applications have been developed. As solely example, a bifunctional dCTPDA:dUTPase in *Mycobacterium tuberculosis* has been reported (Dos Vultos et al., 2009). In this context, studying the DNA repair system of *M. tuberculosis* is of paramount importance because it can provide insights into the pathogen's ability to evolve and develop resistance to antibiotics. Additionally, recent findings have revealed the existence of defensive bacterial dCTPDA proteins that, upon phage infection, catalyze the conversion of dCTP into deoxyuracil nucleotides (Tal et al., 2022). It is well known that DNA viruses and retroviruses consume large quantities of 2′-deoxynucleotides (dNTPs) when replicating. In this context, the depletion of deoxynucleotides is a common strategy used by bacteria to defend against bacteriophage infection.

2.3.4. *Other pyrimidine DAs*

As described for purine DAs (subsection 2.2.4.), the modification of nucleic acids is a trending topic in research in life sciences. Because of this, pyrimidine DAs that catalyze the hydrolytic deamination of nucleic acids are included in a separate subsection.

2.3.4.1. General comments. CydDAs that catalyze the conversion of Cyd to Urd in ssDNA and ssRNA have been intensively studied due to the fundamental physiological functions they fulfill. In vertebrates, this class of CydDA enzymes is identified as the apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) family (Conticello, 2008) (Table 2). Interestingly, up to eleven APOBEC family members have been identified in humans: activation-induced deaminase (AID), APOBEC1, APOBEC2, seven APOBEC3 proteins (APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3D, APOBEC3F, APOBEC3G, APOBEC3H), and APOBEC4 (Salter et al., 2016).

These enzymes serve crucial functions in various biological processes, encompassing lipid metabolism, antibody diversification, as well as both the adaptive and innate immune responses (Bransteitter et al., 2009). APOBEC1, for instance, plays a pivotal role in the deamination of apolipoprotein B (apoB) mRNA, giving rise to two isoforms of the protein responsible for cholesterol and triglyceride transport in the bloodstream. It is worth noting that while APOBEC1 can deaminate ssDNA, in fact it stands as the sole APOBEC family member known to act on ssRNA (Petersen-Mahrt and Neuberger, 2003). More precisely, ApoB mRNA editing is catalyzed by the APOBEC1 homodimer, with the active-site residues also participating in RNA binding (Fig. 20). Specifically, Cyd-to-Urd editing occurs in a Cyd residue (referred to as "C" in Fig. 20) located 3′ near the RNA recognition motif, known as the mooring sequence (Backus and Smith, 1991). In this process, the DA domain of one subunit recognizes the target Cyd, while the DA domain of the other subunit binds to a downstream Urd residue (referred to as "U" in Fig. 20) (Chester et al., 2000). To ensure efficient editing, auxiliary factors such as ACF (APOBEC-1 complementation factor) (Mehta et al., 2000) and ASF (APOBEC-1 stimulating factor) (Lellek et al., 2000) are indispensable. ACF and APOBEC-1 collectively constitute the minimal multiprotein complex required for ApoB mRNA editing *in vitro*, although additional proteins may participate in this process *in vivo* (Mehta et al., 2000).

AID, on the other hand, initiates somatic hypermutation in germinal center B cells, making it a key enzyme for antibody affinity maturation

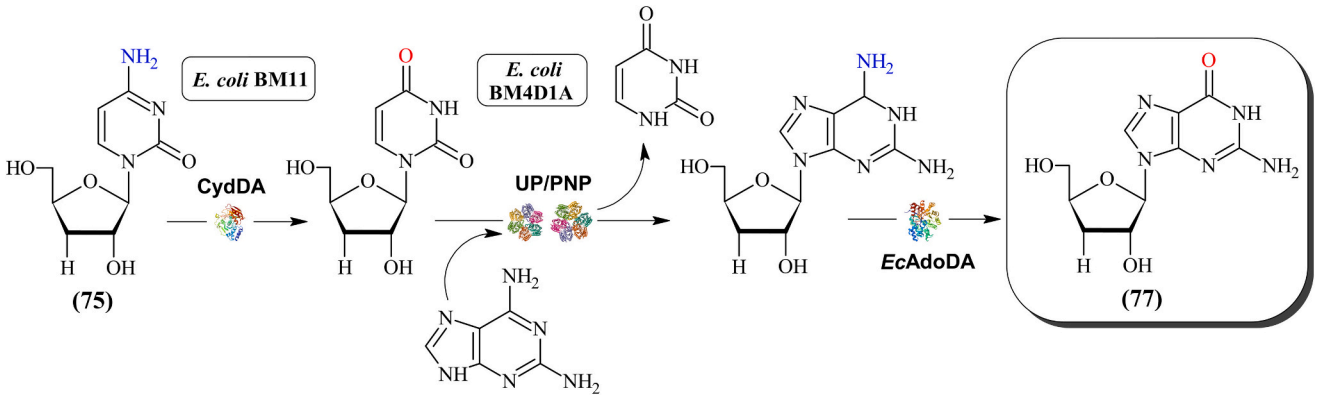


Fig. 18. 3′-Deoxyguanosine synthesis using CydDA/UP/PNP/AdoDA cascade system.

(Muramatsu et al., 2000). In this context, AID plays an indispensable role in the acquired immune system. Conversely, the APOBEC3A-H DAs are integral to the intrinsic immune system, implicated in restricting retrotransposons and inhibiting replication in various retroviruses (*e.g.*, HBV and HIV) and some DNA viruses (*e.g.*, AAV) (Silvas and Schiffer, 2019). In contrast, APOBEC2 and APOBEC4 remain relatively enigmatic, with limited knowledge of their physiological roles. APOBEC2 is mainly expressed in skeletal and cardiac muscle, and its deficiency has been associated with myopathy and muscle atrophy in animal models (Etard et al., 2010; Sato et al., 2018). Intriguingly, although APOBEC2 does not exhibit deaminase activity, it effectively binds to promoter regions in DNA (Lorenzo et al., 2021). Meanwhile, APOBEC4 has been detected in mammalian testicles, where it may play a role in promoter modulation (Marino et al., 2016). In summary, members of the APOBEC family are potent DNA editors, serving essential roles in numerous biological processes. However, when DNA deaminating activity occurs outside their specific targets, APOBEC enzymes could potentially trigger mutagenic events implicated in cancer development (Rebhandl et al., 2015).

The APOBEC family's evolutionary origins date back over 500 million years ago, tracing back to ancestral AID genes present in jawless fish (Conticello et al., 2005). Subsequently, the APOBEC family underwent further expansion in amphibians, birds, and mammals through gene duplication events and by structural and functional diversification from the ancestral AID (Conticello et al., 2005). Despite their diverse functions, APOBEC family members share similar sequence and structural features.

Specifically, all APOBEC DAs contain at least one CDA domain, characterized by the consensus H-[AV]-*E*-x(24–36)-P-C-x(2)-C amino acid sequence motif (where "x" denotes any amino acid) (Jarmuz et al., 2002). This domain, akin to CDA enzymes, comprises a compact catalytic core that exhibits a conserved αβα layered fold, featuring a five-stranded β-sheet surrounded by six α-helices (Yang et al., 2017). Furthermore, the zinc ion within the active site is coordinated by one histidine and two cysteine residues (Salter et al., 2016). In contrast to the rest of APOBEC family members, APOBEC3B and APOBEC3D-G possess two CDA domains. However, in such cases only the CDA domain located in the C-terminal is usually active (Salter et al., 2016). Moreover, further differences among APOBEC family members lay in the length and conformation of the conserved secondary structure elements, particularly concerning the active site loops. These structural differences determine the substrate specificity and the regulation of the deamination reaction (Yang et al., 2017).

In addition to the well-studied APOBEC family of CydDAs in vertebrates, it is crucial to recognize the role of the DYW domain in plant pentatricopeptide repeat (PPR) proteins. The DYW domain functions as a CydDA responsible for RNA editing within plant organelles, such as chloroplasts and mitochondria. This process involves the site-specific deamination of Cyd-to-Urd in mRNA, which is essential for proper gene expression and protein synthesis (Small et al., 2020). Structurally, the DYW domain shares key features with other CydDAs, including a conserved zinc-binding motif crucial for its catalytic activity (Small et al., 2020). However, what sets the DYW domain apart is its association with PPR motifs. These motifs are a series of repetitive amino acid sequences that are responsible for the domain's RNA substrate specificity. The PPR motifs guide the RNA substrate to the catalytic site of the DYW domain, ensuring that editing occurs at the correct locations within the RNA transcript (Wagoner et al., 2015). This targeted editing is vital for the production of functional proteins and the maintenance of organellar genomes (Small et al., 2020). This domain's critical role in plant RNA editing highlights an evolutionary parallel with the APOBEC enzymes in vertebrates, suggesting a shared ancestral origin (Kotera et al., 2005). Over time, these enzymes have diverged and specialized, with the DYW domain evolving to meet the unique demands of organellar gene regulation in plants, a function vital for processes like photosynthesis and overall cellular metabolism.

2.3.4.2. Biotechnological applications. Since ADAR and ADAT enzymes are naturally restricted to RNA editing, the ability of APOBEC family enzymes to act on DNA makes them promising candidates for the development of novel biotechnological tools in genomic engineering. In this sense, the following discussion will cover some of the most pertinent

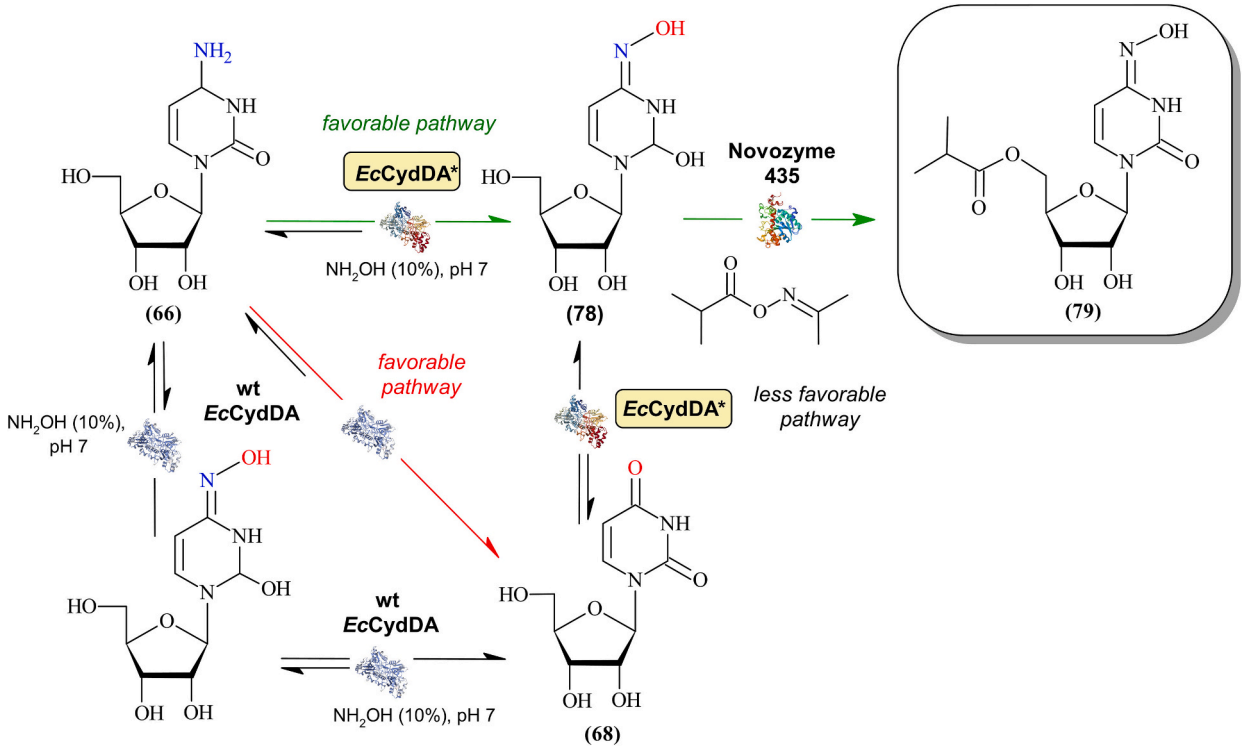


Fig. 19. Two-step synthesis of Molnupiravir. *Ec*CyDA*: engineered *E. coli* cytidine DA; Novozyme 435: immobilized *Candida antarctica* Lipase B.

tools in this category.

2.3.4.2.1. APOBEC-coupled epigenetic sequencing. DNA encompasses various layers of information, among which epigenetic data holds significant value, providing crucial insights into the dynamic regulations governing genome transcription. Among the various epigenetic modifications, one of the most relevant is cytidine methylation within DNA, characterized by the presence of 5-methylcytosine (5-MetCyt) and 5-hydroxymethylcytosine (5-hmCyt) nucleobases in DNA. These modifications play pivotal roles in a wide array of physiological and pathological processes.

In this sense, APOBEC-Coupled Epigenetic Sequencing (ACE-Seq) is widely employed method for epigenetic sequencing (Li et al., 2018c). In this workflow, the first reaction involves ten-eleven translocation 2 (TET2) and T4 Phage β-glucosyltransferase (T4-BGT), which converts 5-MetCyd and 5-hmCyd into products that resist deamination by APOBEC3A. In the subsequent reaction, APOBEC3A deaminates unmodified Cyd nucleosides within DNA, transforming them into Urd. Afterward, the sample is subjected to sequencing and compared to the untreated genome. ACE-Seq is becoming increasingly significant in deciphering the epigenetic code when compared to non-enzymatic methods based on bisulfite oxidation and enzymatic methods based on nucleases (EM-seq). These traditional techniques not only lack the capability to distinguish between 5-MetCyt and 5-hmCyt nucleobase modifications but also fail to identify Cyd-to-Thd (referred to as C-to-T in the literature) conversions, which are the most frequent mutations observed in the mammalian genome and cancer (Schutsky et al., 2018). Nonetheless, ACE-Seq approach requires distinct and parallel workflows, along with separate sequencing steps, to achieve a comprehensive understanding. This may result in elevated sample requirements, increased costs, and prolonged processing times (Füllgrabe et al., 2023). In this context, Füllgrabe et al. (2023) proposed a new five-letter seq workflow comprising five steps. The process involves sonication and ligation of the sample DNA to synthetic DNA hairpin adapters. Subsequently, the strands are separated, and a complementary copy strand is synthesized for each original sample strand. Unmodified Cyd nucleosides within DNA are protected enzymatically through oxidation of 5-MetCyt nucleobase into different derivatives (5-carboxylcytosine, 5-formylcytosine, or 5-hmCyt) by a TET methylcytosine dioxygenase, followed by enzymatic glycosylation of all 5-hmCyt nucleobases by β-glycosyltransferase. The unprotected Cyd are then deaminated to Urd (Cyd-to-Urd) using APOBEC3A (A3A). The deaminated compounds, with reduced duplex stability, are amplified *via* PCR and sequenced in a paired-end format. The data from paired reads are aligned, and the matching residues from both reads are harmonized to generate a cohesive genetic or epigenetic sequence. This approach retains the read while minimizing information loss and maintaining high accuracy, at least in human genome and in cell-free DNA.

2.3.4.2.2. Cytidine base editors for genomic editing. Similar to ABEs, cytosine base editors (CBEs) for single nucleotide modifications in DNA are based on chimeric proteins comprising a CydDA catalytic domain and a dCas9 nuclease (Rees and Liu, 2018). The chimeric protein is guided by a sgRNA, which pairs to target DNA strand displacing a small segment of ssDNA (Nishimasu et al., 2014). Thereupon the CydDA

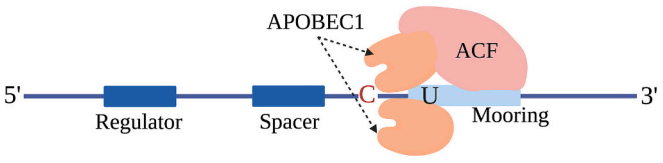


Fig. 20. Schematic representation of the minimal multiprotein complex necessary for apoB mRNA editing. The sequence motifs critical for editing are represented as boxes. The active sites of the APOBEC1 dimer recognize both the target Cyd (C) and the downstream Urd (U). The auxiliary ACF protein is essential for editing and recognizes the editing site by interacting with the mooring sequence. Figure adapted from Gerber and Keller (2001).

enzyme induces Cyd-to-Urd (referred to as "C-to-U" in the literature) modifications in the ssDNA bubble, which, eventually, will end up in Cyd-to-Thd conversion (referred to as "C-to-T" in the literature) through DNA repair and replication processes. As described for ABEs, the major advantage of CBEs relates to the fact that precise point mutations are made with no need for double-strand breaks (DSBs), thereby avoiding undesired byproducts, such as indels, translocations, and rearrangements (Kosicki et al., 2018; Zhang et al., 2015). However, since these modifications are made at the genome level and result in permanent changes, undesired off-target editing is a significant concern that must be thoroughly addressed to ensure successful clinical application (Tang and Xu, 2020).

The first-generation CBE (base editor 1, BE1) (Fig. 21), developed by David R. Liu's group, involved the fusion of APOBEC1 to *Sp*dCas9 (Komor et al., 2016). To enhance the Cyd-to-Urd deamination *in vivo*, authors attached the uracil DNA glycosylase inhibitor (UGI) from bacteriophage PBS1 to the C-terminal of BE1, obtaining a second-generation base editor (BE2). UGI can inhibit human uracil DNA glycosylase (UDG), which is responsible for removing uridine from DNA, thereby triggering base-excision repair (BER) and reverting Urd-Guo pairs back to Cyd-Guo pairs, as elucidated by Kunz et al. (2009). The presence of UGI prevents BER at the target site, leading to a 3-fold increase in editing efficiencies compared to BE1.

To further enhance base editing efficiency in eukaryotic cells, BE2 was optimized by restoring His840 in *Sp*dCas9 (A840H mutation) to create a third-generation base editor known as BE3. So, BE3 comprised a Cas9 with re-established nuclease activity, allowing it to nick (create a single-strand break) in the non-target DNA strand containing the unedited Guo. This nicking of the non-target strand induced eukaryotic mismatch repair (MMR) or long-patch BER, leading to the modification of the Urd-Guo mismatch into a Urd-Ado pair, ultimately resulting in the desired Thd-Ado pair. BE3 was tested across six genomic *loci*, achieving an average editing efficiency of 37 %. Importantly, BE3 retains the D10A mutation in Cas9, which prevents dsDNA cleavage and reduces the likelihood of indel formation. Although BE1 and BE2 produced extremely low indel rates ($\leq$ 0.1 %), BE3 exhibited a slightly higher indel frequency; however, this was still a small frequency, averaging 1.1 % across the six tested *loci*. The D10A mutation in BE3 effectively mitigates double-strand break formation, resulting in these relatively low indel rates.

A similar CBE, known as Target-AID, was developed for introducing single nucleotide modifications in yeast and mammalian cells (Nishida et al., 2016). In this case, the authors utilized an AID ortholog from *Petromyzon marinus* (*Pm*CDA1), instead of APOBEC1, while keeping the remaining components of the system unchanged. This resulted in a fusion protein comprising *Pm*CDA1, Cas9 nickase (D10A variant, nCas9), and UGI to create the nCas9-*Pm*CDA1-UGI editase. All these components made Target-AID highly efficient in yeast, but both deletions and off-target editing were observed in mammalian cells. Moreover, comparing to BE3, the editing window was slightly shifted, which was associated to the different characteristics of *Pm*CDA1 relative to APOBEC1.

Later on, the BE3 system developed by Liu's group was further optimized to create a high-fidelity base editor (HF-BE3) with reduced off-target editing events (Rees et al., 2017). First, modifications were introduced to the original *Sp*Cas9 (N497A, R661A, Q695A and Q926A). This novel variant (*Sp*Cas9*) exhibited reduced affinity for DNA (Kleinstiver et al., 2016), achieving reduced off-target editing but with a slight decrease in on-target editing efficiency. Then, a second strategy was applied for the lipid-mediated delivery of HF-BE3, which increases the specificity of the editing when compared to the plasmid transfection. Thus, HF-BE3 was delivered as ribonucleoprotein complexes into zebrafish embryos and mouse cochlear cells, achieving a highly specific, virus-free and DNA-free *in vivo* DNA editing. More recently, additional modifications were made to BE3 to broaden the number of targetable sites in the genome (Kim et al., 2017), by replacing *Sp*Cas9 with natural

Cas9 from *S. aureus* (*Sa*Cas9) or variant Cas9 nickases (Kleinstiver et al., 2016) with different PAM specificities. Additionally, mutations were also introduced by site-directed mutagenesis in the CydDA domain of APOBEC1 to narrow the width of the editing window. Based on the sequence similarity with APOBEC3G, mutations such as W90Y, W90F, and R126 were made in residues that are crucial for deaminase activity or substrate binding. Combining the window narrowed APOBEC1 enzymes along with the Cas9 variants displaying altered PAM specificities, authors developed novel BE3 systems active over a higher number of disease-related mutations and, in turn, with decreased off-target effects (Kim et al., 2017).

Despite window narrowing mutations in the deaminase domain could reduce the off-target editing to some extent, undesired mutations could still occur if several cytidines are present in the editing window. In this regard, human APOBEC3A (eA3A) was engineered to introduce mutations in the region proximal to the DNA interaction interface, aiming to enhance sequence selectivity and precision in cytidine deamination (Gehrke et al., 2018). Based on co-crystal structures of APOBEC3A and its substrate, specific mutations were introduced at positions N57, K60, and Y130. The designed eA3A-BE3 system displayed reduced bystander cytidine editing while maintaining high activity at target motifs. Interestingly, this system was successfully used for the correction of a disease-related SNP in a human β-thalassemia promoter. When compared to BE3, eA3A-BE3 exhibited over 40-fold higher on-target precision, as well as decreased off-target editing activity.

BE3 was further optimized for the construction of fourth generation base editors (BE4) (Fig. 21) (Komor et al., 2017). BE4 was developed by attaching a second UGI to the C terminal of BE3, as well as by extending both the APOBEC1-nCas9 and UGI-nCas9 linkers. Relative to the previous BE3 version, 1.5-fold higher editing efficiencies, increased product purities, and 2.3-fold lower indel rates were observed across six targeted *loci*. Then, the original *Sp*Cas9 (D10A) was replaced with a smaller nCas9(D10A) from *S. aureus* to generate *Sa*BE4. This way, authors sought to increase the target scope of BE4 based on the alternative PAM requirements of *Sa*nCas9 (Ran et al., 2015). Finally, the Gam protein of bacteriophage Mu was attached to the N-terminal of BE4 to further decrease the indel formation. In this sense, among all the BEs designed in this study, BE4-Gam and *Sa*BE4-Gam averaged the lowest indel frequency and highest product purity throughout the six tested genomic *loci*. Later, a *bp*NLS was introduced at both the N and C terminal of BE4, resulting in the BE4max system with improved nuclear localization (Koblan et al., 2018). Then, the expression of the system was further enhanced by replacing the deaminase portion in BE4max with an APOBEC1 ancestor derived from the ancestral reconstruction of the deaminase component, resulting in the AncBE4max system. Comparing to BE4, in fibroblasts from patients with the glycosylation type 1f disorder (*MPDU1* gene), 2.0- and 2.2-fold higher efficiencies were observed for BE4max and AncBE4max, respectively (Koblan et al., 2018). Similarly, in mouse neuroblastoma cells with mutant *SCN9A* gene encoding the voltage-gated sodium channel NaV1.7 (related to chronic pain), BE4max and AncBE4max displayed 4.2- to 6.0-fold higher editing efficiencies.

Since APOBEC1 is capable of deaminating both DNA and RNA, CBEs comprising APOBEC1 (BE3 and BE4) could cause undesired RNA editing events in human cells (Grünewald et al., 2019a). Within that context, different mutations have been introduced through directed evolution in APOBEC1 enzyme in order to obtain CBE variants with almost negligible RNA off-target activity (Grünewald et al., 2019a; Zhou et al., 2019). So, BE3-R33A/K34A variant displayed significantly reduced RNA editing activity in HEK293T cells, also achieving more accurate on-target DNA editing due to a narrowed editing window (Grünewald et al., 2019a). Similarly, W90Y/R126E mutations in APOBEC1 achieved a reduction of the RNA editing to a base level, while maintaining an efficient DNA editing activity (Zhou et al., 2019). In addition, the replacement of rat APOBEC1 with human APOBEC3A led to a lesser number of RNA editing events (Zhou et al., 2019; Grünewald et al., 2019b). Additionally, this unwanted off-target RNA editing was further reduced through the introduction of R128A or Y130F point mutations (Zhou et al., 2019). Interestingly, as evidenced, the use of DAs different from APOBEC1 broadens the number of BEs to be used with minimal undesired transcriptome editing.

Recently, following a similar approach to that of the previous BE3 system, the potential of APOBEC3A was also investigated within the BE4 framework (Ren et al., 2024). In an interesting development, CBEs based on *Macaca fascicularis* APOBEC3A (BE4-mA3A) exhibited improved properties. Additionally, deletion of Ser-Val-Arg (SVR) in BE4-mA3A led to a significant reduction in DNA and RNA off-target activities, while maintaining the on-target efficiency. Building on these findings, human APOBEC3A was then employed in the creation of a chimeric BE4-hA3A-SVR+ (with SVR inserted in loop 1), demonstrating nearly a 150 % increase in editing efficiency compared to the original BE4-hA3A (Ren et al., 2024).

