The Molecular Basis of AID/APOBEC Deaminase Target Selection

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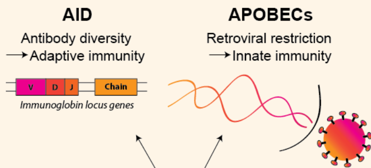
Abstract

AID/APOBEC cytosine deaminases play critical roles in adaptive and innate immunity. AID enzymes target cytosines in host immunoglobin locus genes to initiate class-switch recombination and somatic hypermutation, both of which contribute to antibody diversity. Of the APOBEC family, APOBEC1 targets the mRNA of the ApoB protein transcript to create a truncated protein involved in lipid transport, while APOBEC3 members contribute to innate immunity by targeting retroviruses and retroelements. These deaminases have several promising biotechnological applications, such as pairing with CRISPR systems to achieve more targeted base editing. However, AID/APOBECs have also been associated with pathogenic phenotypes and may play a role in tumor development.



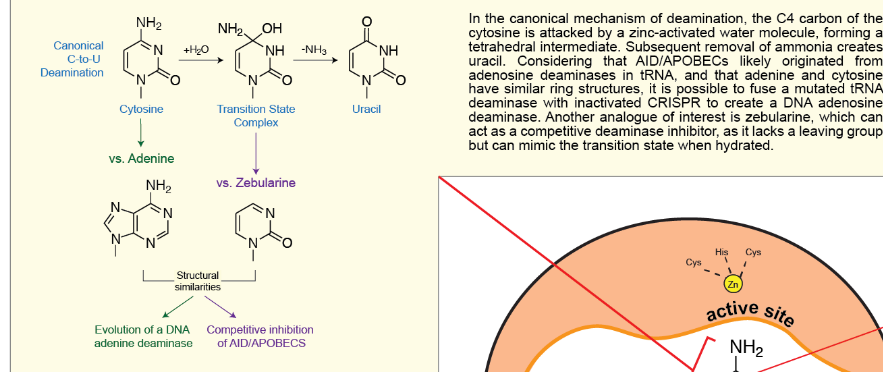
Cytosine Deaminase

Off-target activity → Oncogenesis

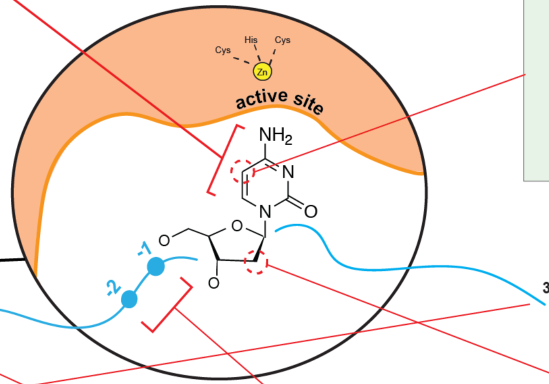
+ CRISPR-Cas9 → Precise, targeted base editing

This project's objectives are to (1) synthesize existing knowledge on the biochemical basis of AID/APOBEC substrate selectivity and (2) decipher how this knowledge can be leveraged to improve biotechnological applications, such as genomic base editors. To do so, we will begin with deamination activity on the target cytosine, focusing on the impact of its structural analogues adenine and zebularine, the relevance of epigenetic modifications at the C5 position, and the identity of the nucleotide sugar. Since the binding of substrate to deaminase implicates more than just the target cytosine, we will then analyze the influence of surrounding nucleotides on deaminase sequence preferences, as well as the influence of the secondary structures in which these nucleotides are found.

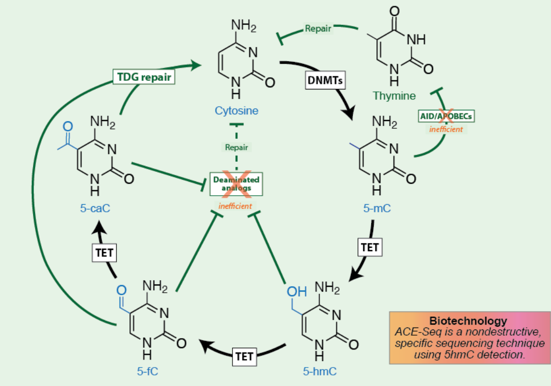
Bases analogous to cytosine offer promising developments for DNA adenine base editing and deaminase inhibition



In the canonical mechanism of deamination, the C4 carbon of the cytosine is attacked by a zinc-activated water molecule, forming a tetrahedral intermediate. Subsequent removal of ammonia creates uracil. Considering that AID/APOBECs likely originated from adenosine deaminases in tRNA, and that adenine and cytosine have similar ring structures, it is possible to fuse a mutated tRNA deaminase with inactivated CRISPR to create a DNA adenosine deaminase. Another analogue of interest is zebularine, which can act as a competitive deaminase inhibitor, as it lacks a leaving group but can mimic the transition state when hydrated.



Deamination activity of modified cytosines elucidates possible demethylation pathways



Cytosine can also experience several epigenetic modifications at its C5 position. Methylation by DNA methyltransferases creates 5-methylcytosine (5-mC), while iterative TET oxidations of 5-mC result in 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxycytosine (5-caC). Of much debate is the mechanism of demethylating these modified cytosines, a process critical to development and gene regulation. AID/APOBECs have been proposed to play a role in demethylation, either through deamination of 5-mC or the TET-oxidized bases. However, poor AID/APOBEC activity on these modified bases has led to the acceptance of demethylation via thymine DNA glycosylase (TDG) excision of 5-fC and 5-caC.

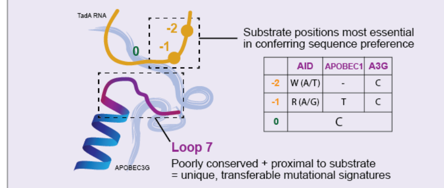
Secondary structures influence deamination activity

	R-Loop	G-Quadruplex	Stem Loop
Structure	Non-template DNA; G-rich RNA; C-rich template DNA	GGGGAGCTGGGG...	Complementary base pairing
Relevant deaminases	AID A3A	AID	A3A
Physiological significance	Transcription-associated mutations	Class-switch recombination of antibodies	Identification in tumor mutagenesis

Although AID/APOBECs preferentially act on ssDNA and RNA, the genome primarily remains in a dsDNA structure. Secondary structures that expose ssDNA are thus important indicators of deamination frequency and can be exploited to alter mutation rates.

Biotechnology
Secondary structure context can help distinguish driver vs. passenger mutations in cancer.

Deaminase sequence preferences can be transferred through loop grafting

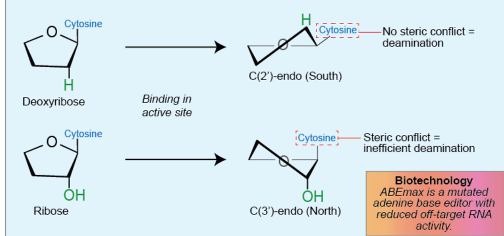


	AID	APOBEC1	A3G
-2	W (A/T)	-	C
-1	R (A/G)	T	C
0		C	

When bound to A3A, ssDNA forms a U-shaped conformation that implicates surrounding bases. A 9-11 amino acid loop, loop 7, is part of the active site pocket and forms many binding interactions with the ssDNA substrate, while also being poorly conserved between AID/APOBEC members. Subsequently, transfer of loop 7 between deaminases has been found to transfer sequence preference of the donor member, confirming the importance of loop 7.

Biotechnology
Loop grafting in Diversifying Base Editors expands sequence targeting abilities.

AID/APOBECs distinguish between DNA and RNA sugar puckers



Although some AID/APOBEC members target RNA in physiological contexts, many, such as AID, have significantly higher activity on ssDNA in vivo. The addition of an OH group on the ribose sugar causes ribose and deoxyribose to form different sugar pucker conformations in the active site pocket. The deoxyribose C(2')-endo conformation places the cytosine nucleobase in an optimal position for deamination, while the additional OH in ribose forces the cytosine in a position that causes steric conflict with the active site and reduced deamination activity. Despite the difference in efficiency, AID/APOBECs are still able to deaminate RNA; for instance, A3A targets RNA in macrophages during M1 maturation and in monocytes in response to hypoxia and interferons. Additionally, off-target RNA deamination has been linked to pathogenic effects.