As demonstrated here, CBEs have been developed and optimized specifically for genomic editing in mammalian cells, distinguishing them from many other systems relevant to research and biotechnology. Consequently, the BE4max and AncBE4max systems have recently undergone optimization to facilitate the introduction of point mutations in biotechnologically significant fish species such as zebrafish (Rosello et al., 2023) and aquaculture species like Atlantic salmon (Raudstein et al., 2024). Additionally, in an interesting development involving another biotechnologically significant animal model, researchers engineered a temperature-tolerant CBE to induce loss- and gain-of-function alleles in *Drosophila melannogaster* (Doll et al., 2023). Despite its widespread use, conventional APOBEC1 displayed reduced activity within the temperature range tolerated by *Drosophila*, spanning from 18 °C to 29 °C. Leveraging the BE4max architecture, researchers replaced APOBEC1 with CydDA from *Petromyzon marinus*, an ectothermic species inhabiting cold to temperate environments. This substitution facilitated robust base editing with exceptionally high efficiency, underscoring the versatility of CBEs for genetic engineering across diverse species (Doll et al., 2023).

Similarly, the thorough study focused on the development and optimization of CBEs has also paved the way for their application in improving crop plants. In particular, BE3 (Li et al., 2017b; Zong et al., 2017; Lu and Zhu, 2017; Ren et al., 2017) and BE4 (Ren et al., 2017) were proven to be effective for the introduction of point mutations in rice. Furthermore, once the capacity of BE3 for base editing in rice was stablished, this very system was also utilized for site-specific editing in wheat and maize (Zong et al., 2017). Similarly, Target-AID was successfully utilized for the introduction of several herbicide-resistance point mutations in rice, as well as for the generation of marker-free tomato plants (Shimatani et al., 2017). In a recent work, an herbicide sensitive rice line (*his1* knockout) was also created by the modification of a start-codon using the Target-AID system (Komatsu et al., 2020). These preliminary studies demonstrated that CBEs serve as an easy-to-use toolkit for target specific base editing in crops. Additionally, CBEs stand out as more economical and efficient systems when compared to homology-directed repair (HDR) and non-homologous end joining (NHEJ) techniques, which have been demonstrated rather ineffective for genome editing in plants (Mao et al., 2013).

However, since APOBEC1 displays a marked preference for Thd-Cyd pairs, BE3 and BE4 applicability in rice, which exhibits high Guo-Cyd content (Wang and Hickey, 2007), is effectively limited. In this context, the efficiency of this novel toolkit was expanded by replacing APOBEC1 with human AID (hAID) (Ren et al., 2018), known to be highly active on Guo-Cyd and Ado-Cyd pairs (Beale et al., 2004). As expected, the developed CBEs displayed higher editing efficiency, favoring the introduction of point mutations in Guo-Cyd pairs, but also resulting highly active on Ado-Cyd, Thd-Cyd, and Cyd-Cyd pairs. In summary, these novel plant CBEs could emerge as suitable editing tools for the generation of gain-of-function and loss-of-function mutations in both monocotyledon and dicotyledon plants.

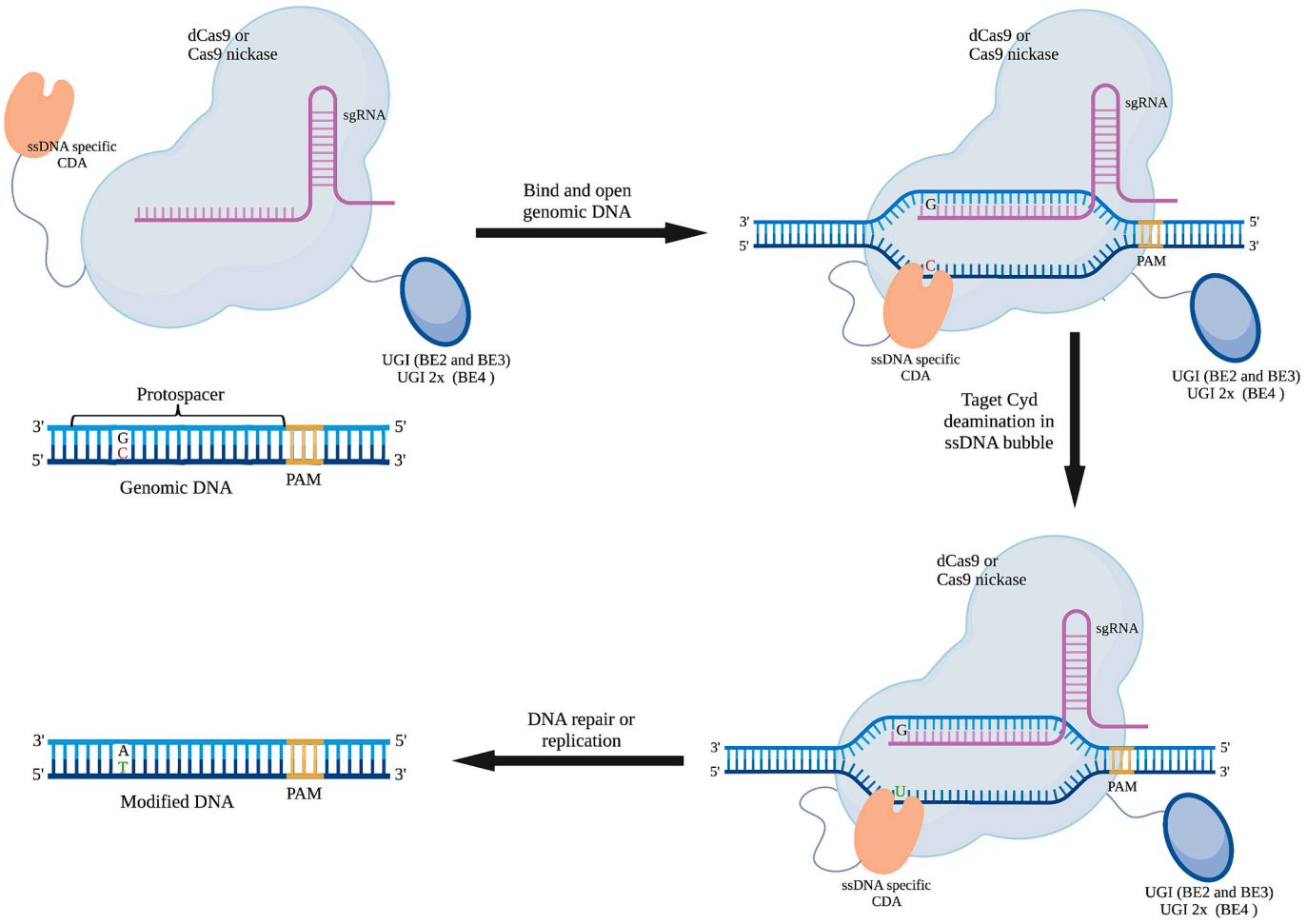


Fig. 21. Schematic representation of CBE-mediated base editing strategy for the conversion of GC base pair to AT pair.

2.3.4.2.3. Evolution of TadA DAs for the development of small and efficient CBEs for genomic editing. As a significant drawback, CBEs can exhibit higher off-target effects and indels and lower on-target editing efficiency compared to ABEs, mainly due to the AID/APOBEC protein family deaminase component. Additionally, the evolved TadA DAs found in ABE systems possess significantly smaller sizes compared to frequently utilized cytidine DAs. This size difference facilitates the delivery of ABEs by viral vectors with restricted cargo capacity, such as AAV. As a matter of fact, the compact nature of TadA has allowed ABEs, though not CBEs, to be successfully delivered into animal tissues *in vivo* utilizing a single AAV (Davis et al., 2022; Zhang et al., 2022). With these advantages in mind, researchers repurposed TadA-8e for Cyd conversion by introducing the N46L mutation (Chen et al., 2023c). This variant eliminated its AdoDA activity and resulted in a TadA-8e-derived Cyd-to-Guo base editor (TadA-CBE). Additionally, by combining with UGI and introducing additional variants, a series of TadA-CBEs with high activity similar to BE4max or more precise than other reported CBEs were obtained. These TadA-CBEs exhibited superior efficiency in producing precise edits within homopolymeric cytosine sites in both cells and mouse embryos, with minimal indel effects and low off-target editing. In a similar manner, the Liu lab employed PACE and PANCE to evolve TadA-8e (E27K/V28A) and develop an additional TadA-CBEs (Neugebauer et al., 2023). This novel TadA-CBE demonstrated exceptional activity and showcased comparable or even greater editing efficiencies when compared to other CBEs. Furthermore, the researchers incorporated the already known V106W mutation (Rees et al., 2019; Richter et al., 2020), which contributed to a higher reduction in off-target editing. This modification fine-tuned the editing window and enhanced the selectivity for Cyd, all while maintaining the on-target editing efficiency. Then, TadA-CBEs were proven capable of efficient editing in primary human T cells and HSPCs, targeting sites of therapeutic significance. Interestingly, PACE and PANCE techniques also allowed the development of a TadA-based dual editor (TadA-DE) with high Ado-to-Guo and Cyd-to-Thd editing efficiency in *E. coli* (Neugebauer et al., 2023). TadA-DE has a smaller size compared to previously reported dual editors that combine CydDAs and AdoDAs with a Cas domain, making it particularly valuable for applications requiring broad mutagenesis, such as genetic screens.

Within the context of minimizing the size of CBEs, an evolved TadA deaminase active on Cyd was obtained through site-directed mutagenesis and subsequently combined with Cas12f to create mini-TadA-CBEs (Zhang et al., 2023a). MiniCBE was able to correct specific pathogenic mutations *in vitro* and *in vivo*, showing reduced off-target editing. Significantly, all the constituents of mini-TadA-CBEs could be delivered within a single AAV system. Further efforts have been also done by screening and engineering TadA orthologs, aiming to create TadA-CBEs that exhibit diminished or minimized off-target effects on both DNA and RNA (Zhang et al., 2023b). Mutations introduced into the *Ec*TadA deaminase enzyme include A106V and D108N, which were identified through directed protein evolution to enhance adenine base editing activity. Additionally, F148A and R153 deletion were introduced to reduce RNA off-target effects, while V82G/W mutations also contributed to minimizing RNA off-target editing. Continuing the effort to minimize off-target editing events, Gaudelli's group designed TadA-CBEs utilizing further engineered versions of TadA (Lam et al., 2023). This newly developed TadA-CBEs demonstrated editing efficiencies comparable to

those reported for BE4, and notably, they showcased more precise editing windows and substantially reduced off-target editing. Additionally, TadA-CBEs were compatible with orthogonal Cas enzymes, thereby potentially expanding their applicability to a wider array of target sites.

Interestingly, the most recent CBE did not evolve from previous TadA-CBEs. Instead, Zhang et al. (2024) introduced novel CBE6 variants *via* PACE, originating from the dual adenine and cytosine base editor TadA-DE (Neugebauer et al., 2023). These new CBE6 variants surpass existing CBE variants in on-target editing efficiency with minimal residual Ado-to-Guo editing. Furthermore, the introduction of the previously known V106W mutation further reduced off-target editing levels without significantly compromising the on-target editing activity. The CBE6 variants efficiently introduced stop codons at genomic loci for PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) knockout in patient-derived fibroblasts (Zhang et al., 2024).

2.3.4.2.4. Organelle specific genomic editing. The current genome editing systems using CRISPR/Cas in the nucleus heavily rely on a crucial RNA component. However, there is a significant challenge in directing RNAs expressed from nuclear transgenes into the DNA-containing organelles, namely mitochondria and chloroplasts (Schmiderer et al., 2022). This obstacle hinders the application of CRISPR/Cas base editing for engineering organellar genomes. Interestingly, in cases where direct transformation of organelles is feasible, and guide RNA genes can be introduced, genome editing becomes much simpler through homologous recombination (HR), rendering CRISPR/Cas unnecessary (Tan et al., 2022). Paradoxically, protein-only genome editing methods have emerged as valuable tools for editing organellar genomes. This is because foreign proteins can be easily targeted to mitochondria and chloroplasts, providing a more practical approach to achieve successful genome editing in these organelles. In this context, the Liu lab was responsible for the development of DddA-derived CBEs (DddA-CBEs), which marked a groundbreaking achievement as the first successful approach to achieve accurate Cyd-to-Thd editing in human mitochondrial DNA (mtDNA) (Mok et al., 2020). The system involves the dsDNA deaminase toxin A (DddA) from *Burkholderia cenocepacia*, which catalyzes Cyd deamination within dsDNA and can be divided into two non-toxic inert fragments. These fragments are then fused to the C-termini of two TALE scaffolds that recognize nearby binding sites. To precisely direct the system to the mitochondria, a mitochondrial targeting signal (MTS) derived from COX8A is fused at the N-terminus. Additionally, the constructions include a UGI inhibitor to prevent mtDNA repair mechanisms. So, the deaminase activity is reactivated when the two DddA fragments converge exclusively at the intended target site (in mtDNA), enabling them to act on the spacer sequence between the two TALE-binding sites. Later, this same laboratory evolved DddA to enhance editing efficiency and surpass the stringent Thd-Cyd sequence-context constraint of DddA (Mok et al., 2022a). So, the DddA6 variant was obtained, displaying an average 3.4-fold improvement in Thd-Cyd editing activity compared to the wild-type DddA. Similarly, a DddA11 variant was also developed to include X-Cyd (X = Ado, Cyd, or Thd) targets for both mitochondrial and nuclear DNA base editing. Since DddA-CBEs inherently need two DddA fragments to function, the size of the system is a limiting factor for packaging in viral vectors with limited cargo space, such as AAV. To tackle this limitation, full-length DddA variants were used to develop monomeric DddA-CBEs (mDddA-CBEs), achieving mtDNA editing with efficiencies up to 50 % (Mok et al., 2022b). DddA-CBEs have been successfully employed for specific mtDNA editing in various organisms, including mice (Lee et al., 2021; Guo et al., 2022), rats (Qi et al., 2021), zebrafish (Guo et al., 2021), plants (Kang et al., 2021), and human embryos (Chen et al., 2022b; Wei et al., 2022).

Lately, Lim et al. (2022) designed another interesting strategy for targeting mtDNA. This novel base editing platform called zinc finger DAs (ZFDAs) comprises custom-designed zinc-finger DNA-binding proteins, the split DddA, and UGI. For its mitochondrial delivery, authors created mitochondria-targeting ZFDAs comprising MTS and NES sequences to the N-terminus. This platform enables precise Cyd-to-Thd base conversions in human cells without inducing undesired small indels. In fact, by assembling plasmids encoding ZFDAs using publicly available zinc finger resources, researchers achieved base editing with frequencies of up to 60 % in nuclear DNA and 30 % in mtDNA. The smaller size of zinc finger arrays compared to TALE arrays offers a distinct advantage for gene delivery by viral vectors with limited cargo space. Unlike TALE arrays, zinc finger arrays lack bulky domains at both the C-terminus and the N-terminus, allowing for easy fusion of split-DddA halves to either terminus of a ZFDA. Moreover, ZFDAs have an intrinsic cell-penetrating activity, which may enable nucleic acid-free gene editing in human cells. These characteristics make ZFDAs an excellent choice as a DNA-binding module for base editing in both nuclear and organelle DNA.

Despite their significant advantages, ZFDAs introduces heteroplasmic mutations non-uniformly within a cell population. To confront this challenge, Liu and colleagues developed a zinc finger DddA-CBEs system (ZF-DddA-CBEs) through structural engineering, defining improved ZF scaffolds, and incorporating mutations in the DddA deaminase components (Willis et al., 2022). Based on previous knowledge, the optimization of the ZF-DddA-CBE architecture, and the creation of versions with enhanced specificities, resulted in reduced off-target editing and enhanced DNA editing in both mitochondria and nucleus. Nonetheless, the still relatively high off-target editing renders ZF-DddA-CBEs unsuitable for therapeutic applications.

Consequently, Lee and coworkers engineered high-fidelity DddA-derived CBEs (HiFi-DddA-CBEs) with minimal off-target activity (Lee et al., 2023). This was achieved by substituting specific amino acid residues with alanine at the interface between the split DddA halves. So, the resulting domains cannot form a functional deaminase without the binding of their linked TALE proteins at adjacent sites on DNA. Whole mitochondrial genome sequencing demonstrated that, in contrast to conventional DddACBEs, HiFi-DddA-CBEs exhibited high efficiency and precision, avoiding collateral off-target mutations. Consequently, HiFi-DddA-CBEs hold great promise for therapeutic applications. In recent developments, the DddA variant in HiFi-DddA-CBEs were replaced with either DddA6 or DddA11 variants, resulting in improved mtDNA editing efficiencies (Wei et al., 2024). This substitution also expanded the potential for Cyd-to-Thd editing at TC, CC, and GC targets in human cell lines. Notably, further mutations introduced in the DddA6 variant enabled the creation of a specialized tool for Ado-to-Guo editing in mtDNA. Additionally, these engineered base editors were used to introduce disease-causing Cyd-to-Thd and Ado-to-Guo mutations into mtDNA in both human cell lines and mouse embryos.

2.3.4.2.5. APOBEC–Cas9 fusion systems for multi-nucleotide deletions. As previously mentioned, the inclusion of UGI within CBE systems is crucial to prevent the activity of UDG (Mol et al., 1995). Without UGI, UDG initiates BER, leading to the reversion of Urd-Guo pairs back to Cyd-Guo pairs (Kunz et al., 2009). However, the BER system, activated by UDG, could be strategically adapted for generating large deletions at designated target sites. So, *Ec*UDG has been employed to the development of APOBEC3A–*Spd*Cas9 fusion-induced deletion (AFID) systems. In these systems, the dCas9 firstly directs the complex to the target, then the deaminase catalyzes the Cyd-to-Urd conversion, and finally, the UDG generates an apurinic/apyrimidinic (AP) site that initiates the BER, allowing the production of large deletions in specific target sites. Later, this system was improved by fusing *E. coli* AP lyase with AFID using the ribosomal skipping peptide (P2A), aiming to enhance the efficiency of AP site removal. By using this improved AFID, about 30.2 % of deletions were created in rice and wheat protoplasts, as well as 34.8 % in regenerated plants. Finally, with the replacement of APOBEC3A to truncated APOBEC3B, more consistent deletions were generated, particularly targeting the preferred Thd-Cyd motif to the double-strand break (Wang et al., 2020).

2.3.4.2.6. CBE systems for Cyd-to-Ado and Cyd-to-Guo transversions.

Similar to ABE systems, in the current state of CBE advancements, the forefront technologies primarily revolve around base transversions. Based in the same idea explored in the AFID systems, instead of a controlled deletion, the DNA repair initiated by UDG could lead to Cyd-to-Ado/Guo editing events at the Urd formed by the deaminase. In this sense, so-called glycosylase base editors (GBEs) have been engineered to achieve Cyd-to-Ado and Cyd-to-Guo transversions in *E. coli* and mammalian cells, respectively (Zhao et al., 2021). In *E.coli*, the GBE comprising the AID-nCas9-UDG editase displayed a remarkable Cyd-to-Ado editing efficiency of 87.2 %. Moreover, by replacing AID to APOBEC1, Cyd-to-Guo transversions were observed in mammalians cells with editing efficiencies ranging from 5.3 % to 53.0 %. These newly developed GBEs complement the existing CBEs and hold significant potential for treating diseases caused by Guo/Cyd mutations.

2.3.4.2.7. CBE systems for random base editing. Methods that induce mutations across the genome have been employed in traditional genetic screening and more recently in the evolutionary engineering of microbes. However, many of these approaches depend on mutagenic chemicals or ultraviolet light, which can cause unintended harm to cellular components, thereby restricting their use in biotechnology (Alcántara-Díaz et al., 2004). Instead of relying on such damaging agents, DNA modification through deaminases offers a flexible alternative that can be used across different microbial species without compromising cell viability. In this sense, CydDA can be fused with DNA transcription or replication related proteins to develop a new toolkit for directed evolution of genomes namely random base editing (rBE) (Pan et al., 2021). In this sense, Wang and coworkers achieved random genomic *loci* for prokaryotic or eukaryotic models using DNAb-AID or MCM5-AID (Mini chromosome maintenance protein 5) fusion proteins, respectively (Wang et al., 2021c). By utilizing this technique, the authors managed to amplify the mutagenicity of the *E. coli* and *S. cerevisiae* wild-type strains by 2.5 × 10^3-fold and 1.68 × 10^3-fold, respectively. Subsequently, these innovative techniques were employed to enhance β-carotene production in modified *E. coli* and *S. cerevisiae* strains by 371.4 % and 75.4 %, respectively.

In a novel approach known as MutaEco, *Pm*CDA1 was combined with the α-subunit of *E. coli* RNA polymerase (Eom et al., 2022). This method increases the mutation rate without compromising cell viability, accelerating the adaptive evolution of *E. coli* for stress tolerance and the utilization of unconventional carbon sources. The main drawback of these technologies is the size of targeted region, which can encompass either the whole genome or a relative narrow window. To address this gap, Zimmermann et al. (2023) developed Confined Mutagenesis using a Type I-E CRISPR-Cas system (CoMuTER). This tool utilizes the targetable helicase Cas3, a hallmark enzyme of the class 1 type I-E CRISPR-Cas system, fused with the *Pm*CDA1 to simultaneously unwind and mutate large DNA stretches, including entire metabolic pathways. By using CoMuTER, the number of mutations in the target region (up to 55 kb) is increased by 350-fold compared to the rest of the genome, resulting in an average of 0.3 mutations per kilobase. In this sense, CoMuTER allowed the authors to produce the double of lycopene compound in *S. cerevisiae* after just one round of mutagenesis.

2.3.4.2.8. CBE systems for in vivo targeted mutagenesis. Global *in vivo* mutagenesis strategies generally produce high mutation rates and create diverse genetic variations. However, the widespread mutations across the genome can pose significant challenges, particularly in directed evolution experiments. Unintended off-target mutations, especially in essential regions of the genome, can be harmful and toxic to the cells (Foster, 1991). Targeted *in vivo* mutagenesis methods offer a solution to these issues by focusing mutations on specific genomic regions. A notable example of gene-specific *in vivo* mutagenesis involves the use of a fusion protein combining CydDA with T7 RNA polymerase. This approach enables precise targeting of genes regulated by T7 promoters, where the CydDA introduces the Cyd-to-Thd mutations. Thus, Moore and colleagues first combined T7 RNA polymerase with rAPOBEC1 for the development of the MutaT7 chimera, allowing for *in vivo* targeted mutagenesis across multi-kb DNA sequences in *E. coli* (Moore et al., 2018). As mentioned, this approach specifically targets sequences that begin with the T7 promoter, enabling *in vivo* mutagenesis and avoiding the toxicity caused by random mutations or insertions and deletions imposed by other techniques on essential regions of the organism's genome.

With this approach in mind, T7 polymerase-driven Continuous Editing method (TRACE) was later developed. TRACE is notable for being the first T7-deaminase system applied in mammalian systems, specifically HEK293T and A375 cells. In fact, the system was shown to induce high rates of mutagenesis over multiple cell generations within genes under the control of a T7 promoter integrated into the genome. Importantly, when introduced into animal models, TRACE does not cause a rise in global mutation rates within 6 days and does not visibly impact cell health for a period of 20 days (Chen et al., 2020). The method was found to be tunable, allowing for adjustments in mutation rates through the engineering of T7 RNA polymerase.

Although the MutaT7 and TRACE systems exhibited high gene-specificity, their mutation rates were relatively low, with MutaT7 showing 0.34 mutations per day per kb (Moore et al., 2018) and TRACE ranging from 0.1 to 0.8 mutations per day per kb (Chen et al., 2020). This limitation reduces their effectiveness when compared to conventional *in vitro* mutagenesis techniques. To address this, PmCDA1 was fused to the N-terminus of T7 RNA polymerase, resulting in the development of enhanced MutaT7 (eMutaT7) (Park and Kim, 2021). This improved system maintained high gene-specificity in *E. coli* while significantly increasing the *in vivo* mutation rate, achieving up to approximately 4 mutations per kb per day.

2.3.4.2.9. CBE systems for RNA editing. Everything discussed so far deals with the use of CBEs exclusively for RNA-guided DNA editing. In this sense, despite the efforts made in this regard, the development of CBEs for RNA editing has proven to be much more complicated. A limitation lies in the fact that RNA deamination catalyzed by APOBEC1, APOBEC3A and APOBEC3G demands the target RNA to adopt certain secondary structure (Sharma and Baysal, 2017; Maris et al., 2005). This could partly explain the difficulty of redesigning these systems for RNA editing. In fact, the first approach for Cyd-to-Urd editing in RNA was based on the rational mutagenesis of ADAR2 deaminase for expanding its substrate scope (Abudayyeh et al., 2019). This process introduced specific mutations to shift the enzyme's activity from Ado-to-Ino modifications to include Cyd-to-Urd changes. Key mutations were strategically introduced both within the catalytic core and throughout the enzyme's structure to enhance its interaction with the RNA substrate and improve overall deamination activity. Notably, these mutations included catalytic core mutations V351G and K350I, as well as RNA contact mutations S486A and S495N. Building on these advancements, the evolved ADAR2 domain was fused with a catalytically inactive Cas13 protein (dCas13) to create a new RNA editing system known as RESCUE (RNA Editing for Specific Cyd-to-Urd Exchange) (Abudayyeh et al., 2019). RESCUE leverages this engineered enzyme to enable precise Cyd-to-Urd editing, while also retaining the capability for Ado-to-Ino modifications, facilitated by specific guide RNAs (gRNAs). To further refine the system and minimize off-target effects, additional modifications were made. RESCUE-S, a variant developed through further rational mutagenesis, features the S375A mutation. This specific alteration was designed to enhance the specificity of C-to-U editing, resulting in approximately 76 % on-target editing while substantially reducing off-target activity compared to the original RESCUE system.

Recently, based on the already described split-ADAR system (Katrekar et al., 2022b), the evolved deaminase domain present in RESCUE was expressed as N-terminal and C-terminal non-functional fragments, each bearing a MCP and λN peptide, respectively. As described earlier, this split-RESCUE system (Katrekar et al., 2022b) uses chimeric gRNAs, which incorporate BoxB and MS2 stem loops for recruiting the two split halves of the deaminase to form the functional editase. As a result, the novel split-RESCUE system exhibited Cyd-to-Urd

editing levels on the endogenous *RAB7A* transcript comparable to those achieved by its full-length counterpart, while also exhibiting high transcriptome-wide specificity. Due to the great outcomes achieved, the evolved deaminase domain found in RESCUE has replaced the $ADAR_{DD}$ in xABE and mxABE (Xu et al., 2021). This substitution serves to broaden the substrate range of these systems, thus obtaining full-length xCBE and mini xCBE (mxCBE), respectively. When tested in HEK293T cells, xCBE and mxCBE demonstrated significantly more efficient Cyd-to-Urd editing compared to the previously reported RESCUE (Xu et al., 2021).

The first cytidine-specific Cyd-to-Urd RNA editor (so-called CURE) was also developed and consists of an APOBEC3A variant (Y132D) fused to dCas13 mutants (Huang et al., 2020). Depending on the system components, three different CUREs were designed, each of them displaying different features. First, APOBEC3A (Y132D) was fused to the C-terminus of PspdCas13b for the construction of CURE-Cytoplasmic (CURE-C), the most active version of CURE. In order to localize CURE to the nucleus, thus reducing the off-target events, an additional NLS was inserted at the C-terminus of the fusion, resulting in CURE-N. Authors followed a second strategy for reducing the off-target effects through the fusion of APOBEC3A (Y132D) to the loop 3 of dCasRx. This last system, CURE-X, was found to be the most specific without any loss of on-target editing activity. It is worth mentioning that the novelty of this RNA editor lies in the use of tailor-made gRNAs capable of inducing loops for the recruitment of the editase to the target sites. In addition, CURE could not deaminate Ado, so it proves more specific than RESCUE, and therefore the risk of off-target missense mutations is highly reduced.

Interestingly, two approaches were developed that set aside the previous CRISPR/Cas-based systems employing APOBEC enzymes (Bhakta et al., 2020; Stroppel et al., 2021). Both systems make use of tagged-gRNAs (Ms2-tagged or SNAP-tagged) to direct the editase to the target mRNA for the introduction specific point mutations. In a first strategy, APOBEC1 was fused with the MS2 coat protein, so using gRNAs carrying the MS2 stem-loop this novel editing tool could be directed to the target RNA sequence (Bhakta et al., 2020). The system was proven successful in the restoration of the GFP reporter in HEK293 cells, suggesting the developed system could be utilized for Cyd-to-Urd editing in transcripts of patients with various diseases. The second strategy was developed in Thorsten Stafforst's group, and it was based on their previous work regarding the SNAP-ADAR editor (Stafforst and Schneider, 2012; Vogel et al., 2018, Vogel and Stafforst, 2019). In this context, the system was expanded to perform both Ado-to-Ino and Cyd-to-Urd base editing in RNA (Stroppel et al., 2021). This could be accomplished using HALO- and SNAP-tag fusions, in combination with their respective gRNAs for the recruitment of both ADAR and APOBEC1 inside the same living cell. Although the off-target activity was much higher than that reported for CURE, as a main advantage, the human origin of SNAP-tag, as well as its small size, facilitates transfection and the subsequent expression of the system components.

2.3.4.2.10. Dual base editors. Considering the capacity of ABE and CBE systems to correct disease-causing single nucleotide polymorphisms, a logical progression is to merge the Ado-to-Guo and Cyd-to-Thd editing capabilities within a single editase (Ado and Cyd base editor, ACBE). In fact, intriguingly, the ability of ABEs to induce not only Ado-to-Guo conversion but also unexpected Cyd-to-Thd conversion was demonstrated in 2019 (Kim et al., 2019). First steps to increase the editing scope of BEs consisted in the combination of *Sp*ABE and *Sa*BE3, resulting in the achievement of both Ado-to-Guo and Cyd-to-Thd editing in mice (Liu et al., 2018). Similarly, Sakata and colleagues designed a fusion editase encompassing *Pm*CDA1, TadA, and a Cas9 nickase (Sakata et al., 2020). The resulting Target-ACEmax exhibited a notable activity in simultaneously editing Cyd-to-Thd and Ado-to-Guo at 47 genomic *loci*. Previously published miniABEmax and Target-AID systems have been also integrated for the design of ACBEs. Thus, the monomeric TadA variant of miniABEmax, the CydDA from Target-AID and two UGIs were combined for the development of SPACE (synchronous programmable Ado and Cyd editor) (Grünewald et al., 2020). SPACE exhibited superior Ado-to-Guo and Cyd-to-Thd conversion rates, as well as a reduced frequency of on-target indels, in comparison to the separate coexpression of the same DAs within miniABEmax and Target-AID systems. Target-AID was also fused to ABE7.10 to generate another ACBE capable of Ado-to-Guo and Cyd-to-Thd editing in both mouse embryonic fibroblasts and porcine fetal fibroblasts (Xie et al., 2020). Yet another ACBE, formed by the fusion of TadA-dCas9-AID, was engineered and applied in *C. glutamicum* (Deng et al., 2020). This dual editase led to a substantial 31.9 % increase in *N*-acetylglucosamine production.

We already mentioned that David Liu's group employed PACE and PANCE to repurpose a TadA-8e deaminase through Cyd-to-Thd editing. In the same study, the authors also developed a dual-base editor (TadDE) capable of achieving equally effective base editing of both Cyd and Ado (Neugebauer et al., 2023). Additional efforts have been made in the development of dual editors by focusing on the evolution of the *Ec*TadA deaminase and its orthologs. In this sense, through the introduction of double mutations, these deaminases were engineered to develop ACBEs with reduced off-target editing (Zhang et al., 2023b). The recently developed ACBEs are expected to broaden the scope of BEs, facilitating their application as the gene therapy tools for the treatment of genetic disorders characterized by heterogeneous point mutations.

3. Conclusion and outlook

In biological contexts, nucleobase, nucleoside, and nucleotide DAs emerge as pivotal players in fundamental cellular processes. These enzymes catalyze essential steps in purine and pyrimidine salvage pathways, contributing to the maintenance of nucleotide pools. In mammals, their functions extend to critical processes such as dendritic ramification, differentiation and maturation of the lymphoid system, along with their contribution to antiviral defense mechanisms, among others. Simultaneously, these ubiquitous enzymes play a role in prokaryotic *quorum sensing* mechanisms, highlighting their versatility across different life forms.

Transitioning from their intrinsic biological roles, this review underscores the diverse biotechnological applications of purine and pyrimidine DAs. Their potential spans pharmaceutical manufacturing, food bioprocessing, and agricultural bioengineering. In biomedicine, these enzymes serve as important therapeutic targets for drug design, and also contribute to disease diagnosis, prognosis, and treatment monitoring. Particularly noteworthy is their role in innovative therapeutic strategies such as GDEPT and as key components in genome and transcriptome BEs, suggesting a promising future for addressing various illnesses and genetic disorders through these enzymatic tools.

DAs have demonstrated their versatility and efficiency as catalysts for the production of NAD-based drugs, offering an eco-friendly alternative to traditional chemical manufacturing processes. Nevertheless, as is common with enzymes, some disadvantages such as narrow range of optimal operating conditions, stability issues and limited range of reactions, constrain their application as industrial catalysts. To overcome these challenges, established methods such as direct evolution and rational design, along with emerging technologies like machine learning and computer-aided retrosynthesis focused on biocatalytic routes, need integration to meet these demands. Furthermore, transitioning from laboratory-scale to industrial-scale production can pose challenges, requiring innovative bioprocess engineering solutions to maintain optimal enzyme activity on a larger scale.

Similarly, in the context of reducing purine content in food, the effectiveness of DAs faces various challenges. Since food and beverages contain diverse purine sources, a combination of DAs with other purine-degrading enzymes like urate oxidase and xanthine oxidase becomes necessary. It is essential that all these enzymes retain their activity and adhere to quality standards when stored together with food products. Lastly, it is crucial to emphasize that any food product undergoing enzymatic modification must comply with food safety regulations.

Continuing in the context of the food industry, plants and cell

cultures expressing DAs show promise as potential and scalable platforms for caffeine production. However, the challenge of limited production yield in plant tissues poses a significant obstacle to large-scale production. The advancement of metabolic engineering has contributed to improve these plant production systems, making them amenable to scaling up in a controlled environment. However, the effectiveness of metabolic engineering strategies is often hampered by pathway compartmentalization, the presence of multiple alkaloid biosynthetic pathways, and intricate regulatory control mechanisms. This cellular complexity can render single gene manipulations ineffective in altering biosynthetic pathways. Promisingly, methods capable of simultaneously modifying multiple metabolic steps (*e.g.*, the use of transcription factors) offer potential solutions to address this complexity. Simultaneously, the construction of plant biosynthetic pathways in microorganisms from the ground up has revealed a novel avenue for production. Microorganisms exhibit significantly lower complexity than plant systems, streamlining the selection of metabolic engineering targets and strategies. Nonetheless, challenges remain to be addressed before microorganisms can serve as an industrial platform for caffeine production. As outlined in this review, the methylation process is crucial for caffeine biosynthesis. Thus, despite the overexpression of highly active DAs, the production of caffeine at high levels is restricted by the intracellular availability of SAM and Xan. Consequently, this bottleneck underscores the need for additional metabolic engineering endeavors aimed at increasing the SAM and Xan pool in microbial cells.

From a biomedical perspective, DAs emerge as potent and versatile enzymes with applications in devising strategies for treating various illnesses and genetic disorders. Deaminase-based GDEPT, for instance, stands out as one of the most viable approaches for precise prodrug activation in tumor cells. This method mitigates toxic effects while enhancing pharmacokinetic properties. Addressing crucial aspects involves the development of effective vectors for efficient and targeted gene transfer to tumor cells, designing prodrugs with a robust bystander effect when converted into active drugs, and engineering DAs with increased affinity for prodrugs.

One of the most groundbreaking applications of DAs is their integration as crucial components in ABEs and CBEs for genome and transcriptome editing. These two classes of BEs have demonstrated effectiveness in editing individual nucleotides across a diverse range of living cells and organisms, establishing themselves as a robust technology that could significantly influence the life sciences and medicine. However, despite the promising advances in base editing, several challenges remain. First, except some few novel systems, most of these editors can only facilitate two of the six possible changes from one base pair to another. So, additional work is essential to develop or further improve BEs capable of inducing transversion mutations at target loci. Achieving success will likely depend on a thorough understanding and innovative manipulation of the cellular mechanisms that regulate base modification and DNA repair in mammalian cells.

Similar to the challenges observed in GDEPT, one significant issue is the delivery of large proteins into specific tissues, which remains a major focus of ongoing research. In the coming years, the development of innovative delivery systems designed to target specific tissues is expected to be a critical area of study. Additionally, it is essential to conduct thorough analyses of off-target editing activities of BEs *in vivo* under various conditions relevant to potential therapeutic applications. These assessments must also include evaluations of the biological impacts of unintended point mutations, especially for DNA base-editing tools. Since DNA BEs induce permanent changes at the genomic level, understanding and mitigating off-target effects are of utmost importance. Despite the availability of several methods to detect off-target effects, there is still a pressing need for a consensus approach that is unbiased, sensitive, rapid, and cost-effective. Current detection methods are varied, have not been extensively compared, and are optimized for only a limited number of experimental systems. A validated and clinically approved method is still absent.

In contrast, RNA base editing offers a reversible and tunable alternative to genome editing, reducing the need for stringent off-target control compared to DNA editing. However, especially when hyperactive deaminase variants like the ADAR E488Q mutant are employed for RNA editing, increased off-target events can still negatively impact health. In this sense, RNA base editors that do not require the introduction of exogenous ADAR proteins circumvent challenges such as widespread off-target effects, immunogenicity, and delivery hurdles. In addition, an important consideration in RNA-targeted gene therapy, particularly when using non-integrating vectors, is the need for repeated administration of effector constructs. This is due to the typically short half-life of edited mRNAs and effector proteins, which necessitates ongoing treatment to sustain therapeutic effectiveness.

As the field progresses, the development of new editing technologies that improve base-editing efficiency, expand targeting capabilities, and minimize off-target effects will be pivotal in advancing towards more sophisticated and ambitious applications. The creation of a diverse array of future ABEs and CBEs, each tailored to convert a specific DNA base pair or RNA base within a defined sequence context or protospacer with a particular PAM, will be instrumental in maximizing the precision and specificity of base editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abudayyeh, O.O., Gootenberg, J.S., Konermann, S., Joung, J., Slaymaker, I.M., Cox, D.B., Shmakov, S., Makarova, K.S., Semenova, E., Minakhin, L., Severinov, K., Regev, A., Lander, E.S., Koonin, E.V., Zhang, F., 2016. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science 353 (6299), aaf5573. https://doi.org/10.1126/science.aaf5573.

Abudayyeh, O.O., Gootenberg, J.S., Franklin, B., Koob, J., Kellner, M.J., Ladha, A., Joung, J., Kirchgatterer, P., Cox, D.B., Zhang, F., 2019. A cytosine deaminase for programmable single-base RNA editing. Science 365 (6451), 382–386. https://doi.org/10.1126/science.aax7063.

Acosta, J., Del Arco, J., Martinez-Pascual, S., Clemente-Suárez, V.J., Fernández-Lucas, J., 2018. One-pot multi-enzymatic production of purine derivatives with application in pharmaceutical and food industry. Catalysts 8 (1), 9. https://doi.org/10.3390/catal8010009.

Acosta, J., Nguyen, K., Spitale, R.C., Fernández-Lucas, J., 2021. Taylor-made production of pyrimidine nucleoside-5′-monophosphate analogues by highly stabilized mutant uracil phosphoribosyltransferase from *toxoplasma gondii*. Bioresour. Technol. 339, 125649. https://doi.org/10.1016/j.biortech.2021.125649.

Alcántara-Díaz, D., Breña-Valle, M., Serment-Guerrero, J., 2004. Divergent adaptation of Escherichia coli to cyclic ultraviolet light exposures. Mutagenesis 19 (5), 349–354. https://doi.org/10.1093/mutage/geh039.

Aldrich, M., Blackburn, M., Kellems, R., 2000. The importance of adenosine deaminase for lymphocyte development and function. Biochem. Biophys. Res. Commun. 272 (2), 311–315. https://doi.org/10.1006/bbrc.2000.2773.

Altaner, C., Altanerova, U., 2019. Mesenchymal stem cell exosome-mediated prodrug gene therapy for cancer. In: Düzgüneş, N. (Ed.), Suicide Gene Therapy. Humana Press, New York, pp. 75–85.

Altanerova, U., Babincova, M., Babinec, P., Benejova, K., Jakubechova, J., Altanerova, V., Zduriencikova, M., Repiska, V., Altaner, C., 2017. Human mesenchymal stem cell-derived iron oxide exosomes allow targeted ablation of tumor cells via magnetic hyperthermia. Int. J. Nanomedicine 12, 7923. https://doi.org/10.2147/IJN.S145096.

Altanerova, U., Jakubechova, J., Benejova, K., Priscakova, P., Pesta, M., Pitule, P., Topolcan, O., Kausitz, J., Zduriencikova, M., Repiska, V., Altaner, C., 2019. Prodrug suicide gene therapy for cancer targeted intracellular by mesenchymal stem cell exosomes. Int. J. Cancer 144 (4), 897–908. https://doi.org/10.1002/ijc.31792.

Altanerova, U., Jakubechova, J., Benejova, K., Priscakova, P., Repiska, V., Babelova, A., Smolkova, B., Altaner, C., 2021. Intracellular prodrug gene therapy for cancer mediated by tumor cell suicide gene exosomes. Int. J. Cancer 148 (1), 128–139. https://doi.org/10.1002/ijc.33188.

Aquino-Jarquin, G., 2020. Novel engineered programmable systems for ADAR-mediated RNA editing. Mol. Ther. Nucleic Acids 19, 1065–1072. https://doi.org/10.1016/j.omtn.2019.12.042.

Atta, S., Kassem, A., Elhadidi, A., El Esawy, H., 2015. The diagnostic value of adenosine deaminase activity in pulmonary tuberculosis: comparison between sputum and serum. Egypt. J. Chest. Dis. Tuberc. 64 (1), 103–107. https://doi.org/10.1016/j.ejcdt.2014.11.004.

Backus, J.W., Smith, H.C., 1991. Apolipoprotein B mRNA sequences 3′ of the editing site are necessary and sufficient for editing and editosome assembly. Nucleic Acids Res. 19, 6781–6786. https://doi.org/10.1093/nar/19.24.6781.

Badat, M., Hua, P., Mettananda, S., Fisher, C., Roy, N., Rice, S., Roy, A., Higgs, D.R., Davies, J., 2021. Base editing repairs the HbE mutation restoring the production of normal globin chains in severe HbE/β-thalassemia patient hematopoietic stem cells and erythroid cells. Blood 138, 2935. https://doi.org/10.1182/blood-2021-153283.

Baek, S.K., Makkouk, A.R., Krasieva, T., Sun, C.H., Madsen, S.J., Hirschberg, H., 2011. Photothermal treatment of glioma; an in vitro study of macrophage-mediated delivery of gold nanoshells. J. Neuro-Oncol. 104 (2), 439–448. https://doi.org/10.1007/s11060-010-0511-3.

Bagheri, S., Saboury, A., Haertlé, T., 2019. Adenosine deaminase inhibition. Int. J. Biol. Macromol. 141, 1246–1257. https://doi.org/10.1016/j.ijbiomac.2019.09.078.

Barai, V.N., Zinchenko, A.I., Eroshevskaya, L.A., Zhernosek, E.V., De Clercq, E., Mikhailopulo, I.A., 2002. Chemo-enzymatic synthesis of 3-deoxy-β-D-ribofuranosyl purines. Helv. Chim. Acta 85 (7), 1893–1900. https://doi.org/10.1002/1522-2675(200207)85:7<1893::AID-HLCA1893>3.0.CO;2-P.

Barai, V.N., Zinchenko, A.I., Eroshevskaya, L.A., Zhernosek, E.V., Balzarini, J., De Clercq, E., Mikhailopulo, I.A., 2003. Chemo-enzymatic synthesis of 3-deoxy-β-D-ribofuranosyl purines and study of their biological properties. Nucleos. Nucleot. Nucl. 22 (5–8), 751–753. https://doi.org/10.1081/NCN-120022626.

Bass, B.L., 2002. RNA editing by adenosine deaminases that act on RNA. Annu. Rev. Biochem. 71 (1), 817–846. https://doi.org/10.1146/annurev.biochem.71.110601.135501.

Bass, B.L., Weintraub, H., 1987. A developmentally regulated activity that unwinds RNA duplexes. Cell 48 (4), 607–613. https://doi.org/10.1016/0092-8674(87)90239-X.

Beach, C., Evans, R., Coleman, M., 1991. Strategy for industrial scale production of dideoxyinosine: enzymatic deamination of dideoxyadenosine by adenosine deaminase. Nucleos. Nucleot. Nucl. 10 (7), 1499–1505. https://doi.org/10.1080/07328319108046678.

Beale, R.C., Petersen-Mahrt, S.K., Watt, I.N., Harris, R.S., Rada, C., Neuberger, M.S., 2004. Comparison of the differential context-dependence of DNA deamination by APOBEC enzymes: correlation with mutation spectra *in vivo*. J. Mol. Biol. 337, 585–596. https://doi.org/10.1016/j.jmb.2004.01.046.

Bhakta, S., Sakari, M., Tsukahara, T., 2020. RNA editing of BFP, a point mutant of GFP, using artificial APOBEC1 deaminase to restore the genetic code. Sci. Rep. 10, 1–12. https://doi.org/10.1038/s41598-020-74374-5.

Bitra, A., Biswas, A., Anand, R., 2013a. Structural basis of the substrate specificity of cytidine deaminase superfamily guanine deaminase. Biochemistry 52 (45), 8106–8114. https://doi.org/10.1021/bi400818e.

Bitra, A., Hussain, B., Tanwar, A.S., Anand, R., 2013b. Identification of function and mechanistic insights of guanine deaminase from Nitrosomonas europaea: role of the C-terminal loop in catalysis. Biochemistry 52 (20), 3512–3522. https://doi.org/10.1021/bi400068g.

Boorstein, R.J., Chiu, L.N., Teebor, G.W., 1992. A mammalian cell line deficient in activity of the DNA repair enzyme 5-hydroxymethyluracil-DNA glycosylase is resistant to the toxic effects of the thymidine analog 5-hydroxymethyl-2′-deoxyuridine. Mol. Cell. Biol. 12, 5536–5540. https://doi.org/10.1128/mcb.12.12.5536-5540.1992.

Brakta, M., Murthy, D., Ellis, L., Phadtare, S., 2002. 9-[(Hydroxymethyl)phenyl] adenines: new aryladenine substrates of adenosine deaminase. Bioorg. Med. Chem. Lett. 12 (11), 1489–1492. https://doi.org/10.1016/s0960-894x(02)00192-0.

Bransteitter, R., Prochnow, C., Chen, X.S., 2009. The current structural and functional understanding of APOBEC deaminases. Cell. Mol. Life Sci. 66, 3137–3147. https://doi.org/10.1007/s00018-009-0070-y.

Buenger, G., Nair, V., 1990. Dideoxygenated purine nucleosides substituted at the 8-position: chemical synthesis and stability. Synthesis 1990 (10), 962–966. https://doi.org/10.1055/s-1990-27066.

Burke, A., Birmingham, W., Zhuo, Y., Thorpe, T., Zucoloto Da Costa, B., Crawshaw, R., Rowles, I., Finnigan, J.D., Young, C., Holgate, G.M., Muldowney, M.P., Charnock, S. J., Lovelock, S., Turner, N., Green, A., 2022. An engineered cytidine deaminase for biocatalytic production of a key intermediate of the Covid-19 antiviral molnupiravir. J. Am. Chem. Soc. 144 (9), 3761–3765. https://doi.org/10.1021/jacs.1c11048.

Burns, C.M., Wortmann, R.L., 2011. Gout therapeutics: new drugs for an old disease. Lancet 377, 165–177. https://doi.org/10.1016/S0140-6736(10)60665-4.

Cao, X., Guo, J., Huang, S., Yu, W., Li, G., An, L., Li, X., Tao, W., Liu, Q., Huang, X., Jin, X., Ma, X., 2022. Engineering of near-PAMless adenine base editor with enhanced editing activity and reduced off-target. Mol. Ther. Nucleic Acids 28, 732–742. https://doi.org/10.1016/j.omtn.2022.04.032.

Cappellacci, L., Barboni, G., Palmieri, M., Pasqualini, M., Grifantini, M., Costa, B., Martini, C., Franchetti, P., 2002. Ribose-modified nucleosides as ligands for adenosine receptors: synthesis, conformational analysis, and biological evaluation of 1'-C-methyl adenosine analogues. J. Med. Chem. 45 (6), 1196–1202. https://doi.org/10.1021/jm0102755.

Carlow, D.C., Carter Jr., C.W., Mejlhede, N., Neuhard, J., Wolfenden, R., 1999. Cytidine deaminases from *B. Subtilis* and *E. coli*: compensating effects of changing zinc coordination and quaternary structure. Biochemistry 38 (38), 12258–12265. https://doi.org/10.1021/bi990819t.

Carter Jr., C.W., 1995. The nucleoside deaminases for cytidine and adenosine: structure, transition state stabilization, mechanism, and evolution. Biochimie 77 (1–2), 92–98. https://doi.org/10.1016/0300-9084(96)88110-7.

Carter, S., Kessler, J., Rankin, C., 1990. Activities of (−)-carbovir and 3′-azido-3′-deoxythymidine against human immunodeficiency virus *in vitro*. Antimicrob. Agents Chemother. 34 (6), 1297–1300. https://doi.org/10.1128/aac.34.6.1297.

Casati, B., Stamkopoulou, D., Tasakis, R.N., Pecori, R., 2021. ADAR-mediated RNA editing and its therapeutic potentials. In: Jurga, S., Barciszewski, J. (Eds.), Epitranscriptomics. Springer, Cham, pp. 471–503.

Charych, E.I., Akum, B.F., Goldberg, J.S., Jörnsten, R.J., Rongo, C., Zheng, J.Q., Firestein, B.L., 2006. Activity-independent regulation of dendrite patterning by postsynaptic density protein PSD-95. J. Neurosci. 26 (40), 10164–10176. https://doi.org/10.1523/JNEUROSCI.2379-06.2006.

Chassy, B., Suhadolnik, R., 1967. Adenosine aminohydrolase. J. Biol. Chem. 242 (16), 3655–3658. https://doi.org/10.1016/s0021-9258(18)95859-x.

Chen, H., Firestein, B.L., 2007. RhoA regulates dendrite branching in hippocampal neurons by decreasing cypin protein levels. J. Neurosci. 27 (31), 8378–8386. https://doi.org/10.1523/JNEUROSCI.0872-07.2007.

Chen, C.X., Cho, D.S.C., Wang, Q., Lai, F., Carter, K.C., Nishikura, K., 2000. A third member of the RNA-specific adenosine deaminase gene family, ADAR3, contains both single-and double-stranded RNA binding domains. RNA 6 (5), 755–767. https://doi.org/10.1017/s1355838200000170.

Chen, Z., Penet, M.F., Krishnamachary, B., Banerjee, S.R., Pomper, M.G., Bhujwalla, Z. M., 2016. PSMA-specific theranostic nanoplex for combination of TRAIL gene and 5-FC prodrug therapy of prostate cancer. Biomaterials 80, 57–67. https://doi.org/10.1016/j.biomaterials.2015.11.048.

Chen, H., Liu, S., Padula, S., Lesman, D., Griswold, K., Lin, A., Zhao, T., Marshall, J.L., Chen, F., 2020. Efficient, continuous mutagenesis in human cells using a pseudo-random DNA editor. Nat. Biotechnol. 38, 165–168. https://doi.org/10.1038/s41587-019-0331-8.

Chen, F., Lian, M., Ma, B., Gou, S., Luo, X., Yang, K., Shi, H., Xie, J., Ge, W., Ouyang, Z., Lai, C., Li, N., Zhang, Q., Jin, Q., Liang, Y., Chen, T., Wang, J., Zhao, X., Li, L., Yu, M., Ye, Y., Wang, K., Wu, H., Lai, L., 2022a. Multiplexed base editing through Cas12a variant-mediated cytosine and adenine base editors. Commun. Biol. 5, 1163. https://doi.org/10.1038/s42003-022-04152-8.

Chen, X., Liang, D., Guo, J., Zhang, J., Sun, H., Zhang, X., Jin, J., Dai, Y., Bao, Q., Qian, X., Tan, L., 2022b. DdCBE-mediated mitochondrial base editing in human 3PN embryos. Cell Discov. 8, 8. https://doi.org/10.1038/s41421-021-00358-y.

Chen, L., Zhang, S., Xue, N., Hong, M., Zhang, X., Zhang, D., Yang, J., Bai, S., Huang, Y., Meng, H., Wu, H., Luan, C., Zhu, B., Ru, G., Gao, H., Zhong, L., Liu, M., Liu, M., Cheng, Y., Yi, C., Wang, L., Zhao, Y., Song, G., Li, D., 2023a. Engineering a precise adenine base editor with minimal bystander editing. Nat. Chem. Biol. 19, 101–110. https://doi.org/10.1038/s41589-022-01163-8.

Chen, L., Hong, M., Luan, C., Gao, H., Ru, G., Guo, X., Zhang, D., Zhang, S., Li, C., Wu, J., Randolph, P.B., Sousa, A.A., Qu, C., Zhu, Y., Guan, Y., Wang, L., Liu, M., Feng, B., Song, G., Liu, D.R., Li, D., 2023b. Adenine transversion editors enable precise, efficient A•T-to-C•G base editing in mammalian cells and embryos. Nat. Biotechnol. 1-13. https://doi.org/10.1038/s41587-023-01821-9.

Chen, L., Zhu, B., Ru, G., Meng, H., Yan, Y., Hong, M., Zhang, D., Luan, C., Zhang, S., Wu, H., Gao, H., 2023c. Re-engineering the adenine deaminase TadA-8e for efficient and specific CRISPR-based cytosine base editing. Nat. Biotechnol. 41, 663–672. https://doi.org/10.1038/s41587-022-01532-7.

Chester, A., Scott, J., Anant, S., Navaratnam, N., 2000. RNA editing: cytidine to uridine conversion in apolipoprotein B mRNA. Biochim. Biophys. Acta Gene Struct. Expr. 1494, 1–13. https://doi.org/10.1016/S0167-4781(00)00219-0.

Chew, B.L., Fisk, I.D., Fray, R., Tucker, G.A., Bodi, Z., Ferguson, A., Xia, W., Seymour, G. B., 2017. The effect of adenosine monophosphate deaminase overexpression on the accumulation of umami-related metabolites in tomatoes. Plant Cell Rep. 36, 81–87. https://doi.org/10.1007/s00299-016-2058-z.

Chhabra, E.S., Moore, N., Furcht, C., Holthaus, A.M., Liu, J., Liu, T., Schellenberger, V., Kulman, J., Salas, J., Peters, R., 2015. Evaluation of enhanced *in vitro* plasma stability of a novel long acting recombinant FVIIIFc-VWF-XTEN fusion protein. Blood 126 (23), 2279. https://doi.org/10.1182/blood.V126.23.2279.2279.

Choi, H.K., Curhan, G., 2004. Beer, liquor, and wine consumption and serum uric acid level: the third national health and nutrition examination survey. Arthritis Rheum. 51 (6), 1023–1029. https://doi.org/10.1002/art.20821.

Choi, H.K., Atkinson, K., Karlson, E.W., Willett, W., Curhan, G., 2004. Purine-rich foods, dairy and protein intake, and the risk of gout in men. N. Engl. J. Med. 350 (11), 1093–1103. https://doi.org/10.1056/NEJMoa035700.

Cihova, M., Altanerova, V., Altaner, C., 2011. Stem cell based cancer gene therapy. Mol. Pharm. 8 (5), 1480–1487. https://doi.org/10.1021/mp200151a.

Ciuffreda, P., Casati, S., Santaniello, E., 1999. Lipase-catalyzed protection of the hydroxy groups of the nucleosides inosine and 2′-deoxyinosine: A new chemoenzymatic synthesis of the antiviral drug 2′,3′-dideoxyinosine. Bioorg. Med. Chem. Lett. 9 (11), 1577–1582. https://doi.org/10.1016/s0960-894x(99)00228-0.

Ciuffreda, P., Loseto, A., Santaniello, E., 2002a. Stereoselective deamination of (5′ *RS*)-5′-methyl-2′, 3′-isopropylidene adenosine catalyzed by adenosine deaminase: preparation of diastereomerically pure 5′-methyl adenosine and inosine derivatives. Tetrahedron Asymmetry 13 (3), 239–241. https://doi.org/10.1016/S0957-4166(02)00080-0.

Ciuffreda, P., Loseto, A., Santaniello, E., 2002b. Deamination of 5′-substituted-2′, 3′-isopropylidene adenosine derivatives catalyzed by adenosine deaminase (ADA, EC 3.5. 4.4) and complementary enzymatic biotransformations catalyzed by adenylate deaminase (AMPDA, EC 3.5. 4.6): a viable route for the preparation of 5′-substituted inosine derivatives. Tetrahedron 58 (29), 5767–5771. https://doi.org/10.1016/S0040-4020(02)00575-6.

Ciuffreda, P., Loseto, A., Alessandrini, L., Terraneo, G., Santaniello, E., 2003. Adenylate deaminase (5′-adenylic acid deaminase, AMPDA)-catalyzed deamination of 5′-deoxy-5′-substituted and 5′-protected adenosines: a comparison with the catalytic activity of adenosine deaminase (ADA). Eur. J. Org. Chem. 24, 4748–4751. https://doi.org/10.1002/ejoc.200300435.

Ciuffreda, P., Loseto, A., Santaniello, E., 2004. Stereoselective adenylate deaminase (5′-adenylic acid deaminase, AMPDA)-catalyzed deamination of 5′-alkyl substituted adenosines: a comparison with the action of adenosine deaminase (ADA). Tetrahedron Asymmetry 15 (2), 203–206. https://doi.org/10.1016/j.tetasy.2003.11.007.

Conticello, S.G., 2008. The AID/APOBEC family of nucleic acid mutators. Genome Biol. 9, 1–10. https://doi.org/10.1186/gb-2008-9-6-229.

Conticello, S.G., Thomas, C.J., Petersen-Mahrt, S.K., Neuberger, M.S., 2005. Evolution of the AID/APOBEC family of polynucleotide (deoxy) cytidine deaminases. Mol. Biol. Evol. 22, 367–377. https://doi.org/10.1093/molbev/msi026.

Costanzi, S., Vincenzetti, S., Vita, A., Lambertucci, C., Taffi, S., Volpini, R., Vittori, S., Cristalli, G., 2003. Human cytidine deaminase: understanding the catalytic mechanism. Nucleos. Nucleot. Nucl. 22 (5–8), 1539–1543. https://doi.org/10.1081/NCN-120023029.

Cox, D.B., Gootenberg, J.S., Abudayyeh, O.O., Franklin, B., Kellner, M.J., Joung, J., Zhang, F., 2017. RNA editing with CRISPR-Cas13. Science 358 (6366), 1019–1027. https://doi.org/10.1126/science.aaq0180.

Cristalli, G., Costanzi, S., Lambertucci, C., Lupidi, G., Vittori, S., Volpini, R., Camaioni, E., 2001. Adenosine deaminase: functional implications and different classes of inhibitors. Med. Res. Rev. 21 (2), 105–128. https://doi.org/10.1002/1098-1128(200103)21:2<105::aid-med1002>3.0.co;2-.

Crudele, J.M., Chamberlain, J.S., 2018. Cas9 immunity creates challenges for CRISPR gene editing therapies. Nat. Commun. 9 (1), 1–3. https://doi.org/10.1038/s41467-018-05843-9.

Cruz, G., Saiz, L.P., Bilal, M., Eltoukhy, L., Loderer, C., Fernández-Lucas, J., 2022. Magnetic multi-enzymatic system for cladribine manufacturing. Int. J. Mol. Sci. 23 (21), 13634. https://doi.org/10.3390/ijms232113634.

Dahncke, K., Witte, C.P., 2013. Plant purine nucleoside catabolism employs a guanosine deaminase required for the generation of xanthosine in Arabidopsis. Plant Cell 25 (10), 4101–4109. https://doi.org/10.1105/tpc.113.117184.

Dai, S., Carcamo, R., Zhang, Z., Chen, S., Beachy, R.N., 2001. The bacterial cytosine deaminase gene used as a conditional negative selection marker in transgenic rice plants. Plant Cell Rep. 20, 738–743. https://doi.org/10.1007/s002990100390.

Davis, J.R., Wang, X., Witte, I.P., Huang, T.P., Levy, J.M., Raguram, A., Banskota, S., Seidah, N.G., Musunuru, K., Liu, D.R., 2022. Efficient *in vivo* base editing via single adeno-associated viruses with size-optimized genomes encoding compact adenine base editors. Nat. Biomed. Eng. 6, 1272–1283. https://doi.org/10.1038/s41551-022-00911-4.

Del Arco, J., Fernández-Lucas, J., 2018. Purine and pyrimidine salvage pathway in thermophiles: a valuable source of biocatalysts for the industrial production of nucleic acid derivatives. Appl. Microbiol. Biotechnol. 102, 7805–7820. https://doi.org/10.1007/s00253-018-9242-8.

Del Arco, J., Cejudo-Sanches, J., Esteban, I., Clemente-Suárez, V.J., Hormigo, D., Perona, A., Fernández-Lucas, J., 2017. Enzymatic production of dietary nucleotides from low-soluble purine bases by an efficient, thermostable and alkali-tolerant biocatalyst. Food Chem. 237, 605–611. https://doi.org/10.1016/j.foodchem.2017.05.136.

Del Arco, J., Jordaan, J., Moral-Dardé, V., Fernández-Lucas, J., 2019a. Sustainable production of nucleoside analogues by a high-efficient purine 2′-deoxyribosyltransferase immobilized onto Ni2+ chelate magnetic microparticles. Bioresour. Technol. 289, 121772. https://doi.org/10.1016/j.biortech.2019.121772.

Del Arco, J., Pérez, E., Naitow, H., Kunishima, N., Fernández-Lucas, J., 2019b. Structural and functional characterization of thermostable biocatalysts for the synthesis of 6-aminopurine nucleoside-5′-monophospate analogues. Bioresour. Technol. 276, 244–252. https://doi.org/10.1016/j.biortech.2018.12.120.

Del Arco, J., Acosta, J., Fernández-Lucas, J., 2021. New trends in the biocatalytic production of nucleosidic active pharmaceutical ingredients using 2′-deoxyribosyltransferases. Biotechnol. Adv. 51, 107701. https://doi.org/10.1016/j.biotechadv.2021.107701.

Deng, C., Lv, X., Li, J., Liu, Y., Du, G., Liu, L., 2020. Development of a DNA double-strand break-free base editing tool in *Corynebacterium glutamicum* for genome editing and metabolic engineering. Metab. Eng. Commun. 11, e00135. https://doi.org/10.1016/j.mec.2020.e00135.

Denoeud, F., Carretero-Paulet, L., Dereeper, A., Droc, G., Guyot, R., Pietrella, M., Zheng, C., Alberti, A., Anthony, F., Aprea, G., Aury, J.M., Bento, P., Bernard, M., Bocs, S., Campa, C., Cenci, A., Combes, M.C., Crouzillat, D., Da Silva, C., Daddiego, L., De Bellis, F., Dussert, S., Garsmeur, O., Gayraud, T., Guignon, V., Jahn, K., Jamilloux, V., Joët, T., Labadie, K., Lan, T., Leclercq, J., Lepelley, M., Leroy, T., Li, L.T., Librado, P., Lopez, L., Muñoz, A., Noel, B., Pallavicini, A., Perrotta, G., Poncet, V., Pot, D., Priyono, H., Rigoreau, M., Rouard, M., Rozas, J., Tranchant-Dubreuil, C., VanBuren, R., Zhang, Q., Andrade, A.C., Argout, X., Bertrand, B., de Kochko, A., Graziosi, G., Henry, R.J., Jayarama, S., Ming, R., Nagai, C., Rounsley, S., Sankoff, D., Giuliano, G., Albert, V.A., Wincker, P., Lashermes, P., 2014. The coffee genome provides insight into the convergent evolution of caffeine biosynthesis. Science 345 (6201), 1181–1184. https://doi.org/10.1126/science.1255274.

Desterro, J.M., Keegan, L.P., Lafarga, M., Berciano, M.T., O'Connell, M., Carmo-Fonseca, M., 2003. Dynamic association of RNA-editing enzymes with the nucleolus. J. Cell Sci. 116 (9), 1805–1818. https://doi.org/10.1242/jcs.00371.

Díaz-Rodríguez, A., Sanghvi, Y., Fernández, S., Schinazi, R., Theodorakis, E., Ferrero, M., Gotor, V., 2009. Synthesis and anti-HIV activity of conformationally restricted bicyclic hexahydroisobenzofuran nucleoside analogs. Org. Biomol. Chem. 7 (7), 1415–1423. https://doi.org/10.1039/b818707j.

Ding, Y., Fan, J., Deng, L., Huang, B., Zhou, B., 2020. Antitumor efficacy of cytosine deaminase-armed vaccinia virus plus 5-fluorocytosine in colorectal cancers. Cancer Cell Int. 20 (1), 1–12. https://doi.org/10.1186/s12935-020-01340-6.

Dolezal, T., Dolezelova, E., Zurovec, M., Bryant, P., 2005. A role for adenosine deaminase in Drosophila larval development. PLoS Biol. 3 (7), e201. https://doi.org/10.1371/journal.pbio.0030201.

Doll, R.M., Boutros, M., Port, F., 2023. A temperature-tolerant CRISPR base editor mediates highly efficient and precise gene editing in Drosophila. Sci. Adv. 9 (35), eadj1568. https://doi.org/10.1126/sciadv.adj1568.

Dore-Savard, L., Chen, Z., Winnard, P.T., Krishnamachary, B., Raman, V., Black, M.E., Bhujwalla, Z.M., 2017. Delayed progression of lung metastases following delivery of a prodrug-activating enzyme. Anticancer Res. 37 (5), 2195–2200. https://doi.org/10.21873/anticanres.11554.

Dos Vultos, T., Mestre, O., Tonjum, T., Gicquel, B., 2009. DNA repair in *mycobacterium tuberculosis* revisited. FEMS Microbiol. Rev. 33 (3), 471–487. https://doi.org/10.1111/j.1574-6976.2009.00170.x.

Driscoll, J., Siddiqui, M., Ford, H., Kelley, J., Roth, J., Mitsuya, H., Tanaka, M., Marquez, V.E., 1996. Lipophilic, acid-stable, adenosine deaminase-activated anti-HIV prodrugs for central nervous system delivery. 3. 6-amino prodrugs of 2′-β-fluoro-2′,3′-dideoxyinosine. J. Med. Chem. 39 (8), 1619–1625. https://doi.org/10.1021/jm9509197.

Eggebeen, A.T., 2007. Gout: an update. Am. Fam. Physician 76 (6), 801–808.

Eid, A., Alshareef, S., Mahfouz, M.M., 2018. CRISPR base editors: genome editing without double-stranded breaks. Biochem. J. 475, 1955–1964. https://doi.org/10.1042/BCJ20170793.

El Kouni, M.H., 2003. Potential chemotherapeutic targets in the purine metabolism of parasites. Pharmacol. Ther. 99 (3), 283–309. https://doi.org/10.1016/s0163-7258(03)00071-8.

Ellims, P.H., Kao, A.Y., Chabner, B.A., 1981. Deoxycytidylate deaminase. Purification and some properties of the enzyme isolated from human spleen. J. Biol. Chem. 256 (12), 6335–6340. https://doi.org/10.1016/s0021-9258(19)69167-2.

Emamian, M., Abbaspour, A., Shahani, T., Biglari, A., Sharafi, A., 2021. Non-viral suicide gene therapy: cytosine deaminase gene directed by VEGF promoter and 5-fluorocytosine as a gene directed enzyme/prodrug system in breast cancer model. Drug Res. 71 (07), 395–406. https://doi.org/10.1055/a-1488-6054.

Eom, G.E., Lee, H., Kim, S., 2022. Development of a genome-targeting mutator for the adaptive evolution of microbial cells. Nucleic Acids Res. 50 (7), e38. https://doi.org/10.1093/nar/gkab1244.

Etard, C., Roostalu, U., Strähle, U., 2010. Lack of Apobec2-related proteins causes a dystrophic muscle phenotype in zebrafish embryos. J. Cell Biol. 189, 527–539. https://doi.org/10.1083/jcb.200912125.

Faivre-Nitschke, S.E., Grienenberger, J.M., Gualberto, J.M., 1999. A prokaryotic-type cytidine deaminase from *Arabidopsis thaliana*: gene expression and functional characterization. Eur. J. Biochem. 263 (3), 896–903. https://doi.org/10.1046/j.1432-1327.1999.00591.x.

Fernández, J.R., Sweet, E.S., Welsh, W.J., Firestein, B.L., 2010. Identification of small molecule compounds with higher binding affinity to guanine deaminase (cypin) than guanine. Bioorg. Med. Chem. 18 (18), 6748–6755. https://doi.org/10.1016/j.bmc.2010.07.054.

Fernández-Lucas, J., 2015. Multienzymatic synthesis of nucleic acid derivatives: a general perspective. Appl. Microbiol. Biotechnol. 99 (11), 4615–4627. https://doi.org/10.1007/s00253-015-6642-x.

Fernández-Lucas, J., Camarasa, M.J., 2019. Enzymatic and Chemical Synthesis of Nucleic Acid Derivatives, first ed. John Wiley & Sons, Hoboken. https://doi.org/10.1002/9783527812103.

Ford, H., Siddiqui, M., Driscoll, J., Marquez, V., Kelley, J., Mitsuya, H., Shirasaka, T., 1995. Anti-HIV prodrugs for central nervous system delivery. 2. 6-halo- and 6-alkoxy prodrugs of 2′-.beta.-fluoro-2′,3′-dideoxyinosine. J. Med. Chem. 38 (7), 1189–1195. https://doi.org/10.1021/jm00007a015.

Foster, P.L., 1991. In vivo mutagenesis. Methods Enzymol. 204, 114–125. https://doi.org/10.1016/0076-6879(91)04007-B.

Frances, A., Cordelier, P., 2020. The emerging role of cytidine deaminase in human diseases: a new opportunity for therapy? Mol. Ther. 28 (2), 357–366. https://doi.org/10.1016/j.ymthe.2019.11.026.

Frisch, J., Maršić, T., Loderer, C.A., 2021. Novel one-pot enzyme cascade for the biosynthesis of cladribine triphosphate. Biomolecules 11 (3), 346. https://doi.org/10.3390/biom11030346.

Fu, J., Li, Q., Liu, X., Tu, T., Lv, X., Yin, X., Lv, J., Song, Z., Qu, J., Zhang, J., Li, J., Gu, F., 2021. Human cell based directed evolution of adenine base editors with improved efficiency. Nat. Commun. 12, 5897. https://doi.org/10.1038/s41467-021-26211-0.

Fuchita, M., Ardiani, A., Zhao, L., Serve, K., Stoddard, B.L., Black, M.E., 2009. Bacterial cytosine deaminase mutants created by molecular engineering show improved 5-fluorocytosine–mediated cell killing in vitro and in vivo. Cancer Res. 69 (11), 4791–4799. https://doi.org/10.1158/0008-5472.CAN-09-0615.

Fukuda, M., Umeno, H., Nose, K., Nishitarumizu, A., Noguchi, R., Nakagawa, H., 2017. Construction of a guide-RNA for site-directed RNA mutagenesis utilising intracellular A-to-I RNA editing. Sci. Rep. 7, 1–13. https://doi.org/10.1038/srep41478.

Füllgrabe, J., Gosal, W.S., Creed, P., Liu, S., Lumby, C.K., Morley, D.J., Ost, T.W., Vilella, A.J., Yu, S., Bignell, H., Burns, P., 2023. Simultaneous sequencing of genetic and epigenetic bases in DNA. Nat. Biotechnol. 6, 1–8. https://doi.org/10.1038/s41587-022-01652-0.

Gaded, V., Anand, R., 2018. Nucleobase deaminases: a potential enzyme system for new therapies. RSC Adv. 8. https://doi.org/10.1039/C8RA04112A, 23567–2.

Garcia-Gil, M., Tozzi, M., Balestri, F., Colombaioni, L., Camici, M., 2015a. Mitochondrial damage and apoptosis induced by adenosine deaminase inhibition and deoxyadenosine in human neuroblastoma cell lines. J. Cell. Biochem. 117 (7), 1671–1679. https://doi.org/10.1002/jcb.25460.

Garcia-Gil, M., Tozzi, M., Varani, S., Della Verde, L., Petrotto, E., Balestri, F., Colombaioni, L., Camici, M., 2015b. The combination of adenosine deaminase inhibition and deoxyadenosine induces apoptosis in a human astrocytoma cell line. Neurochem. Int. 80, 14–22. https://doi.org/10.1016/j.neuint.2014.11.005.

Gaspar, H.B., Kinnon, C., 2020. Gene therapy for adenosine deaminase deficiency. In: Lemoine, N.R., Cooper, D.N. (Eds.), Gene Therapy. Garland Science, London, pp. 225–239.

Gaudelli, N.M., Komor, A.C., Rees, H.A., Packer, M.S., Badran, A.H., Bryson, D.I., Liu, D. R., 2017. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature 551, 464–471. https://doi.org/10.1038/nature24644.

Gaudelli, N.M., Lam, D.K., Rees, H.A., Solá-Esteves, N.M., Barrera, L.A., Born, D.A., Edwards, A., Gehrke, J.M., Lee, S.J., Liquori, A.J., Murray, R., Packer, M.S., Rinaldi, C., Slaymaker, I.M., Yen, J., Young, L.E., Ciaramella, G., 2020. Directed evolution of adenine base editors with increased activity and therapeutic application. Nat. Biotechnol. 38, 892–900. https://doi.org/10.1038/s41587-020-0491-6.

Gehrke, J.M., Cervantes, O., Clement, M.K., Wu, Y., Zeng, J., Bauer, D.E., Pinello, L., Joung, J.K., 2018. An APOBEC3A-Cas9 base editor with minimized bystander and off-target activities. Nat. Biotechnol. 36, 977–982. https://doi.org/10.1038/nbt.4199.

Gerber, A.P., Keller, W., 2001. RNA editing by base deamination: more enzymes, more targets, new mysteries. Trends Biochem. Sci. 26 (6), 376–384. https://doi.org/10.1016/s0968-0004(01)01827-8.

Giannecchini, M., D'Innocenzo, B., Pesi, R., Sgarrella, F., Iorio, M., Collecchi, P., Tozzi, M.G., Camici, M., 2003. 2'-Deoxyadenosine causes apoptotic cell death in a human colon carcinoma cell line. J. Biochem. Mol. Toxicol. 17 (6), 329–337. https://doi.org/10.1002/jbt.10095.

Goble, A.M., Zhang, Z., Sauder, J.M., Burley, S.K., Swaminathan, S., Raushel, F.M., 2011. Pa0148 from *Pseudomonas aeruginosa* catalyzes the deamination of adenine. Biochemistry 50, 6589–6597. https://doi.org/10.1021/bi200868u.

Goble, A.M., Toro, R., Li, X., Ornelas, A., Fan, H., Eswaramoorthy, S., Patskovsky, Y., Hillerich, B., Seidel, R., Sali, A., Shoichet, B.K., Almo, S.C., Swaminathan, S., Tanner, M.E., Raushel, F.M., 2013. Deamination of 6-aminodeoxyfutalosine in menaquinone biosynthesis by distantly related enzymes. Biochemistry 52 (37), 6525–6536. https://doi.org/10.1021/bi400750a.

Gotarkar, D., Longkumer, T., Yamamoto, N., Nanda, A.K., Iglesias, T., Li, L.F., Miro, B., Blanco Gonzalez, E., Montes Bayon, M., Olsen, K.M., Hsing, Y.C., Kohli, A., 2021. A drought-responsive rice amidohydrolase is the elusive plant guanine deaminase with the potential to modulate the epigenome. Physiol. Plant. 172 (4), 1853–1866. https://doi.org/10.1111/ppl.13392.

Gracia, E., Farré, D., Cortés, A., Ferrer-Costa, C., Orozco, M., Mallol, J., Lluís, C., Canela, E.I., McCormick, P.J., Franco, R., Fanelli, F., Casadó, V., 2012. The catalytic site structural gate of adenosine deaminase allosterically modulates ligand binding to adenosine receptors. FASEB J. 27 (3), 1048–1061. https://doi.org/10.1096/fj.12-212621.

Grünewald, J., Zhou, R., Garcia, S.P., Iyer, S., Lareau, C.A., Aryee, M.J., Joung, J.K., 2019a. Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors. Nature 569, 433–437. https://doi.org/10.1038/s41586-019-1161-z.

Grünewald, J., Zhou, R., Iyer, S., Lareau, C.A., Garcia, S.P., Aryee, M.J., Joung, J.K., 2019b. CRISPR DNA base editors with reduced RNA off-target and self-editing activities. Nat. Biotechnol. 37, 1041–1048. https://doi.org/10.1038/s41587-019-0236-6.

Grünewald, J., Zhou, R., Lareau, C.A., Garcia, S.P., Iyer, S., Miller, B.R., Langner, L.M., Hsu, J.Y., Aryee, M.J., Joung, J.K., 2020. A dual-deaminase CRISPR base editor enables concurrent adenine and cytosine editing. Nat. Biotechnol. 38, 861–864. https://doi.org/10.1038/s41587-020-0535-y.

Guan, R., Ho, M.C., Fröhlich, R.F., Tyler, P.C., Almo, S.C., Schramm, V.L., 2012. Methylthioadenosine deaminase in an alternative *quorum sensing* pathway in *Pseudomonas aeruginosa*. Biochemistry 51 (45), 9094–9103. https://doi.org/10.1021/bi301062y.

Guo, J., Zhang, X., Chen, X., Sun, H., Dai, Y., Wang, J., Qian, X., Tan, L., Lou, X., Shen, B., 2021. Precision modeling of mitochondrial diseases in zebrafish via DdCBE-mediated mtDNA base editing. Cell Discov. 7, 78. https://doi.org/10.1038/s41421-021-00307-9.

Guo, J., Chen, X., Liu, Z., Sun, H., Zhou, Y., Dai, Y., Ma, Y., He, L., Qian, X., Wang, J., Zhang, J., Zhu, Y., Zhang, J., Shen, B., Zhou, F., 2022. DdCBE mediates efficient and inheritable modifications in mouse mitochondrial genome. Mol. Ther. Nucleic Acids 27, 73–80. https://doi.org/10.1016/j.omtn.2021.11.016.

Gupta, M., Nair, V., 2006. Adenosine deaminase in nucleoside synthesis. A review. Collect. Czechoslov. Chem. Commun. 71 (6), 769–787. https://doi.org/10.1135/cccc20060769.

Halim, N.S.S.A., Fakiruddin, K.S., Ali, S.A., Yahaya, B.H., 2014. A comparative study of non-viral gene delivery techniques to human adipose-derived mesenchymal stem cell. Int. J. Mol. Sci. 15 (9), 15044–15060. https://doi.org/10.3390/ijms150915044.

Hall, R.S., Fedorov, A.A., Marti-Arbona, R., Fedorov, E.V., Kolb, P., Sauder, J.M., Burley, S.K., Shoichet, B.K., Almo, S.C., Raushel, F.M., 2010. The hunt for 8-oxo-guanine deaminase. J. Am. Chem. Soc. 132 (6), 1762–1763. https://doi.org/10.1021/ja909817d.

Hall, R.S., Fedorov, A.A., Xu, C., Fedorov, E.V., Almo, S.C., Raushel, F.M., 2011. Three-dimensional structure and catalytic mechanism of cytosine deaminase. Biochemistry 50 (22), 5077–5085. https://doi.org/10.1021/bi200483k.

Han, W., Huang, W., Wei, T., Ye, Y., Mao, M., Wang, Z., 2022a. Programmable RNA base editing with a single gRNA-free enzyme. Nucleic Acids Res. 50, 9580–9595. https://doi.org/10.1093/nar/gkac713.

Han, H., Wu, Z., Zheng, L., Han, J., Zhang, Y., Li, J., Zhang, S., Li, G., Ma, C., Wang, P., 2022b. Generation of a high-efficiency adenine base editor with TadA8e for developing wheat dinitroaniline-resistant germplasm. Crop J. 10, 368–374. https://doi.org/10.1016/j.cj.2021.08.006.

Hanson, R., Shi, Z., Brzozowski, D., Banerjee, A., Kissick, T., Singh, J., Pullockaran, A.J., North, J.T., Fan, J., Howell, J., Durand, S.C., Montana, M.A., Kronenthal, D.R., Mueller, R.H., Patel, R.N., 2000. Regioselective enzymatic aminoacylation of lobucavir to give an intermediate for lobucavir prodrug. Bioorg. Med. Chem. 8 (12), 2681–2687. https://doi.org/10.1016/s0968-0896(00)00209-1.

Harguindey, A., Roy, S., Harris, A.W., Fairbanks, B.D., Goodwin, A.P., Bowman, C.N., Cha, J.N., 2019. Click nucleic acid mediated loading of prodrug activating enzymes in PEG–PLGA nanoparticles for combination chemotherapy. Biomacromolecules 20 (4), 1683–1690. https://doi.org/10.1021/acs.biomac.9b00040.

Hashimoto, R.Y., Menck, C.F.M., Van Sluys, M.A., 1999. Negative selection driven by cytosine deaminase gene in Lycopersicon esculentum hairy roots. Plant Sci. 141 (2), 175–181. https://doi.org/10.1016/S0168-9452(98)00242-8.

Herbert, A., Rich, A., 2001. The role of binding domains for dsRNA and Z-DNA in the in vivo editing of minimal substrates by ADAR1. Proc. Natl. Acad. Sci. 98 (21), 12132–12137. https://doi.org/10.1073/pnas.211419898.

Herbert, A., Alfken, J., Kim, Y.G., Mian, I.S., Nishikura, K., Rich, A., 1997. A Z-DNA binding domain present in the human editing enzyme, double-stranded RNA adenosine deaminase. Proc. Natl. Acad. Sci. 94 (16), 8421–8426. https://doi.org/10.1073/pnas.94.16.8421.

Hermann, J.C., Marti-Arbona, R., Fedorov, A.A., Fedorov, E., Almo, S.C., Shoichet, B.K., Raushel, F.M., 2007. Structure-based activity prediction for an enzyme of unknown function. Nature 448 (7155), 775. https://doi.org/10.1038/nature05981.

Hershfield, M.S., Buckley, R.H., Greenberg, M.L., Melton, A.L., Schiff, R., Hatem, C., Kurtzberg, J., Markert, L., Kobayashi, R.H., Kobayashi, A.L., Abuchowski, A., 1987. Treatment of adenosine deaminase deficiency with polyethylene glycol–modified adenosine deaminase. N. Engl. J. Med. 316 (10), 589–596. https://doi.org/10.1056/nejm198703053161005.

Hess, G.T., Tycko, J., Yao, D., Bassik, M.C., 2017. Methods and applications of CRISPR-mediated base editing in eukaryotic genomes. Mol. Cell 68, 26–43. https://doi.org/10.1016/j.molcel.2017.09.029.

Hitchcock, D.S., Fan, H., Kim, J., Vetting, M., Hillerich, B., Seidel, R.D., Almo, S.C., Shoichet, B.K., Sali, A., Raushel, F.M., 2013. Structure-guided discovery of new deaminase enzymes. J. Am. Chem. Soc. 135 (37), 13927–13933. https://doi.org/10.1021/ja4066078.

Hitchcock, D.S., Fedorov, A.A., Fedorov, E.V., Almo, S.C., Raushel, F.M., 2014. Discovery of a bacterial 5-methylcytosine deaminase. Biochemistry 53 (47), 7426–7435. https://doi.org/10.1021/bi5012767.

Ho, Y.K., Woo, J.Y., Tu, G.X.E., Deng, L.W., Too, H.P., 2020. A highly efficient non-viral process for programming mesenchymal stem cells for gene directed enzyme prodrug cancer therapy. Sci. Rep. 10 (1), 1–15. https://doi.org/10.1038/s41598-020-71224-2.

Holm, L., Sander, C., 1997. An evolutionary treasure: unification of a broad set of amidohydrolases related to urease. Proteins 28 (1), 72–82.

Hong, R., He, X., 2023. Development of a high-fidelity Cas9-dependent adenine base editor (ABE) system for genome editing with high-fidelity Cas9 variants. Genes Dis. 10, 705–707. https://doi.org/10.1016/j.gendis.2022.07.022.

Hu, J.H., Miller, S.M., Geurts, M.H., Tang, W., Chen, L., Sun, N., Zeina, C.M., Gao, X., Rees, H.A., Lin, Z., Liu, D.R., 2018. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature 556, 57–63. https://doi.org/10.1038/nature26155.

Hu, Z., Wang, S., Zhang, C., Gao, N., Li, M., Wang, D., Wang, D., Liu, D., Liu, H., Ong, S. G., Wang, H., Wang, Y., 2020. A compact Cas9 ortholog from staphylococcus Auricularis (SauriCas9) expands the DNA targeting scope. PLoS Biol. 18, e3000686. https://doi.org/10.1371/journal.pbio.3000686.

Hua, K., Tao, X., Zhu, J.K., 2019. Expanding the base editing scope in rice by using Cas9 variants. Plant Biotechnol. J. 17, 499–504. https://doi.org/10.1111/pbi.12993.

Huang, W., Xu, Y., Zhang, Y., Zhang, P., Zhang, Q., Zhang, Z., Xu, F., 2019a. Metabolomics-driven identification of adenosine deaminase as therapeutic target in a mouse model of Parkinson's disease. J. Neurochem. 150 (3), 282–295. https://doi.org/10.1111/jnc.14774.

Huang, T.P., Zhao, K.T., Miller, S.M., Gaudelli, N.M., Oakes, B.L., Fellmann, C., Savage, D.F., Liu, D.R., 2019b. Circularly permuted and PAM-modified Cas9 variants broaden the targeting scope of base editors. Nat. Biotechnol. 37, 626–631. https://doi.org/10.1038/s41587-019-0134-y.

Huang, X., Lv, J., Li, Y., Mao, S., Li, Z., Jing, Z., Sun, Y., Zhang, X., Shen, S., Wang, X., Di, M., Ge, J., Huang, X., Zuo, E., Chi, T., 2020. Programmable C-to-U RNA editing using the human APOBEC3A deaminase. EMBO J. 39, e108209. https://doi.org/10.15252/embj.2020104741.

Hubert, L., Sutton, V., 2017. Disorders of purine and pyrimidine metabolism. In: Garg, U., Smith, L.D. (Eds.), Biomarkers in Inborn Errors of Metabolism. Elsevier, Amsterdam, pp. 283–299. https://doi.org/10.1016/b978-0-12-802896-4.00009-2.

Huffman, J.L., Li, H., White, R.H., Tainer, J.A., 2003. Structural basis for recognition and catalysis by the bifunctional dCTP deaminase and dUTPase from *Methanococcus jannaschii*. J. Mol. Biol. 331 (4), 885–896. https://doi.org/10.1016/s0022-2836(03)00789-7.

Hundley, H.A., Bass, B.L., 2010. ADAR editing in double-stranded UTRs and other noncoding RNA sequences. Trends Biochem. Sci. 35 (7), 377–383. https://doi.org/10.1016/j.tibs.2010.02.008.

Ihry, R.J., Worringer, K.A., Salick, M.R., Frias, E., Ho, D., Theriault, K., Kommineni, S., Chen, J., Sondey, M., Ye, C., Randhawa, R., Kulkarni, T., Yang, Z., McAllister, G., Russ, C., Reece-Hoyes, J., Forrester, W., Hoffman, G.R., Dolmetsch, R., Kaykas, A., 2018. p53 inhibits CRISPR–Cas9 engineering in human pluripotent stem cells. Nat. Med. 24, 939–946. https://doi.org/10.1038/s41591-018-0050-6.

Ireton, G.C., McDermott, G., Black, M.E., Stoddard, B.L., 2002. The structure of *Escherichia coli* cytosine deaminase. J. Mol. Biol. 315 (4), 687–697. https://doi.org/10.1006/jmbi.2001.5277.

Iyer, L.M., Zhang, D., Rogozin, I.B., Aravind, L., 2011. Evolution of the deaminase fold and multiple origins of eukaryotic editing and mutagenic nucleic acid deaminases from bacterial toxin systems. Nucleic Acids Res. 39, 9473–9497. https://doi.org/10.1093/nar/gkr691.

James, S.J., Melnyk, S., Pogribna, M., Pogribny, I.P., Caudill, M.A., 2002. Elevation in S-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology. J. Nutr. 132 (8), 2361S–2366S. https://doi.org/10.1093/jn/132.8.2361S.

Jankowska, D.A., Faulwasser, K., Trautwein-Schult, A., Cordes, A., Hoferichter, P., Klein, C., Bode, R., Baronian, K., Kunze, G., 2013. *Arxula adeninivorans* recombinant adenine deaminase and its application in the production of food with low purine content. J. Appl. Microbiol. 115 (5), 1134–1146. https://doi.org/10.1159/000357674.

Jankowska, D.A., Trautwein-Schult, A., Cordes, A., Bode, R., Baronian, K., Kunze, G., 2015. A novel enzymatic approach in the production of food with low purine content using *Arxula adeninivorans* endogenous and recombinant purine deg-radative enzymes. Bioengineered 6 (1), 20–25. https://doi.org/10.4161/21655979.2014.991667.

Jarmuz, A., Chester, A., Bayliss, J., Gisbourne, J., Dunham, I., Scott, J., Navaratnam, N., 2002. An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. Genomics 79, 285–296. https://doi.org/10.1006/geno.2002.6718.

Jeong, Y.K., Lee, S., Hwang, G.H., Hong, S.A., Park, S.E., Kim, J.S., Woo, J.S., Bae, S., 2021. Adenine base editor engineering reduces editing of bystander cytosines. Nat. Biotechnol. 39, 1426–1433. https://doi.org/10.1038/s41587-021-00943-2.

Jiang, Z., Wang, C., Wu, Z., Chen, K., Yang, W., Deng, H., Song, H., Zhou, X., 2021. Enzymatic deamination of the epigenetic nucleoside N6-methyladenosine regulates gene expression. Nucleic Acids Res. 49 (21), 12048–12068. https://doi.org/10.1093/nar/gkab1124.

Kalkan, A., Bulut, V., Erel, O., Avci, S., Bingol, N.K., 1999. Adenosine deaminase and guanosine deaminase activities in sera of patients with viral hepatitis. Mem. Inst. Oswaldo Cruz 94 (3), 383–3866. https://doi.org/10.1590/s0074-02761999000300018.

Kamat, S.S., Raushel, F.M., 2011. Adenine deaminase. In: Scott, R.A. (Ed.), Encyclopedia of Inorganic and Bioinorganic Chemistry. John Wiley & Sons, Hoboken, pp. 1–9. https://doi.org/10.1002/9781119951438.eibc2172.

Kamat, S.S., Bagaria, A., Desigan, K., Holmes-Hampton, G.P., Fan, H., Sali, A., Sauder, J. M., Burley, S.K., Lindahl, P.A., Swaminathan, S., Raushel, F.M., 2011a. Catalytic mechanism and three dimensional structure of adenine deaminase. Biochemistry 50, 1917–1927. https://doi.org/10.1021/bi101788n.

Kamat, S.S., Fan, H., Sauder, J.M., Burley, S.K., Shoichet, B.K., Sali, A., Raushel, F.M., 2011b. Enzymatic deamination of the epigenetic base N-6-methyladenine. J. Am. Chem. Soc. 133 (7), 2080–2083. https://doi.org/10.1021/ja110157u.

Kamel, S., Weiß, M., Klare, H.F., Mikhailopulo, I.A., Neubauer, P., Wagner, A., 2018. Chemo-enzymatic synthesis of α-D-pentofuranose-1-phosphates using thermostable pyrimidine nucleoside phosphorylases. Mol. Catal. 458, 52–59. https://doi.org/10.1016/j.mcat.2018.07.028.

Kang, B.C., Yun, J.Y., Kim, S.T., Shin, Y., Ryu, J., Choi, M., Woo, J.W., Kim, J.S., 2018. Precision genome engineering through adenine base editing in plants. Nat. Plants 4, 427–431. https://doi.org/10.1038/s41477-018-0178-x.

Kang, B.C., Bae, S.J., Lee, S., Lee, J.S., Kim, A., Lee, H., Baek, G., Seo, H., Kim, J., Kim, J. S., 2021. Chloroplast and mitochondrial DNA editing in plants. Nat. Plants 7, 899–905. https://doi.org/10.1038/s41477-021-00943-9.

Katagiri, N., Kokufuda, H., Makino, M., Vince, R., Kaneko, C., 1998. Deamination of cyclaradine by adenosine deaminase under high pressure. Nucleosides Nucleotides Nucleic Acids 17 (1), 81–89. https://doi.org/10.1080/07328319808005159.

Katrekar, D., Chen, G., Meluzzi, D., Ganesh, A., Worlikar, A., Shih, Y.R., Varghese, S., Mali, P., 2019. *In vivo* RNA editing of point mutations via RNA-guided adenosine deaminases. Nat. Methods 16 (3), 239–242. https://doi.org/10.1038/s41592-019-0323-0.

Katrekar, D., Yen, J., Xiang, Y., Saha, A., Meluzzi, D., Savva, Y., Mali, P., 2022a. Efficient in vitro and in vivo RNA editing via recruitment of endogenous ADARs using circular guide RNAs. Nat. Biotechnol. 40, 938–945. https://doi.org/10.1038/s41587-021-01171-4.

Katrekar, D., Xiang, Y., Palmer, N., Saha, A., Meluzzi, D., Mali, P., 2022b. Comprehensive interrogation of the ADAR2 deaminase domain for engineering enhanced RNA editing activity and specificity. Elife 11, e75555. https://doi.org/10.7554/eLife.75555.

Katsuragi, T., Sakai, T., Tonomura, K., 1987. Implantable enzyme capsules for cancer chemotherapy from bakers' yeast cytosine deaminase immobilized on epoxy-acrylic resin and urethane prepolymer. Appl. Biochem. Biotechnol. 16 (1), 61–69. https://doi.org/10.1007/BF02798356.

Keegan, L.P., Gallo, A., O'Connell, M.A., 2001. The many roles of an RNA editor. Nat. Rev. Genet. 2 (11), 869–878. https://doi.org/10.1038/35098584.

Keegan, L.P., Leroy, A., Sproul, D., O'Connell, M.A., 2004. Adenosine deaminases acting on RNA (ADARs): RNA-editing enzymes. Genome Biol. 5, 1–10. https://doi.org/10.1186/gb-2004-5-2-209.

Keppler, A., Gendreizig, S., Gronemeyer, T., Pick, H., Vogel, H., Johnsson, K., 2003. A general method for the covalent labeling of fusion proteins with small molecules in vivo. Nat. Biotechnol. 21 (1), 86–89. https://doi.org/10.1038/nbt765.

Kim, J.S., 2018. Precision genome engineering through adenine and cytosine base editing. Nat. Plants 4, 148–151. https://doi.org/10.1038/s41477-018-0115-z.

Kim, D., Kim, H., Chae, Y., 1994a. Design and synthesis of 6-fluoropurine acyclonucleosides : potential prodrugs of acyclovir and ganciclovir. Bioorg. Med. Chem. Lett. 4 (11), 1309–1312. https://doi.org/10.1016/s0960-894x(01)80350-4.

Kim, U., Wang, Y., Sanford, T., Zeng, Y., Nishikura, K., 1994b. Molecular cloning of cDNA for double-stranded RNA adenosine deaminase, a candidate enzyme for nuclear RNA editing. Proc. Natl. Acad. Sci. 91 (24), 11457–11461. https://doi.org/10.1073/pnas.91.24.11457.

Kim, Y.B., Komor, A.C., Levy, J.M., Packer, M.S., Zhao, K.T., Liu, D.R., 2017. Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. Nat. Biotechnol. 35, 371–376. https://doi.org/10.1038/nbt.3803.

Kim, H.S., Jeong, Y.K., Hur, J.K., Kim, J.S., Bae, S., 2019. Adenine base editors catalyze cytosine conversions in human cells. Nat. Biotechnol. 37, 1145–1148. https://doi.org/10.1038/s41587-019-0254-4.

Kim, D.Y., Chung, Y., Lee, Y., Jeong, D., Park, K.H., Chin, H.J., Lee, J.M., Park, S., Ko, S., Ko, J.H., Kim, Y.S., 2022. Hypercompact adenine base editors based on a Cas12f variant guided by engineered RNA. Nat. Chem. Biol. 18, 1005–1013. https://doi.org/10.1038/s41589-022-01077-5.

Kleinstiver, B.P., Pattanayak, V., Prew, M.S., Tsai, S.Q., Nguyen, N.T., Zheng, Z., Joung, J.K., 2016. High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. Nature 529, 490–495. https://doi.org/10.1038/nature16526.

Ko, T.P., Lin, J.J., Hu, C.Y., Hsu, Y.H., Wang, A.H.J., Liaw, S.H., 2003. Crystal structure of yeast cytosine deaminase: in-sights into enzyme mechanism and evolution. J. Biol. Chem. 278 (21), 19111–19117. https://doi.org/10.1074/jbc.M300874200.

Koblan, L.W., Doman, J.L., Wilson, C., Levy, J.M., Tay, T., Newby, G.A., Maianti, J.P., Raguram, A., Liu, D.R., 2018. Improving cytidine and adenine base editors by expression optimization and ancestral reconstruction. Nat. Biotechnol. 36, 843–846. https://doi.org/10.1038/nbt.4172.

Kode, N., Phadtare, S., 2011. Synthesis and cytotoxic activity of some new 2,6-substituted purines. Molecules 16 (7), 5840–5860. https://doi.org/10.3390/molecules16075840.

Kohgo, S., Yamada, K., Kitano, K., Iwai, Y., Sakata, S., Ashida, N., Hayakawa, H., Nameki, D., Kodama, E., Matsuoka, M., Mitsuya, H., Ohrui, H., 2004. Design, efficient synthesis, and anti-hiv activity of 4′-C-cyano- and 4′-C-ethynyl-2′-deoxy purine nucleosides. Nucleosides Nucleotides Nucleic Acids 23 (4), 671–690. https://doi.org/10.1081/ncn-120037508.

Kohgo, S., Imoto, S., Tokuda, R., Takamatsu, Y., Higashi-Kuwata, N., Aoki, M., Amano, M., Kansui, H., Onitsuka, K., Maeda, K., Mitsuya, H., 2018. Synthesis of 4′-substituted purine 2′-deoxynucleosides and their activity against human immunodeficiency virus type 1 and hepatitis B virus. ChemistrySelect 3 (11), 3313–3317. https://doi.org/10.1002/slct.201800527.

Komatsu, A., Ohtake, M., Shimatani, Z., Nishida, K., 2020. Production of herbicide-sensitive strain to prevent volunteer rice infestation using a CRISPR-Cas9 cytidine deaminase fusion. Front. Plant Sci. 11, 925. https://doi.org/10.3389/fpls.2020.00925.

Komor, A.C., Kim, Y.B., Packer, M.S., Zuris, J.A., Liu, D.R., 2016. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 533, 420–424. https://doi.org/10.1038/nature17946.

Komor, A.C., Zhao, K.T., Packer, M.S., Gaudelli, N.M., Waterbury, A.L., Koblan, L.W., Kim, Y.B., Badran, A.H., Liu, D.R., 2017. 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. 3, eaao4774. https://doi.org/10.1126/sciadv.aao4774.

Kosicki, M., Tomberg, K., Bradley, A., 2018. Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. Nat. Biotechnol. 36, 765–771. https://doi.org/10.1038/nbt.4192.

Kotchoni, S.O., Gachomo, E.W., Slobodenko, K., Shain, D.H., 2016. AMP deaminase suppression increases biomass, cold tolerance and oil content in green algae. Algal Res. 16, 473–480. https://doi.org/10.1016/j.algal.2016.04.007.

Kotera, E., Tasaka, M., Shikanai, T., 2005. A pentatricopeptide repeat protein is essential for RNA editing in chloroplasts. Nature 433 (7023), 326–330. https://doi.org/10.1038/nature03229.

Kozaeva, E., Nielsen, Z.S., Nieto-Domínguez, M., Nikel, P.I., 2024. The pAblo· pCasso self-curing vector toolset for unconstrained cytidine and adenine base-editing in gram-negative bacteria. Nucleic Acids Res. 52 (4), e19. https://doi.org/10.1093/nar/gkad1236.

Krishnamurthy, S., Traore, S., Cooney, A.L., Brommel, C.M., Kulhankova, K., Sinn, P.L., Newby, G.A., Liu, D.R., McCray Jr., P.B., 2021. Functional correction of CFTR mutations in human airway epithelial cells using adenine base editors. Nucleic Acids Res. 49, 10558–10572. https://doi.org/10.1093/nar/gkab788.

Kubo, S., Takagi-Kimura, M., Tagawa, M., Kasahara, N., 2019. Dual-vector prodrug activator gene therapy using retroviral replicating vectors. Cancer Gene Ther. 26 (5), 128–135. https://doi.org/10.1038/s41417-018-0051-0.

Kucerova, L., Matuskova, M., Hlubinova, K., Altanerova, V., Altaner, C., 2010. Tumor cell behaviour modulation by mesenchymal stromal cells. Mol. Cancer 9 (1), 1–15. https://doi.org/10.1186/1476-4598-9-129.

Kumar, M., Carmichael, G.G., 1997. Nuclear antisense RNA induces extensive adenosine modifications and nuclear retention of target transcripts. Proc. Natl. Acad. Sci. 94 (8), 3542–3547. https://doi.org/10.1073/pnas.94.8.3542.

Kunz, C., Saito, Y., Schär, P., 2009. DNA repair in mammalian cells. Cell. Mol. Life Sci. 66, 1021–1038. https://doi.org/10.1007/s00018-009-8739-9.

Kutryb-Zajac, B., Mierzejewska, P., Slominska, E.M., Smolenski, R.T., 2020. Therapeutic perspectives of adenosine deaminase inhibition in cardiovascular diseases. Molecules 25 (20), 4652. https://doi.org/10.3390/molecules25204652.
Kuttan, A., Bass, B.L., 2012. Mechanistic insights into editing-site specificity of ADARs. Proc. Natl. Acad. Sci. 109 (48), E3295–E3304. https://doi.org/10.1073/pnas.1212548109.
Kutzing, M.K., Firestein, B.L., 2008. Altered uric acid levels and disease states. J. Pharmacol. Exp. Ther. 324, 1–7. https://doi.org/10.1124/jpet.107.129031.
Lachmann, N., Brennig, S., Pfaff, N., Schermeier, H., Dahlmann, J., Phaltane, R., Gruh, I., Modlich, U., Schambach, A., Baum, C., Moritz, T., 2013a. Efficient *in vivo* regulation of cytidine deaminase expression in the haematopoietic system using a doxycycline-inducible lentiviral vector system. Gene Ther. 20 (3), 298–307. https://doi.org/10.1038/gt.2012.40.
Lachmann, N., Brennig, S., Phaltane, R., Flasshove, M., Dilloo, D., Moritz, T., 2013b. Myeloprotection by cytidine deaminase gene transfer in antileukemic therapy. Neoplasia 15 (3), 239–248. https://doi.org/10.1593/neo.121954.
Lam, D.K., Feliciano, P.R., Arif, A., Bohnuud, T., Fernandez, T.P., Gehrke, J.M., Grayson, P., Lee, K.D., Ortega, M.A., Sawyer, C., Schwaegerle, N.D., Peraro, L., Young, L., Lee, S.J., Ciaramella, G., Gaudelli, N.M., 2023. Improved cytosine base editors generated from TadA variants. Nat. Biotechnol. 41, 686–697. https://doi.org/10.1038/s41587-022-01611-9.
Lapinaite, A., Knott, G.J., Palumbo, C.M., Lin-Shiao, E., Richter, M.F., Zhao, K.T., Beal, P. A., Liu, D.R., Doudna, J.A., 2020. DNA capture by a CRISPR-Cas9–guided adenine base editor. Science 369 (6503), 566–571. https://doi.org/10.1126/science.abb1390.
Lapponi, M.J., Rivero, C.W., Zinni, M.A., Britos, C.N., Trelles, J.A., 2016. New developments in nucleoside analogues biosynthesis: A review. J. Mol. Catal. B Enzym. 133, 218–233. https://doi.org/10.1016/j.molcatb.2016.08.015.
Lee, H., Lee, S., Baek, G., Kim, A., Kang, B.C., Seo, H., Kim, J.S., 2021. Mitochondrial DNA editing in mice with DddA-TALE fusion deaminases. Nat. Commun. 12, 1190. https://doi.org/10.1038/s41467-021-21464-1.
Lee, S., Lee, H., Baek, G., Kim, J.S., 2023. Precision mitochondrial DNA editing with high-fidelity DddA-derived base editors. Nat. Biotechnol. 41, 378–386. https://doi.org/10.1038/s41587-022-01486-w.
Legault, P., Li, J., Mogridge, J., Kay, L.E., Greenblatt, J., 1998. NMR structure of the bacteriophage λ N peptide/boxB RNA complex: recognition of a GNRA fold by an arginine-rich motif. Cell 93, 289–299. https://doi.org/10.1016/S0092-8674(00)81579-2.
Lehouritis, P., Springer, C., Tangney, M., 2013. Bacterial-directed enzyme prodrug therapy. J. Control. Release 170 (1), 120–131. https://doi.org/10.1016/j.jconrel.2013.05.005.
Leija, C., Rijo-Ferreira, F., Kinch, L.N., Grishin, N.V., Nischan, N., Kohler, J.J., Hu, Z., Phillips, M.A., 2016. Pyrimidine salvage enzymes are essential for *de novo* biosynthesis of deoxypyrimidine nucleotides in *Trypanosoma brucei*. PLoS Pathog. 12 (11), e1006010. https://doi.org/10.1371/journal.ppat.1006010.
Lellek, H., Kirsten, R., Diehl, I., Apostel, F., Buck, F., Greeve, J., 2000. Purification and molecular cloning of a novel essential component of the apolipoprotein B mRNA editing enzyme-complex. J. Biol. Chem. 275, 19848–19856. https://doi.org/10.1074/jbc.M001786200.
Leone, R.D., Emens, L.A., 2018. Targeting adenosine for cancer immunotherapy. J. Immunother. Cancer 6 (57), 1–9. https://doi.org/10.1186/s40425-018-0360-8.
Leonhardt, N., Divol, F., Chiarenza, S., Deschamps, S., Renaud, J., Giacalone, C., Nussaume, L., Berthomé, R., Péret, B., 2020. Tissue-specific inactivation by cytosine deaminase/uracil phosphoribosyl transferase as a tool to study plant biology. Plant J. 101 (3), 731–741. https://doi.org/10.1111/tpj.14569.
Levy, J.M., Yeh, W.H., Pendse, N., Davis, J.R., Hennessey, E., Butcher, R., Koblan, L.W., Comander, J., Liu, Q., Liu, D.R., 2020. Cytosine and adenine base editing of the brain, liver, retina, heart and skeletal muscle of mice via adeno-associated viruses. Nat. Biomed. Eng. 4, 97–110. https://doi.org/10.1038/s41551-019-0501-5.
Lewkowicz, E.S., Iribarren, A.M., 2017. Whole cell biocatalysts for the preparation of nucleosides and their derivatives. Curr. Pharm. Des. 23, 6851–6878. https://doi.org/10.2174/1381612823666171011101133.
Li, N., Smith, T., Zong, M., 2010. 2010. Biocatalytic transformation of nucleoside derivatives. Biotechnol. Adv. 28 (3), 348–366. https://doi.org/10.1016/j.biotechadv.2010.01.006.
Li, M., Sun, Y., Pan, S.A., Deng, W.W., Yu, O., Zhang, Z., 2017a. Engineering a novel biosynthetic pathway in *Escherichia coli* for the production of caffeine. RSC Adv. 7 (89), 56382–56389. https://doi.org/10.1039/C7RA10986E.
Li, J., Sun, Y., Du, J., Zhao, Y., Xia, L., 2017b. Generation of targeted point mutations in rice by a modified CRISPR/Cas9 system. Mol. Plant 10, 526–529. https://doi.org/10.1016/j.molp.2016.12.001.
Li, C., Zong, Y., Wang, Y., Jin, S., Zhang, D., Song, Q., Zhang, R., Gao, C., 2018a. Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion. Genome Biol. 19, 1–9. https://doi.org/10.1186/s13059-018-1443-z.
Li, X., Wang, Y., Liu, Y., Yang, B., Wang, X., Wei, J., Lu, Z., Zhang, Y., Wu, J., Huang, X., Yang, L., Chen, J., 2018b. Base editing with a Cpf1–cytidine deaminase fusion. Nat. Biotechnol. 36, 324–327. https://doi.org/10.1038/nbt.4102.
Li, Q.Y., Xie, N.B., Xiong, J., Yuan, B.F., Feng, Y.Q., 2018c. Single-nucleotide resolution analysis of 5-Hydroxymethylcytosine in DNA by enzyme-mediated deamination in combination with sequencing. Anal. Chem. 18, 14622–14628. https://doi.org/10.1021/acs.analchem.8b04833.
Li, J., Yu, L., Li, J., Xie, L., Zhang, R., Wang, H., 2019. Establishment of a high throughput-screening system for nucleoside deoxyribosyltransferase II mutant enzymes with altered substrate specificity. J. Biosci. Bioeng. 128, 22–27. https://doi.org/10.1016/j.jbiosc.2019.01.002.
Li, Y., Li, S., Li, C., Zhang, C., Yan, L., Li, J., He, Y., Guo, Y., Lin, Y., Zhang, Y., Xia, L., 2023. Engineering a plant A-to-K base editor with improved performance by fusion with a transactivation module. Plant Commun. 4 (6). https://doi.org/10.1016/j.xplc.2023.100667.
Li, X., Xie, J., Dong, C., Zheng, Z., Shen, R., Cao, X., Chen, X., Wang, M., Zhu, J.K., Tian, Y., 2024. Efficient and heritable A-to-K base editing in rice and tomato. Hortic. Res. 11 (1), uhad250. https://doi.org/10.1093/hr/uhad250.
Liang, P., Sun, H., Zhang, X., Xie, X., Zhang, J., Bai, Y., Ouyang, X., Zhi, S., Xiong, Y., Ma, W., Liu, D., Huang, J., Songyang, Z., 2018. Effective and precise adenine base editing in mouse zygotes. Protein Cell 9, 808–813. https://doi.org/10.1007/s13238-018-0566-z.
Liaw, S.H., Chang, Y.J., Lai, C.T., Chang, H.C., Chang, G.G., 2004. Crystal structure of *Bacillus subtilis* guanine deaminase: the first domain-swapped structure in the cytidine deaminase superfamily. J. Biol. Chem. 279 (34), 35479–35485. https://doi.org/10.1074/jbc.M405304200.
Lim, K., Cho, S.I., Kim, J.S., 2022. Nuclear and mitochondrial DNA editing in human cells with zinc finger deaminases. Nat. Commun. 13, 366. https://doi.org/10.1038/s41467-022-27962-0.
Liu, Z., Lu, Z., Yang, G., Huang, S., Li, G., Feng, S., Liu, Y., Li, J., Yu, W., Zhang, Y., Chen, J., Sun, Q., Huang, X., 2018. Efficient generation of mouse models of human diseases via ABE-and BE-mediated base editing. Nat. Commun. 9, 2338. https://doi.org/10.1038/s41467-018-04768-7.
Longley, D.B., Harkin, D.P., Johnston, P.G., 2003. 5-fluorouracil: mechanisms of action and clinical strategies. Nat. Rev. Cancer 3 (5), 330–338. https://doi.org/10.1038/nrc1074.
Lonkar, P., Dedon, P.C., 2011. Reactive species and DNA damage in chronic inflammation: reconciling chemical mechanisms and biological fates. Int. J. Cancer 128 (9), 1999–2009. https://doi.org/10.1002/ijc.25815.
Lorenzo, J.P., Molla, L., Ibarra, I.L., Ruf, S., Ridani, J., Subramani, P.G., Boulais, J., Harjanto, D., Vonica, A., Di Noia, J.M., Dieterich, C., Zaugg, J.B., Papavasiliou, F.N., 2021. APOBEC2 is a transcriptional repressor required for proper myoblast differentiation. bioRxiv. https://doi.org/10.1101/2020.07.29.223594, 2020-07.
Losey, H.C., Ruthenburg, A.J., Verdine, G.L., 2006. Crystal structure of Staphylococcus aureus tRNA adenosine deaminase TadA in complex with RNA. Nat. Struct. Mol. Biol. 13, 153–159. https://doi.org/10.1038/nsmb1047.
Lu, Y., Zhu, J.K., 2017. Precise editing of a target base in the rice genome using a modified CRISPR/Cas9 system. Mol. Plant 10, 523–525. https://doi.org/10.1016/j.molp.2016.11.013.
Ma, Y., Yu, L., Zhang, X., Xin, C., Huang, S., Bai, L., Chen, W., Gao, R., Li, J., Pan, S., Qi, X., Huang, X., Zhang, L., 2018. Highly efficient and precise base editing by engineered dCas9-guide tRNA adenosine deaminase in rats. Cell Discov. 4, 1–4. https://doi.org/10.1038/s41421-018-0047-9.
Maas, S., Rich, A., Nishikura, K., 2003. A-to-I RNA editing: recent news and residual mysteries. J. Biol. Chem. 278, 1391–1394. https://doi.org/10.1074/jbc.R200025200.
Macbeth, M.R., Schubert, H.L., VanDemark, A.P., Lingam, A.T., Hill, C.P., Bass, B.L., 2005. Inositol hexakisphosphate is bound in the ADAR2 core and required for RNA editing. Science 309 (5740), 1534–1539. https://doi.org/10.1126/science.1113150.
Mahmoudian, M., Baines, B.S., Drake, C.S., Hale, R.S., Jones, P., Piercey, J.E., Montgomery, D.S., Purvis, I.J., Storer, R., Dawson, M.J., Lawrence, G.C., 1993. Enzymatic production of optically pure (2′R-cis)-2′-deoxy-3′-thiacytidine (3TC, lamivudine): a potent anti-HIV agent. Enzym. Microb. Technol. 15 (9), 749–755. https://doi.org/10.1016/0141-0229(93)90005-m.
Mahor, D., Prasad, G.S., 2018. Biochemical characterization of *Kluyveromyces lactis* adenine deaminase and guanine deaminase and their potential application in lowering purine content in beer. Front. Bioeng. Biotechnol. 6, 180. https://doi.org/10.3389/fbioe.2018.00180.
Majumdar, P., Wu, H., Tipton, P., Glaser, R., 2005. Oxanosine is a substrate of adenosine deaminase. Implications for the quest for a toxicological marker for nitrosation activity. Chem. Res. Toxicol. 18 (12), 1830–1841. https://doi.org/10.1021/tx050232h.
Maley, F., Maley, G.F., 1990. A tale of two enzymes, deoxycytidylate deaminase and thymidylate synthase. Prog. Nucleic Acid Res. Mol. Biol. 39, 49–80. https://doi.org/10.1016/S0079-6603(08)60623-6.
Maley, G.F., Lobo, A.P., Maley, F., 1993. Properties of an affinity-column-purified human deoxycytidylate deaminase. Biochim. Biophys. Acta 1162 (1–2), 161–170. https://doi.org/10.1016/0167-4838(93)90143-F.
Manta, B., Raushel, F.M., Himo, F., 2014. Reaction mechanism of zinc-dependent cytosine deaminase from *Escherichia coli*: A quantum-chemical study. J. Phys. Chem. B 118 (21), 5644–5652. https://doi.org/10.1021/jp501228s.
Mao, Y., Zhang, H., Xu, N., Zhang, B., Gou, F., Zhu, J.K., 2013. Application of the CRISPR–Cas system for efficient genome engineering in plants. Mol. Plant 6, 2008–2011. https://doi.org/10.1093/mp/sst121.
Marino, D., Perković, M., Hain, A., Jaguva Vasudevan, A.A., Hofmann, H., Hanschmann, K.M., Mühlebach, M.D., Schumann, G.G., König, R., Cichutek, K., Häussinger, D., Münk, C., 2016. APOBEC4 enhances the replication of HIV-1. PLoS One 11, e0155422. https://doi.org/10.1371/journal.pone.0155422.
Maris, C., Masse, J., Chester, A.N.N., Navaratnam, N., Allain, F.H.T., 2005. NMR structure of the apoB mRNA stem–loop and its interaction with the C to U editing APOBEC1 complementary factor. RNA 11, 173–186. https://doi.org/10.1261/rna.7190705.
Marquez, V.E., Tseng, C.K., Mitsuya, H., Aoki, S., Kelley, J.A., Ford Jr., H., Roth, J.S., Broder, S., Johns, D.G., Driscoll, J.S., 1990. Acid-stable 2′-fluoro purine dideoxynucleosides as active agents against HIV. J. Med. Chem. 33 (3), 978–985. https://doi.org/10.1021/jm00165a015.

Martín-Nieves, V., Sanghvi, Y.S., Fernández, S., Ferrero, M., 2022. Sustainable protocol for the synthesis of 2′, 3′-dideoxynucleoside and 2′, 3′-didehydro-2′, 3′-dideoxynucleoside derivatives. Molecules 27 (13), 3993. https://doi.org/10.3390/molecules27133993.

Matsoukas, I.G., 2018. Commentary: programmable base editing of A·T to G·C in genomic DNA without DNA cleavage. Front. Genet. 9, 21. https://doi.org/10.3389/fgene.2018.00021.

Maury, G., Daiboun, T., Elalaoui, A., Genu-Dellac, C., Perigaud, C., Bergogne, C., Gosselin, G., Imbach, J.L., 1991. Inhibition and substrate specificity of adenosine deaminase. Interaction with 2′-, 3′-and/or 5′-substituted adenine nucleoside derivatives. Nucleosides Nucleotides Nucleic Acids 10 (8), 1677–1692.

Médici, R., Lewkowicz, E., Iribarren, A., 2008. *Arthrobacter oxydansas* a biocatalyst for purine deamination. FEMS Microbiol. Lett. 289 (1), 20–26. https://doi.org/10.1111/j.1574-6968.2008.01349.x.

Megati, S., Goren, Z., Silverton, J.V., Orlin, J., Nishimura, H., Shirasaki, T., Mitsuya, H., Zemlicka, J., 1992. (R)-(−)- and (S)-(+)-Adenallene: synthesis, absolute configuration, enantioselectivity of antiretroviral effect, and enzymic deamination. J. Med. Chem. 35 (22), 4098–4114. https://doi.org/10.1021/jm00100a016.

Mehta, A., Kinter, M.T., Sherman, N.E., Driscoll, D.M., 2000. Molecular cloning of apobec-1 complementation factor, a novel RNA-binding protein involved in the editing of apolipoprotein B mRNA. Mol. Cell. Biol. 20, 1846–1854. https://doi.org/10.1128/MCB.20.5.1846-1854.2000.

Melcher, T., Maas, S., Herb, A., Sprengel, R., Seeburg, P.H., Higuchi, M., 1996. A mammalian RNA editing enzyme. Nature 379 (6564), 460–464. https://doi.org/10.1038/379460a0.

Merkle, T., Merz, S., Reautschnig, P., Blaha, A., Li, Q., Vogel, P., Wettengel, J., Li, J.B., Stafforst, T., 2019. Precise RNA editing by recruiting endogenous ADARs with antisense oligonucleotides. Nat. Biotechnol. 37 (2), 133–138. https://doi.org/10.1038/s41587-019-0013-6.

Mikhailopulo, I.A., 2007. Biotechnology of nucleic acid constituents-state of the art and perspectives. Curr. Org. Chem. 11 (4), 317–335. https://doi.org/10.2174/138527207780059330.

Mikhailopulo, I.A., Miroshnikov, A.I., 2011. Biologically important nucleosides: modern trends in biotechnology and application. Mendeleev Commun. 21 (2), 57–69. https://doi.org/10.1016/j.mencom.2011.03.001.

Miller, E., Maier, R., 2013. Identification of a new class of adenosine deaminase from *Helicobacter pylori* with homologs among diverse taxa. J. Bacteriol. 195 (18), 4154–4160. https://doi.org/10.1128/jb.00587-13.

Miller, D., O'Brien, K., Xu, H., White, R., 2013. Identification of a 5′-deoxyadenosine deaminase in *Methanocaldococcus jannaschii* and its possible role in recycling the radical S-adenosylmethionine enzyme reaction product 5′-deoxyadenosine. J. Bacteriol. 196 (5), 1064–1072. https://doi.org/10.1128/jb.01308-13.

Mistry, J., Chuguransky, S., Williams, L., Qureshi, M., Salazar, G.A., Sonnhammer, E.L., Tosatto, S.C.E., Paladin, L., Raj, S., Richardson, L.J., Finn, R.D., Bateman, A., 2021. Pfam: the protein families database in 2021. Nucleic Acids Res. 49 (D1), D412–D419. https://doi.org/10.1093/nar/gkaa913.

Mok, B.Y., de Moraes, M.H., Zeng, J., Bosch, D.E., Kotrys, A.V., Raguram, A., Hsu, F., Radey, M.C., Peterson, S.B., Mootha, V.K., Mougous, J.D., 2020. A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. Nature 583, 631–637. https://doi.org/10.1038/s41586-020-2477-4.

Mok, B.Y., Kotrys, A.V., Raguram, A., Huang, T.P., Mootha, V.K., Liu, D.R., 2022a. CRISPR-free base editors with enhanced activity and expanded targeting scope in mitochondrial and nuclear DNA. Nat. Biotechnol. 40, 1378–1387. https://doi.org/10.1038/s41587-022-01256-8.

Mok, Y.G., Lee, J.M., Chung, E., Lee, J., Lim, K., Cho, S.I., Kim, J.S., 2022b. Base editing in human cells with monomeric DddA-TALE fusion deaminases. Nat. Commun. 13, 4038. https://doi.org/10.1038/s41467-022-31745-y.

Mol, C.D., Arvai, A.S., Sanderson, R.J., Slupphaug, G., Kavli, B., Krokan, H.E., Mosbaugh, D.W., Tainer, J.A., 1995. Crystal structure of human uracil-DNA glycosylase in complex with a protein inhibitor: protein mimicry of DNA. Cell 82, 701–708. https://doi.org/10.1016/0092-8674(95)90467-0.

Monian, P., Shivalila, C., Lu, G., Shimizu, M., Boulay, D., Bussow, K., Byrne, M., Bezigian, A., Chatterjee, A., Chew, D., Desai, J., Favaloro, F., Godfrey, J., Hoss, A., Iwamoto, N., Kawamoto, T., Kumarasamy, J., Lamattina, A., Lindsey, A., Liu, F., Looby, R., Marappan, S., Metterville, J., Murphy, R., Rossi, J., Pu, T., Bhattarai, B., Standley, S., Tripathi, S., Yang, H., Yin, Y., Yu, H., Zhou, C., Apponi, L.H., Kandasamy, P., Vargeese, C., 2022. Endogenous ADAR-mediated RNA editing in non-human primates using stereopure chemically modified oligonucleotides. Nat. Biotechnol. 40, 1093–1102. https://doi.org/10.1038/s41587-022-01225-1.

Montiel-Gonzalez, M.F., Vallecillo-Viejo, I., Yudowski, G.A., Rosenthal, J.J., 2013. Correction of mutations within the cystic fibrosis transmembrane conductance regulator by site-directed RNA editing. Proc. Natl. Acad. Sci. 110 (45), 18285–18290. https://doi.org/10.1073/pnas.1306243110.

Montiel-González, M.F., Vallecillo-Viejo, I.C., Rosenthal, J.J., 2016. An efficient system for selectively altering genetic information within mRNAs. Nucleic Acids Res. 44 (21), e157. https://doi.org/10.1093/nar/gkw738.

Moore, C.L., Papa III, L.J., Shoulders, M.D., 2018. A processive protein chimera introduces mutations across defined DNA regions in vivo. J. Am. Chem. Soc. 140, 11560–11564. https://doi.org/10.1021/jacs.8b04001.

Mullen, C.A., Kilstrup, M., Blaese, R.M., 1992. Transfer of the bacterial gene for cytosine deaminase to mammalian cells confers lethal sensitivity to 5-fluorocytosine: a negative selection system. Proc. Natl. Acad. Sci. 89 (1), 33–37. https://doi.org/10.1073/pnas.89.1.33.

Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y., Honjo, T., 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell 102, 553–563. https://doi.org/10.1016/S0092-8674(00)00078-7.

Naguib, F.N.M., Wilson, C.M., El Kouni, M.H., 2018. Enzymes of pyrimidine salvage pathways in intraerythrocytic *plasmodium falciparum*. Int. J. Biochem. Cell Biol. 105, 115–122. https://doi.org/10.1016/j.biocel.2018.10.007.

Nair, V., Turner, G., Chamberlain, S., 1987. Novel approaches to functionalized nucleosides via palladium-catalyzed cross coupling with organostannanes. J. Am. Chem. Soc. 109 (23), 7223–7224. https://doi.org/10.1021/ja00257a071.

Nair, V., Bera, B., Kern, E., 2003. Synthesis and biological activities of 2-functionalized purine nucleosides. Nucleosides Nucleotides Nucleic Acids 22 (2), 115–127. https://doi.org/10.1081/ncn-120019498.

Navaratnam, N., Sarwar, R., 2006. An overview of cytidine deaminases. Int. J. Hematol. 83 (3), 195–200. https://doi.org/10.1532/IJH97.06032.

Nemani, K.V., Ennis, R.C., Griswold, K.E., 2015. Gimi, B. Magnetic nanoparticle hyperthermia induced cytosine deaminase expression in microencapsulated *E. coli* for enzyme–prodrug therapy. J. Biotechnol. 203, 32–40. https://doi.org/10.1016/j.jbiotec.2015.03.008.

Neugebauer, M.E., Hsu, A., Arbab, M., Krasnow, N.A., McElroy, A.N., Pandey, S., Doman, J.L., Huang, T.P., Raguram, A., Banskota, S., Newby, G.A., 2023. Evolution of an adenine base editor into a small, efficient cytosine base editor with low off-target activity. Nat. Biotechnol. 41, 673–685. https://doi.org/10.1038/s41587-022-01533-6.

Nishida, K., Arazoe, T., Yachie, N., Banno, S., Kakimoto, M., Tabata, M., Mochizuki, M., Miyabe, A., Araki, M., Hara, K.Y., Shimatani, Z., Kondo, A., 2016. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. Science 353 (6305), aaf8729. https://doi.org/10.1126/science.aaf872.

Nishikura, K., 2010. Functions and regulation of RNA editing by ADAR deaminases. Annu. Rev. Biochem. 79, 321–349. https://doi.org/10.1146/annurev-biochem-060208-105251.

Nishikura, K., 2016. A-to-I editing of coding and non-coding RNAs by ADARs. Nat. Rev. Mol. Cell Biol. 17 (2), 83–96. https://doi.org/10.1038/nrm.2015.4.

Nishimasu, H., Ran, F.A., Hsu, P.D., Konermann, S., Shehata, S.I., Dohmae, N., Ishitani, R., Zhang, F., Nureki, O., 2014. Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell 156, 935–949. https://doi.org/10.1016/j.cell.2014.02.001.

Nishiyama, T., Kawamura, Y., Kawamoto, K., Matsumura, H., Yamamoto, N., Ito, T., Ohyama, A., Katsuragi, T., Sakai, T., 1985. Antineoplastic effects in rats of 5-fluorocytosine in combination with cytosine deaminase capsules. Cancer Res. 45 (4), 1753–1761.

Niu, M., Wang, Y., Wang, C., Lyu, J., Wang, Y., Dong, H., Long, W., Wang, D., Kong, W., Wang, L., Guo, X., Sun, L., Hu, T., Zhai, H., Wang, H., Wan, J., 2017. ALR encoding dCMP deaminase is critical for DNA damage repair, cell cycle progression and plant development in rice. J. Exp. Bot. 68 (21−22), 5773–5786. https://doi.org/10.1093/jxb/erx380.

Oehlenschlæger, C.B., Løvgreen, M.N., Reinauer, E., Lehtinen, E., Pind, M.L.L., Harris, P., Martinussen, J., Willemoës, M., 2015. *Bacillus halodurans* strain C125 encodes and synthesizes enzymes from both known pathways to form dUMP directly from cytosine deoxyribonucleotides. Appl. Environ. Microbiol. 81 (10), 3395–3404. https://doi.org/10.1128/AEM.00268-15.

Oestreicher, N., 2003. Sub-families of α/β barrel enzymes: a new adenine deaminase family. J. Mol. Biol. 334 (5), 1117–1131. https://doi.org/10.1016/j.jmb.2003.10.005.

Oestricher, N., Ribard, C., Scazzocchio, C., 2008. The nadA gene of *Aspergillus nidulans*, encoding adenine deaminase, is subject to a unique regulatory pattern. Fungal Genet. Biol. 45, 760–775. https://doi.org/10.1016/j.fgb.2007.10.015.

Ogilvie, K.K., Nguyen-Ba, N., Gillen, M.F., Radatus, B.K., Cheriyan, U.O., Hanna, H.R., Smith, K.O., Galloway, K.S., 1984. Synthesis of a purine acyclonucleoside series having pronounced antiviral activity. The glyceropurines. Can. J. Chem. 62 (2), 241–252. https://doi.org/10.1139/v84-039.

Pal, S., Bera, B., Nair, V., 2002. Inhibition of inosine monophosphate dehydrogenase (IMPDH) by the antiviral compound, 2-vinylinosine monophosphate. Bioorg. Med. Chem. 10 (11), 3615–3618. https://doi.org/10.1016/s0968-0896(02)00247-x.

Paletzki, R.F., 2002. Cloning and characterization of guanine deaminase from mouse and rat brain. Neuroscience 109 (1), 15–26. https://doi.org/10.1016/s0306-4522(01)00352-9.

Pan, S.A., Sun, Y., Li, M., Deng, W.W., Zhang, Z.Z., 2019. Guanine deaminase provides evidence of the increased caffeine content during the piling process of pu'erh tea. R. Soc. Chem. Adv. 9 (62), 36136–36143. https://doi.org/10.1039/C9RA05655F.

Pan, Y., Xia, S., Dong, C., Pan, H., Cai, J., Huang, L., Xu, Z., Lian, J., 2021. Random base editing for genome evolution in Saccharomyces cerevisiae. Am. Chem. Soc. Synth. Biol. 10, 2440–2446. https://doi.org/10.1021/acssynbio.1c00217.

Park, H., Kim, S., 2021. Gene-specific mutagenesis enables rapid continuous evolution of enzymes in vivo. Nucleic Acids Res. 49 (6), e32. https://doi.org/10.1093/nar/gkaa1231.

Patel, R.N., 2008. Synthesis of chiral pharmaceutical intermediates by biocatalysis. Coord. Chem. Rev. 252 (5–7), 659. https://doi.org/10.1016/j.ccr.2007.10.031.

Pecori, R., Di Giorgio, S., Lorenzo, J.P., Papavasiliou, F.N., 2022. Functions and consequences of AID/APOBEC-mediated DNA and RNA deamination. Nat. Rev. Genet. 1-14. https://doi.org/10.1038/s41576-022-00459-8.

Peters, C.W., Hanlon, K.S., Ivanchenko, M.V., Zinn, E., Linarte, E.F., Li, Y., Levy, J.M., Liu, D.R., Kleinstiver, B.P., Indzhykulian, A.A., Corey, D.P., 2023. Rescue of hearing by adenine base editing in a humanized mouse model of Usher syndrome type 1F. Mol. Ther. https://doi.org/10.1016/j.ymthe.2023.06.007. In Press.

Petersen-Mahrt, S.K., Neuberger, M.S., 2003. In vitro deamination of cytosine to uracil in single-stranded DNA by apolipoprotein B editing complex catalytic subunit 1

(APOBEC1). J. Biol. Chem. 278, 19583–19586. https://doi.org/10.1074/jbc.C300114200.
Porter, D.J., Austin, E.A., 1993. Cytosine deaminase. The roles of divalent metal ions in catalysis. J. Biol. Chem. 268 (32), 24005–24011.
Pospísilová, H., Šebela, M., Novák, O., Frébort, I., 2008. Hydrolytic cleavage of N 6-substituted adenine derivatives by eukaryotic adenine and adenosine deaminases. Biosci. Rep. 28 (6), 335–347. https://doi.org/10.1042/BSR20080081.
Qi, X., Chen, X., Guo, J., Zhang, X., Sun, H., Wang, J., Qian, X., Li, B., Tan, L., Yu, L., Chen, W., Zhang, L., Ma, Y., Shen, B., 2021. Precision modeling of mitochondrial disease in rats via DdCBE-mediated mtDNA editing. Cell Discov. 7 (1), 95. https://doi.org/10.1038/s41421-021-00325-7.
Qin, W., Lu, X., Liu, Y., Bai, H., Li, S., Lin, S., 2018. Precise A•T to G•C base editing in the zebrafish genome. BMC Biol. 16, 1–8. https://doi.org/10.1186/s12915-018-0609-1.
Qu, L., Yi, Z., Zhu, S., Wang, C., Cao, Z., Zhou, Z., Yuan, P., Yu, Y., Tian, F., Liu, Z., Bao, Y., Zhao, Y., Wei, W., 2019. Programmable RNA editing by recruiting endogenous ADAR using engineered RNAs. Nat. Biotechnol. 37 (9), 1059–1069. https://doi.org/10.1038/s41587-019-0292-y.
Rabie, A., 2022. Potent inhibitory activities of the adenosine analogue cordycepin on SARS-CoV-2 replication. Am. Chem. Soc. Omega 7 (3), 2960–2969. https://doi.org/10.1021/acsomega.1c05998.
Ran, F.A., Hsu, P.D., Lin, C.Y., Gootenberg, J.S., Konermann, S., Trevino, A.E., Scott, D. A., Inoue, A., Matoba, S., Zhang, Y., 2013. Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell 154, 1380–1389. https://doi.org/10.1016/j.cell.2013.08.021.
Ran, F.A., Cong, L., Yan, W.X., Scott, D.A., Gootenberg, J.S., Kriz, A.J., Zetsche, B., Shalem, O., Wu, X., Makarova, K.S., Koonin, E.V., Sharp, P.A., Zhang, F., 2015. In vivo genome editing using *Staphylococcus aureus* Cas9. Nature 520, 186–191. https://doi.org/10.1038/nature14299.
Rauch, S., He, E., Srienc, M., Zhou, H., Zhang, Z., Dickinson, B.C., 2019. Programmable RNA-guided RNA effector proteins built from human parts. Cell 178, 122–134. https://doi.org/10.1016/j.cell.2019.05.049.
Raudstein, M., Straume, A.H., Kjærner-Semb, E., Barvik, M., Ellingsen, S., Edvardsen, R. B., 2024. Highly efficient in vivo C-to-T base editing in Atlantic salmon (Salmo salar)–A step towards aquaculture precision breeding. Aquaculture 581, 740487. https://doi.org/10.1016/j.aquaculture.2023.740487.
Reautschnig, P., Wahn, N., Wettengel, J., Schulz, A.E., Latifi, N., Vogel, P., Kang, T., Pfeiffer, L.S., Zarges, C., Naumann, U., Zender, L., Stafforst, T., 2022. CLUSTER guide RNAs enable precise and efficient RNA editing with endogenous ADAR enzymes in vivo. Nat. Biotechnol. 40, 759–768. https://doi.org/10.1038/s41587-021-01105-0.
Rebhandl, S., Huemer, M., Greil, R., Geisberger, R., 2015. AID/APOBEC deaminases and cancer. Oncoscience 2, 320–333. https://doi.org/10.18632/oncoscience.155.
Rees, H.A., Liu, D.R., 2018. Base editing: precision chemistry on the genome and transcriptome of living cells. Nat. Rev. Genet. 19, 770–788. https://doi.org/10.1038/s41576-018-0059-1.
Rees, H.A., Komor, A.C., Yeh, W.H., Caetano-Lopes, J., Warman, M., Edge, A.S., Liu, D.R., 2017. Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery. Nat. Commun. 8, 1–10. https://doi.org/10.1038/ncomms15790.
Rees, H.A., Wilson, C., Doman, J.L., Liu, D.R., 2019. Analysis and minimization of cellular RNA editing by DNA adenine base editors. Sci. Adv. 5, eaax5717. https://doi.org/10.1126/sciadv.aax5717.
Reizer, J., Buskirk, S., Reizer, A., Saier Jr., M.H., Bairoch, A., 1994. A novel zinc-binding motif found in two ubiquitous deaminase families. Protein Sci. 3 (5), 853–856. https://doi.org/10.1002/pro.5560030515.
Ren, B., Yan, F., Kuang, Y., Li, N., Zhang, D., Lin, H., Zhou, H., 2017. A CRISPR/Cas9 toolkit for efficient targeted base editing to induce genetic variations in rice. Sci. China Life Sci. 60, 516–519. https://doi.org/10.1007/s11427-016-0406-x.
Ren, B., Yan, F., Kuang, Y., Li, N., Zhang, D., Zhou, X., Lin, H., Zhou, H., 2018. Improved base editor for efficiently inducing genetic variations in rice with CRISPR/Cas9-guided hyperactive hAID mutant. Mol. Plant 11, 623–626. https://doi.org/10.1016/j.molp.2018.01.005.
Ren, C.Y., Liu, Y.S., He, Y.S., Zhang, L.P., Rao, J.H., Rao, Y., Chen, J.H., 2024. Engineered CBEs based on Macaca fascicularis A3A with improved properties for precise genome editing. Cell Rep. 43 (3), 113878. https://doi.org/10.1016/j.celrep.2024.113878.
Richette, P., Bardin, T., 2010. Gout. Lancet 375 (9711), 318–328. https://doi.org/10.1016/S0140-6736(09)60883-7.
Richter, M.F., Zhao, K.T., Eton, E., Lapinaite, A., Newby, G.A., Thuronyi, B.W., Wilson, C., Koblan, L.W., Zeng, J., Bauer, D.E., Doudna, J.A., Liu, D.R., 2020. Phage-assisted evolution of an adenine base editor with improved Cas domain compatibility and activity. Nat. Biotechnol. 38, 883–891. https://doi.org/10.1038/s41587-020-0453-z.
Rinaldi, F., Fernández-Lucas, J., de la Fuente, D., Zheng, C., Bavaro, T., Peters, B., Massolini, G., Annunziata, F., Conti, P., De la Mata, I., Terreni, M., Calleri, E., 2020. Immobilized enzyme reactors based on nucleoside phosphorylases and 2′-deoxyribosyltransferase for the in-flow synthesis of pharmaceutically relevant nucleoside analogues. Bioresour. Technol. 307, 123258. https://doi.org/10.1016/j.biortech.2020.123258.
Robins, M., Hatfield, P., 1982. Nucleic acid related compounds. 37. Convenient and high-yield syntheses of N-[(2-hydroxyethoxy)methyl] heterocycles as "acyclic nucleoside" analogues. Can. J. Chem. 60 (5), 547–553. https://doi.org/10.1139/v82-081.
Rocha, P.S., Sheikh, M., Melchiorre, R., Fagard, M., Boutet, S., Loach, R., Moffatt, B., Wagner, C., Vaucheret, H., Furner, I., 2005. The Arabidopsis homology-dependent gene silencing gene codes for an S-adenosyl-L-homocysteine hydrolase required for DNA methylation-dependent gene silencing. Plant Cell 17 (2), 404–417. https://doi.org/10.1105/tpc.104.028332.
Romena, G., Nguyen, L., Berg, K., Madsen, S.J., Hirschberg, H., 2021. Enhanced gene transfection of macrophages by photochemical internalization: potential for gene-directed enzyme prodrug therapy of gliomas. Photodiagn. Photodyn. Ther. 33, 102098. https://doi.org/10.1016/j.pdpdt.2020.102098.
Rosello, M., Serafini, M., Concordet, J.P., Del Bene, F., 2023. Precise mutagenesis in zebrafish using cytosine base editors. Nat. Protoc. 18 (9), 2794–2813. https://doi.org/10.1038/s41596-023-00854-3.
Rothgangl, T., Dennis, M.K., Lin, P.J., Oka, R., Witzigmann, D., Villiger, L., Qi, W., Hruzova, M., Kissling, L., Lenggenhager, D., Borrelli, C., Egli, S., Frey, N., Bakker, N., Walker II, J.A., Kadina, A.P., Victorov, D.V., Pacesa, M., Kreutzer, S., Kontarakis, Z., Moor, A., Jinek, M., Weissman, D., Stoffel, M., van Boxtel, R., Holden, K., Pardi, N., Thöny, B., Häberle, J., Tam, Y.K., Semple, S.C., Schwank, G., 2021. In vivo adenine base editing of PCSK9 in macaques reduces LDL cholesterol levels. Nat. Biotechnol. 39, 949–957. https://doi.org/10.1038/s41587-021-00933-4.
Rovira, A., Fin, A., Tor, Y., 2017. Expanding a fluorescent RNA alphabet: synthesis, photophysics and utility of isothiazole-derived purine nucleoside surrogates. Chem. Sci. 8 (4), 2983–2993. https://doi.org/10.1039/c6sc05354h.
Ryu, S.M., Koo, T., Kim, K., Lim, K., Baek, G., Kim, S.T., Kim, H.S., Kim, D., Lee, H., Chung, E., Kim, J.S., 2018. Adenine base editing in mouse embryos and an adult mouse model of Duchenne muscular dystrophy. Nat. Biotechnol. 36, 536–539. https://doi.org/10.1038/nbt.4148.
Saag, K.G., Choi, H., 2006. Epidemiology, risk factors, and lifestyle modifications for gout. Arthritis Res. Ther. 8, 1–7. https://doi.org/10.1186/ar1907.
Sakai, T., Yu, T.S., Taniguchi, K., Omata, S., 1975. Purification of cytosine deaminase from Pseudomonas aureofaciens. Agric. Biol. Chem. 39 (10), 2015–2020. https://doi.org/10.1080/00021369.1975.10861902.
Sakata, R.C., Ishiguro, S., Mori, H., Tanaka, M., Tatsuno, K., Ueda, H., Yamamoto, S., Seki, M., Masuyama, N., Nishida, K., Nishimasu, H., Arakawa, K., Kondo, A., Nureki, O., Tomita, M., Aburatani, H., Yachie, N., 2020. Base editors for simultaneous introduction of C-to-T and A-to-G mutations. Nat. Biotechnol. 38, 865–869. https://doi.org/10.1038/s41587-020-0509-0.
Salter, J.D., Bennett, R.P., Smith, H.C., 2016. The APOBEC protein family: united by structure, divergent in function. Trends Biochem. Sci. 41, 578–594. https://doi.org/10.1016/j.tibs.2016.05.001.
Santos, L., Alves, J., Farinha, C.M., Harrison, P.T., 2023. Improved adenine base editing approach to correct W1282X-CFTR. J. Cyst. Fibros. 22, S32. https://doi.org/10.1016/S1569-1993(23)00278-3.
Sato, Y., Ohtsubo, H., Nihei, N., Kaneko, T., Sato, Y., Adachi, S.I., Kondo, S., Nakamura, M., Mizunoya, W., Iida, H., Tatsumi, R., Rada, C., Yoshizawa, F., 2018. Apobec2 deficiency causes mitochondrial defects and mitophagy in skeletal muscle. Fed. Am. Soc. Exp. Biol. J. 32, 1428–1439. https://doi.org/10.1096/fj.201700493R.
Savić, N., Schwank, G., 2016. Advances in therapeutic CRISPR/Cas9 genome editing. Transl. Res. 168, 15–21. https://doi.org/10.1016/j.trsl.2015.09.008.
Savva, Y.A., Rieder, L.E., Reenan, R.A., 2012. The ADAR protein family. Genome Biol. 13 (12), 1–10. https://doi.org/10.1186/gb-2012-13-12-252.
Schaub, M., Keller, W., 2002. RNA editing by adenosine deaminases generates RNA and protein diversity. Biochimie 84 (8), 791–803. https://doi.org/10.1016/s0300-9084(02)01446-3.
Schlesinger, N., Dalbeth, N., Perez-Ruiz, F., 2009. Gout–what are the treatment options? Expert. Opin. Pharmacother. 10, 1319–1328. https://doi.org/10.1517/14656560902950742.
Schmiderer, L., Yudovich, D., Oburoglu, L., Hjort, M., Larsson, J., 2022. Site-specific CRISPR-based mitochondrial DNA manipulation is limited by gRNA import. Sci. Rep. 12, 18687. https://doi.org/10.1038/s41598-022-21794-0.
Schneider, M.F., Wettengel, J., Hoffmann, P.C., Stafforst, T., 2014. Optimal guideRNAs for re-directing deaminase activity of hADAR1 and hADAR2 in trans. Nucleic Acids Res. 42 (10), e87.
Schutsky, E.K., DeNizio, J.E., Hu, P., Liu, M.Y., Nabel, C.S., Fabyanic, E.B., Hwang, Y., Bushman, F.D., Wu, H., Kohli, R.M., 2018. Nondestructive, base-resolution sequencing of 5-hydroxymethylcytosine using a DNA deaminase. Nat. Biotechnol. 36, 1083–1090. https://doi.org/10.1038/nbt.4204.
Scortecci, J.F., Serrão, V.H.B., Cheleski, J., Torini, J.R., Romanello, L., DeMarco, R., Pereira, H.D.M., 2017. Spectroscopic and calorimetric assays reveal dependence on dCTP and two metals (Zn^{2+} Mg^{2+}) for enzymatic activity of *Schistosoma mansoni* deoxycytidylate (dCMP) deaminase. Biochim. Biophys. Acta, Proteins Proteomics 1865 (11), 1326–1335. https://doi.org/10.1016/j.bbapap.2017.07.015.
Secord, E., Hartog, N.L., 2022. Review of treatment for adenosine deaminase deficiency (ADA) severe combined immunodeficiency (SCID). Ther. Clin. Risk Manag. 18, 939–944. https://doi.org/10.2147/tcrm.s350762.
Secrist, J., Montgomery, J., Shealy, Y., O'Dell, C., Clayton, S., 1987. Resolution of racemic carbocyclic analogs of purine nucleosides through the action of adenosine deaminase. Antiviral activity of the carbocyclic 2′-deoxyguanosine enantiomers. J. Med. Chem. 30 (4), 746–749. https://doi.org/10.1021/jm00387a032.
Seela, F., Kaiser, K., 1988. 8-Aza-7-deaza-2′,3′-dideoxyadenosine: Synthesis and conversion into allopurinol 2′,3′-dideoxyribofuranoside. Chem. Pharm. Bull. 36 (10), 4153–4156. https://doi.org/10.1248/cpb.36.4153.
Seffernick, J.L., Dodge, A.G., Sadowsky, M.J., Bumpus, J.A., Wackett, L.P., 2010. Bacterial ammeline metabolism via guanine deaminase. J. Bacteriol. 192, 1106–1112. https://doi.org/10.1128/JB.01243-09.
Seibert, C.M., Raushel, F.M., 2005. Structural and catalytic diversity within the amidohydrolase superfamily. Biochemistry 44, 6383–6391. https://doi.org/10.1021/bi047326v.
Shao, M., Michno, J.M., Hotton, S.K., Blechl, A., Thomson, J., 2015. A bacterial gene codA encoding cytosine deaminase is an effective conditional negative selectable marker in Glycine max. Plant Cell Rep. 34, 1707–1716. https://doi.org/10.1007/s00299-015-1818-5.

Sharma, S., Baysal, B.E., 2017. Stem-loop structure preference for site-specific RNA editing by APOBEC3A and APOBEC3G. PeerJ 5, e4136. https://doi.org/10.7717/peerj.4136.
Shek, R., Hilaire, T., Sim, J., French, J.B., 2019. Structural determinants for substrate selectivity in guanine deaminase enzymes of the amidohydrolase superfamily. Biochemistry 58 (30), 3280–3292. https://doi.org/10.1021/acs.biochem.9b00341.
Shimada, N., Hasegawa, S., Saito, S., Nishikiori, T., Fujii, A., Takita, T., 1987. Derivatives of oxetanocin: Oxetanocins H, X and G, and 2-aminooxetanocin A. J. Antibiot. 40 (12), 1788–1790. https://doi.org/10.7164/antibiotics.40.1788.
Shimatani, Z., Kashojiya, S., Takayama, M., Terada, R., Arazoe, T., Ishii, H., Teramura, H., Yamamoto, T., Komatsu, H., Miura, K., Ezura, H., Nishida, K., Ariizumi, T., Kondo, A., 2017. Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. Nat. Biotechnol. 35, 441–443. https://doi.org/10.1038/nbt.3833.
Silvas, T.V., Schiffer, C.A., 2019. APOBEC3s: DNA-editing human cytidine deaminases. Protein Sci. 28, 1552–1566. https://doi.org/10.1002/pro.3670.
Simić, S., Zukić, E., Schmermund, L., Faber, K., Winkler, C.K., Kroutil, W., 2021. Shortening synthetic routes to small molecule active pharmaceutical ingredients employing biocatalytic methods. Chem. Rev. 122 (1), 1052–1126. https://doi.org/10.1021/acs.chemrev.1c00574.
Sinkeldam, R., McCoy, L., Shin, D., Tor, Y., 2013. Enzymatic interconversion of isomorphic fluorescent nucleosides: adenosine deaminase transforms an adenosine analogue into an inosine analogue. Angew. Chem. 125 (52), 14276–14280. https://doi.org/10.1002/ange.201307064.
Sinnamon, J.R., Kim, S.Y., Corson, G.M., Song, Z., Nakai, H., Adelman, J.P., Mandel, G., 2017. Site-directed RNA repair of endogenous Mecp2 RNA in neurons. Proc. Natl. Acad. Sci. 114 (44), E9395–E9402. https://doi.org/10.1073/pnas.1715320114.
Sinnamon, J.R., Kim, S.Y., Fisk, J.R., Song, Z., Nakai, H., Jeng, S., McWeeney, S.K., Mandel, G., 2020. *In vivo* repair of a protein underlying a neurological disorder by programmable RNA editing. Cell Rep. 32 (2), 107878. https://doi.org/10.1016/j.celrep.2020.107878.
Skaldin, M., Tuittila, M., Zavialov, A., Zavialov, A., 2018. Secreted bacterial adenosine deaminase is an evolutionary precursor of adenosine deaminase growth factor. Mol. Biol. Evol. 35 (12), 2851–2861. https://doi.org/10.1093/molbev/msy193.
Slagman, S., Fessner, W.D., 2021. Biocatalytic routes to anti-viral agents and their synthetic intermediates. Chem. Soc. Rev. 50, 1968–2009. https://doi.org/10.1039/D0CS00763C.
Small, I.D., Schallenberg-Rüdinger, M., Takenaka, M., Mireau, H., Ostersetzer-Biran, O., 2020. Plant organellar RNA editing: what 30 years of research has revealed. Plant J. 101 (5), 1040–1056. https://doi.org/10.1111/tpj.14578.
Song, C.Q., Jiang, T., Richter, M., Rhym, L.H., Koblan, L.W., Zafra, M.P., Schatoff, E.M., Doman, J.L., Cao, Y., Dow, L.E., Zhu, L.J., Anderson, D.G., Liu, D.R., Yin, H., Xue, W., 2020. Adenine base editing in an adult mouse model of tyrosinaemia. Nat. Biomed. Eng. 4, 125–130. https://doi.org/10.1038/s41551-019-0357-8.
Stafforst, T., Schneider, M.F., 2012. An RNA–deaminase conjugate selectively repairs point mutations. Angew. Chem. Int. Ed. Eng. 51 (44), 11166–11169. https://doi.org/10.1002/anie.201206489.
Stefl, R., Oberstrass, F.C., Hood, J.L., Jourdan, M., Zimmermann, M., Skrisovska, L., Maris, C., Peng, L., Hofr, C., Emerson, R.B., Allain, F.H.T., 2010. The solution structure of the ADAR2 dsRBM-RNA complex reveals a sequence-specific readout of the minor groove. Cell 143 (2), 225–237. https://doi.org/10.1016/j.cell.2010.09.026.
Sternberg, S.H., Redding, S., Jinek, M., Greene, E.C., Doudna, J.A., 2014. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. Nature 507, 62–67. https://doi.org/10.1038/nature13011.
Stroppel, A.S., Latifi, N., Hanswillemenke, A., Tasakis, R.N., Papavasiliou, F.N., Stafforst, T., 2021. Harnessing self-labeling enzymes for selective and concurrent A-to-I and C-to-U RNA base editing. Nucleic Acids Res 49 (16), e95. https://doi.org/10.1093/nar/gkab541.
Sugiyama, T., Schweinberger, E., Kazimierczuk, Z., Ramzaeva, N., Rosemeyer, H., Seela, F., 2000. 2-aza-2′-deoxyadenosine: synthesis, base-pairing selectivity, and stacking properties of oligonucleotides. Chem. Eur. J. 6 (2), 369–378. https://doi.org/10.1002/(SICI)1521-3765(20000117)6:2<369::AID-CHEM369>3.0.CO;2-2.
Suleyman, O.N.A.L., 2012. Adenosine deaminase and Guanase deaminase activities in serum of patients with rheumatoid arthritis. Cell Membr. Free. Radic. Res. 4 (3), 215–219.
Sun, X., Shen, X., Jain, R., Lin, Y., Wang, J., Sun, J., Wang, J., Yan, Y., Yuan, Q., 2015. Synthesis of chemicals by metabolic engineering of microbes. Chem. Soc. Rev. 44 (11), 3760–3785. https://doi.org/10.1039/C5CS00159E.
Sürün, D., Schneider, A., Mircetic, J., Neumann, K., Lansing, F., Paszkowski-Rogacz, M., Hänchen, V., Lee-Kirsch, M.A., Buchholz, F., 2020. Efficient generation and correction of mutations in human iPS cells utilizing mRNAs of CRISPR base editors and prime editors. Genes 11, 511. https://doi.org/10.3390/genes11050511.
Taj, S., Narayanan, S., Meenakshi, S., Sanghvi, Y., Ross, B., Ravikumar, V., 2008. Process research on the preparation of DMT protected 2′-O-methoxyethylguanosine for oligonucleotide synthesis in therapeutic applications. Nucleosides Nucleotides Nucleic Acids 27 (9), 1024–1033. https://doi.org/10.1080/15257770802271748.
Tal, N., Millman, A., Stokar-Avihail, A., Fedorenko, T., Leavitt, A., Melamed, S., Yirmiya, E., Avraham, C., Brandis, A., Mehlman, T., Amitai, G., Sorek, R., 2022. Bacteria deplete deoxynucleotides to defend against bacteriophage infection. Nat. Microbiol. 7, 1200–1209. https://doi.org/10.1038/s41564-022-01158-0.
Tan, J., Forner, J., Karcher, D., Bock, R., 2022. DNA base editing in nuclear and organellar genomes. Trends Genet. 38, 1147–1169. https://doi.org/10.1016/j.tig.2022.06.015.
Tang, R., Xu, Z., 2020. Gene therapy: a double-edged sword with great powers. Mol. Cell. Biochem. 474, 73–81. https://doi.org/10.1007/s11010-020-03834-3.
Tao, J., Bauer, D.E., Chiarle, R., 2023. Assessing and advancing the safety of CRISPR-Cas tools: from DNA to RNA editing. Nat. Commun. 14 (1), 212. https://doi.org/10.1038/s41467-023-35886-6.
Tatusov, R.L., Natale, D.A., Garkavtsev, I.V., Tatusova, T.A., Shankavaram, U.T., Rao, B. S., Kiryutin, B., Galperin, M.Y., Fedorova, N.D., Koonin, E.V., 2001. The COG database: new developments in phylogenetic classification of proteins from complete genomes. Nucleic Acids Res. 29 (1), 22–28. https://doi.org/10.1093/nar/29.1.22.
Tausche, A.-K., Jansen, T.L., Schröder, H.-E., Bornstein, S.R., Aringer, M., Müller-Ladner, U., 2010. Gout – current diagnosis and treatment. Dtsch Ärztebl Int. 106 (34–35), 549–555. https://doi.org/10.3238/arztebl.2009.0549.
Terkeltaub, R., 2010. Update on gout: new therapeutic strategies and options. Nat. Rev. Rheumatol. 6 (1), 30–38. https://doi.org/10.1038/nrrheum.2009.236.
Thomas, C.E., Ehrhardt, A., Kay, M.A., 2003. Progress and problems with the use of viral vectors for gene therapy. Nat. Rev. Genet. 4 (5), 346–358. https://doi.org/10.1038/nrg1066.
Tohama, T., Sakari, M., Tsukahara, T., 2020. Development of a single construct system for site-directed RNA editing using MS2-ADAR. Int. J. Mol. Sci. 21 (14), 4943. https://doi.org/10.3390/ijms21144943.
Tong, H., Wang, X., Liu, Y., Liu, N., Li, Y., Luo, J., Ma, Q., Wu, D., Li, J., Xu, C., Yang, H., 2023. Programmable A-to-Y base editing by fusing an adenine base editor with an N-methylpurine DNA glycosylase. Nat. Biotechnol. 1-5. https://doi.org/10.1038/s41587-022-01595-6.
Trautwein-Schult, A., Jankowska, D., Cordes, A., Hoferichter, P., Klein, C., Matros, A., Mock, H.-P., Baronian, K., Bode, R., Kunze, G., 2014. *Arxula adeninivorans* recombinant guanine deaminase and its application in the production of food with low purine content. J. Mol. Microbiol. Biotechnol. 24 (2), 67–81. https://doi.org/10.1159/000357674.
Trelles, J., Rivero, C., Britos, C., Lapponi, M., 2018. Enzymatic synthesis of nucleic acid derivatives by immobilized cells. In: Fernández-Lucas, J., Camarasa, M.J. (Eds.), Enzymatic and Chemical Synthesis of Nucleic Acid Derivatives. Wiley-VCH Verlag GmbH & Co. KGaA Weinheim, Germany, pp. 79–106. https://doi.org/10.1002/9783527812103.ch4.
Tritsch, D., Jung, P., Burger, A., Biellmann, J., 2000. 3′-β-ethynyl and 2′-deoxy-3′-β-ethynyl adenosines: first 3′-β-branched-adenosines substrates of adenosine deaminase. Bioorg. Med. Chem. Lett. 10 (2), 139–141. https://doi.org/10.1016/s0960-894x(99)00639-3.
Urbelienė, N., Tiškus, M., Tamulaitienė, G., Gasparavičiūtė, R., Lapinskaitė, R., Jauniškis, V., Sūdžius, J., Meškienė, R., Tauraitė, D., Skrodenytė, E., Urbelis, G., Vaitekūnas, J., Meškys, R., 2023. Cytidine deaminases catalyze the conversion of N (S, O) 4-substituted pyrimidine nucleosides. Sci. Adv. 9 (5), eade4361. https://orcid.org/0000-0002-8331-2469.
Valable, S., Barbier, E.L., Bernaudin, M., Roussel, S., Segebarth, C., Petit, E., Rémy, C., 2007. *In vivo* MRI tracking of exogenous monocytes/macrophages targeting brain tumors in a rat model of glioma. Neuroimage 37, S47–S58. https://doi.org/10.1016/j.neuroimage.2007.05.041.
Vallecillo-Viejo, I.C., Liscovitch-Brauer, N., Montiel-Gonzalez, M.F., Eisenberg, E., Rosenthal, J.J., 2018. Abundant off-target edits from site-directed RNA editing can be reduced by nuclear localization of the editing enzyme. RNA Biol. 15 (1), 104–114. https://doi.org/10.1080/15476286.2017.1387711.
Villiger, L., Schmidheini, L., Mathis, N., Rothgangl, T., Marquart, K., Schwank, G., 2021. Replacing the SpCas9 HNH domain by deaminases generates compact base editors with an alternative targeting scope. Mol. Ther. Nucleic Acids 26, 502–510. https://doi.org/10.1016/j.omtn.2021.08.025.
Vincenzetti, S., Mariani, P.L., Cammertoni, N., Polzonetti, V., Natalini, P., Quadrini, B., Volpini, R., Vita, A., 2004. Isoenzymatic forms of human cytidine deaminase. Protein Eng. Des. Sel. 17 (12), 871–877. https://doi.org/10.1093/protein/gzh101.
Vistoli, G., Pedretti, A., Alessandrini, L., Casati, S., Ciuffreda, P., Meroni, G., Santaniello, E., 2009. Enhanced activity or resistance of adenosine derivatives towards adenosine deaminase-catalyzed deamination: influence of ribose modifications. Bioorg. Med. Chem. Lett. 19 (10), 2877–2879. https://doi.org/10.1016/j.bmcl.2009.03.084.
Vogel, P., Stafforst, T., 2019. Critical review on engineering deaminases for site-directed RNA editing. Curr. Opin. Biotechnol. 55, 74–80. https://doi.org/10.1016/j.copbio.2018.08.006.
Vogel, P., Schneider, M.F., Wettengel, J., Stafforst, T., 2014. Improving site-directed RNA editing in vitro and in cell culture by chemical modification of the guideRNA. Angew. Chem. Int. Ed. Eng. 53 (24), 6267–6271. https://doi.org/10.1002/anie.201402634.
Vogel, P., Moschref, M., Li, Q., Merkle, T., Selvasaravanan, K.D., Li, J.B., Stafforst, T., 2018. Efficient and precise editing of endogenous transcripts with SNAP-tagged ADARs. Nat. Methods 15 (7), 535–538. https://doi.org/10.1038/s41592-018-0017-z.
Wagoner, J.A., Sun, T., Lin, L., Hanson, M.R., 2015. Cytidine deaminase motifs within the DYW domain of two pentatricopeptide repeat-containing proteins are required for site-specific chloroplast RNA editing. J. Biol. Chem. 290 (5), 2957–2968. https://doi.org/10.1074/jbc.M114.622084.
Wang, H.C., Hickey, D.A., 2007. Rapid divergence of codon usage patterns within the rice genome. BMC Evol. Biol. 7, 1–10. https://doi.org/10.1186/1471-2148-7-S1-S6.
Wang, X., Wan, X., Hu, S., Pan, C., 2008. Study on the increase mechanism of the caffeine content during the fermentation of tea with microorganisms. Food Chem. 107 (3), 1086–1091. https://doi.org/10.1016/j.foodchem.2007.09.023.
Wang, Y., Zheng, Y., Beal, P.A., 2017. Adenosine deaminases that act on RNA (ADARs). Enzymes 41, 215–268. https://doi.org/10.1016/bs.enz.2017.03.006.
Wang, Y., Liu, Y., Li, J., Yang, Y., Ni, X., Cheng, H., Huang, T., Guo, Y., Ma, H., Zheng, P., Wang, M., Sun, J., Ma, Y., 2019. Expanding targeting scope, editing window, and

base transition capability of base editing in *Corynebacterium glutamicum*. Biotechnol. Bioeng. 116, 3016–3029. https://doi.org/10.1002/bit.27121.
Wang, S., Zong, Y., Lin, Q., Zhang, H., Chai, Z., Zhang, D., Chen, K., Qiu, J.L., Gao, C., 2020. Precise, predictable multi-nucleotide deletions in rice and wheat using APOBEC–Cas9. Nat. Biotechnol. 38, 1460–1465. https://doi.org/10.1038/s41587-020-0566-4.
Wang, L., Xiao, Y., Wei, X., Pan, J., Duanmu, D., 2021a. Highly efficient CRISPR-mediated base editing in *Sinorhizobium meliloti*. Front. Microbiol. 12, 686008. https://doi.org/10.3389/fmicb.2021.686008.
Wang, Z., Liu, X., Xie, X., Deng, L., Zheng, H., Pan, H., Li, D., Li, L., Zhong, C., 2021b. ABE8e with polycistronic tRNA-gRNA expression cassette significantly improves adenine base editing efficiency in *Nicotiana benthamiana*. Int. J. Mol. Sci. 22, 5663. https://doi.org/10.3390/ijms22115663.
Wang, J., Zhao, D., Li, J., Hu, M., Xin, X., Price, M.A., Li, Q., Liu, L., Li, S., Rosser, S.J., Zhang, C., 2021c. Helicase-AID: A novel molecular device for base editing at random genomic loci. Metab. Eng. 67, 396–402. https://doi.org/10.1016/j.ymben.2021.08.005.
Wang, G., Xu, Z., Wang, F., Huang, Y., Xin, Y., Liang, S., Li, B., Si, H., Sun, L., Wang, Q., Ding, X., Zhu, X., Chen, L., Yu, L., Lindsey, K., Zhang, X., Jin, S., 2022. Development of an efficient and precise adenine base editor (ABE) with expanded target range in allotetraploid cotton (Gossypium hirsutum). BMC Biol. 20, 1–15. https://doi.org/10.1186/s12915-022-01232-3.
Wang, Y., Tang, S., Guo, N., An, R., Ren, Z., Hu, S., Wei, X., Jiao, G., Xie, L., Wang, L., Chen, Y., Zhao, F., Hu, P., Sheng, Z., Tang, S., 2023. Base editing of EUI1 improves the elongation of the uppermost internode in two-line male sterile rice lines. Agriculture 13, 693. https://doi.org/10.3390/agriculture13030693.
Warf, M.B., Shepherd, B.A., Johnson, W.E., Bass, B.L., 2012. Effects of ADARs on small RNA processing pathways in C. Elegans. Genome Res. 22 (8), 1488–1498. https://doi.org/10.1101/gr.134841.111.
Wei, Y., Xu, C., Feng, H., Xu, K., Li, Z., Hu, J., Zhou, L., Wei, Y., Zuo, Z., Zuo, E., Li, W., Yang, H., Zhang, M., 2022. Human cleaving embryos enable efficient mitochondrial base-editing with DdCBE. Cell Discov. 8 (1), 7. https://doi.org/10.1038/s41421-021-00372-0.
Wei, Y., Jin, M., Huang, S., Yao, F., Ren, N., Xu, K., Li, S., Gao, P., Zhou, Y., Chen, Y., Yang, H., Li, W., Xu, C., Zhang, M., Wang, X., 2024. Enhanced C-to-T and A-To-G Base editing in mitochondrial DNA with engineered DdCBE and TALED. Adv. Sci. 11 (3), 2304113. https://doi.org/10.1002/advs.202304113.
Wettengel, J., Reautschnig, P., Geisler, S., Kahle, P.J., Stafforst, T., 2017. Harnessing human ADAR2 for RNA repair–recoding a PINK1 mutation rescues mitophagy. Nucleic Acids Res. 45 (5), 2797–2808. https://doi.org/10.1093/nar/gkw911.
Willis, J.C., Silva-Pinheiro, P., Widdup, L., Minczuk, M., Liu, D.R., 2022. Compact zinc finger base editors that edit mitochondrial or nuclear DNA *in vitro* and *in vivo*. Nat. Commun. 13, 7204. https://doi.org/10.1038/s41467-022-34784-7.
Witte, C.P., Herde, M., 2020. Nucleotide Metabolism in Plants. Plant Physiol 182 (1), 63–78. https://doi.org/10.1104/pp.19.00955.
Wolf, J., Gerber, A.P., Keller, W., 2002. tadA, an essential tRNA-specific adenosine deaminase from Escherichia coli. EMBO J. 21 (14), 3841–3851. https://doi.org/10.1093/emboj/cdf362.
Wu, X., Ren, B., Liu, L., Qiu, S., Li, P., Yan, F., Lin, H., Zhou, X., Zhang, D., Zhou, H., 2023. Adenine base editor incorporating the N-methylpurine DNA glycosylase MPGv3 enables efficient A-to-K base editing in rice. Plant Commun. 4 (6). https://doi.org/10.1016/j.xplc.2023.100668.
Xia, Y., Sun, L., Liang, Z., Guo, Y., Li, J., Tang, D., Huo, Y.X., Guo, S., 2023. The construction of a PAM-less base editing toolbox in Bacillus subtilis and its application in metabolic engineering. Chem. Eng. J. 469, 143865. https://doi.org/10.1016/j.cej.2023.143865.
Xiao, Y.L., Wu, Y., Tang, W., 2024. An adenine base editor variant expands context compatibility. Nat. Biotechnol. 1-12. https://doi.org/10.1038/s41587-023-01994-3.
Xie, J., Huang, X., Wang, X., Gou, S., Liang, Y., Chen, F., Li, N., Ouyang, Z., Zhang, Q., Ge, W., Jin, Q., Shi, H., Zhuang, Z., Zhao, X., Lian, M., Wang, J., Ye, Y., Quan, L., Wu, H., Wang, K., Lai, L., 2020. ACBE, a new base editor for simultaneous C-to-T and A-to-G substitutions in mammalian systems. BMC Biol. 18, 1–14. https://doi.org/10.1186/s12915-020-00866-5.
Xu, Q., Guo, H., 2004. Quantum mechanical/molecular mechanical molecular dynamics simulations of cytidine deaminase: from stabilization of transition state analogues to catalytic mechanisms. J. Phys. Chem. B 108 (7), 2477–2483. https://doi.org/10.1021/jp037529d.
Xu, J., Deng, Y., Li, Q., Zhu, X., He, Z., 2014. STRIPE2 encodes a putative dCMP deaminase that plays an important role in chloroplast development in rice. J. Genet. Genom. 41 (10), 539–548. https://doi.org/10.1016/j.jgg.2014.05.008.
Xu, C., Zhou, Y., Xiao, Q., He, B., Geng, G., Wang, Z., Cao, B., Dong, X., Bai, W., Wang, Y., Wang, X., Zhou, D., Yuan, T., Huo, X., Lai, J., Yang, H., 2021. Programmable RNA editing with compact CRISPR–Cas13 systems from uncultivated microbes. Nat. Methods 18, 499–506. https://doi.org/10.1038/s41592-021-01124-4.
Yan, W.X., Chong, S., Zhang, H., Makarova, K.S., Koonin, E.V., Cheng, D.R., Scott, D.A., 2018a. Cas13d is a compact RNA-targeting type VI CRISPR effector positively modulated by a WYL-domain-containing accessory protein. Mol. Cell 70 (2), 327–339. https://doi.org/10.1016/j.molcel.2018.02.028.
Yan, F., Kuang, Y., Ren, B., Wang, J., Zhang, D., Lin, H., Yang, B., Zhou, X., Zhou, H., 2018b. Highly efficient A·T to G·C base editing by Cas9n-guided tRNA adenosine deaminase in rice. Mol. Plant 11, 631–634. https://doi.org/10.1016/j.molp.2018.02.008.
Yang, B., Li, X., Lei, L., Chen, J., 2017. APOBEC: from mutator to editor. J. Genet. Genom. 44 (9), 423–437. https://doi.org/10.1016/j.jgg.2017.04.009.
Yata, V.K., Gopinath, P., Ghosh, S.S., 2012. Emerging implications of nonmammalian cytosine deaminases on cancer therapeutics. Appl. Biochem. Biotechnol. 167 (7), 2103–2116. https://doi.org/10.1007/s12010-012-9746-0.
Yi, Z., Qu, L., Tang, H., Liu, Z., Liu, Y., Tian, F., Wang, Z., Zhang, X., Feng, Z., Yu, Y., Yuan, P., Yi, Z., Zhao, Y., Wei, W., 2022. Engineered circular ADAR-recruiting RNAs increase the efficiency and fidelity of RNA editing *in vitro* and *in vivo*. Nat. Biotechnol. 40, 946–955. https://doi.org/10.1038/s41587-021-01180-3.
Zauri, M., Berridge, G., Thézénas, M.L., Thézénas, M.-L., Pugh, K.M., Goldin, R., Kessler, B.M., Kriaucionis, S., 2015. CDA directs metabolism of epigenetic nucleosides revealing a therapeutic window in cancer. Nature 524, 114–118. https://doi.org/10.1038/nature14948.
Zeng, H., Yuan, Q., Peng, F., Ma, D., Lingineni, A., Chee, K., Gilberd, P., Osikpa, E.C., Sun, Z., Gao, X., 2023. A split and inducible adenine base editor for precise in vivo base editing. Nat. Commun. 14 (1), 5573. https://doi.org/10.1038/s41467-023-41331-5.
Zhang, Y., Maley, F., Maley, G.F., Duncan, G., Dunigan, D.D., Van Etten, J.L., 2007. Chloroviruses encode a bifunctional dCMP-dCTP deaminase that produces two key intermediates in dTTP formation. J. Virol. 81 (14), 7662–7671. https://doi.org/10.1128/JVI.00186-07.
Zhang, L., Jia, R., Palange, N.J., Satheka, A.C., Togo, J., An, Y., Humphrey, M., Ban, L., Ji, Y., Jin, H., Feng, X., Zheng, Y., 2015. Large genomic fragment deletions and insertions in mouse using CRISPR/Cas9. PLoS One 10, e0120396. https://doi.org/10.1371/journal.pone.0120396.
Zhang, X.K., Nie, M.Y., Chen, J., Wei, L.J., Hua, Q., 2019. Multicopy integrants of crt genes and co-expression of AMP deaminase improve lycopene production in *Yarrowia lipolytica*. J. Biotechnol. 289, 46–54. https://doi.org/10.1016/j.algal.2016.04.007.
Zhang, Y., Zhang, H., Wang, Z., Wu, Z., Wang, Y., Tang, N., Xu, X., Zhao, S., Chen, W., Ji, Q., 2020. Programmable adenine deamination in bacteria using a Cas9–adenine-deaminase fusion. Chem. Sci. 11, 1657–1664. https://doi.org/10.1039/C9SC03784E.
Zhang, H., Bamidele, N., Liu, P., Ojelabi, O., Gao, X.D., Rodriguez, T., Cheng, H., Kelly, K., Watts, J.K., Xie, J., Gao, G., Wolfe, S.A., Xue, W., Sontheimer, E.J., 2022. Adenine base editing in vivo with a single adeno-associated virus vector. GEN Biotechnol. 1, 285–299. https://doi.org/10.1089/genbio.2022.0015.
Zhang, S., Song, L., Yuan, B., Zhang, C., Cao, J., Chen, J., Qiu, J., Tai, Y., Chen, J., Qiu, Z., Zhao, X.M., Cheng, T.L., 2023a. TadA reprogramming to generate potent miniature base editors with high precision. Nat. Commun. 14, 413. https://doi.org/10.1038/s41467-023-36004-2.
Zhang, S., Yuan, B., Cao, J., Song, L., Chen, J., Qiu, J., Qiu, Z., Zhao, X.M., Chen, J., Cheng, T.L., 2023b. TadA orthologs enable both cytosine and adenine editing of base editors. Nat. Commun. 14, 414. https://doi.org/10.1038/s41467-023-36003-3.
Zhang, E., Neugebauer, M.E., Krasnow, N.A., Liu, D.R., 2024. Phage-assisted evolution of highly active cytosine base editors with enhanced selectivity and minimal sequence context preference. Nat. Commun. 15 (1), 1697. https://doi.org/10.1038/s41467-024-45969-7.
Zhao, D., Li, J., Li, S., Xin, X., Hu, M., Price, M.A., Rosser, S.J., Bi, C., Zhang, X., 2021. New base editors change C to A in bacteria and C to G in mammalian cells. Nat. Biotechnol. 39, 35–40. https://doi.org/10.1038/s41587-020-0592-2.
Zheng, Y., Lorenzo, C., Beal, P.A., 2017. DNA editing in DNA/RNA hybrids by adenosine deaminases that act on RNA. Nucleic Acids Res. 45, 3369–3377. https://doi.org/10.1093/nar/gkx050.
Zhou, Q., Yang, D., Ombrello, A., Zavialov, A., Toro, C., Zavialov, A., Stone, D.L., Chae, J. J., Rosenzweig, S.D., Bishop, K., Barron, K.S., Kuehn, H.S., Hoffmann, P., Negro, A., Tsai, W.L., Cowen, E.W., Pei, W., Milner, J.D., Silvin, C., Heller, T., Chin, D.T., Patronas, N.J., Barber, J.S., Lee, C.C., Wood, G.M., Ling, A., Kelly, S.J., Kleiner, D.E., Mullikin, J.C., Ganson, N.J., Kong, H.H., Hambleton, S., Candotti, F., Quezado, M. M., Calvo, K.R., Alao, H., Barham, B.K., Jones, A., Meschia, J.F., Worrall, B.B., Kasner, S.E., Rich, S.S., Goldbach-Mansky, R., Abinun, M., Chalom, E., Gotte, A.C., Punaro, M., Pascual, V., Verbsky, J.W., Torgerson, T.R., Singer, N.G., Gershon, T.R., Ozen, S., Karadag, O., Fleisher, T.A., Remmers, E.F., Burgess, S.M., Moir, S.L., Gadina, M., Sood, R., Hershfield, M.S., Boehm, M., Kastner, D.L., Aksentijevich, I., 2014. Early-onset stroke and vasculopathy associated with mutations in ADA2. N. Engl. J. Med. 370 (10), 911–920. https://doi.org/10.1056/nejmoa1307361.
Zhou, C., Sun, Y., Yan, R., Liu, Y., Zuo, E., Gu, C., Linxiao, H., Wei, Y., Hu, X., Zeng, R., Li, Y., Zhou, H., Guo, F., Yang, H., 2019. Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis. Nature 571, 275–278. https://doi.org/10.1038/s41586-019-1314-0.
Zhu, H., Yang, S.M., Yuan, Z.M., Ban, R., 2015. Metabolic and genetic factors affecting the productivity of pyrimidine nucleoside in *Bacillus subtilis*. Microb. Cell Factories 14 (54), 1–12. https://doi.org/10.1186/s12934-015-0237-1.

Zhu, H., Jiang, X., Wang, L., Qin, Q., Song, M., Huang, Q., 2023. Directed-evolution mutations of adenine base editor ABE8e improve its DNA-binding affinity and protein stability. bioRxiv. https://doi.org/10.1101/2023.05.09.539947, 2023-05.

Zimmermann, A., Prieto-Vivas, J.E., Cautereels, C., Gorkovskiy, A., Steensels, J., Van de Peer, Y., Verstrepen, K.J., 2023. A Cas3-base editing tool for targetable in vivo mutagenesis. Nat. Commun. 14, 3389. https://doi.org/10.1038/s41467-023-39087-z.

Zinchenko, A.I., Barai, V.N., Bokut, S.B., Kvasyuk, E.I., Mikhailopulo, I.A., 1990. Synthesis of 9-(beta-D-arabinofuranosyl)guanine using whole cells of *Escherichia coli*. Appl. Microbiol. Biotechnol. 32 (6), 658–661. https://doi.org/10.1007/BF00164735.

Zong, Y., Wang, Y., Li, C., Zhang, R., Chen, K., Ran, Y., Qiu, J.L., Wang, D., Gao, C., 2017. Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. Nat. Biotechnol. 35, 438–440. https://doi.org/10.1038/nbt.3811.

Zulty, J.J., Speedie, M.K., 1989. Purification and characterization of S-adenosylhomocysteine deaminase from streptonigrin-producing Streptomyces flocculus. J. Bacteriol. 171 (12), 6840–6844. https://doi.org/10.1128/jb.171.12.6840-6844.1989.