胞嘧啶碱基编辑蛋白APOBEC的系统发生与结构分析

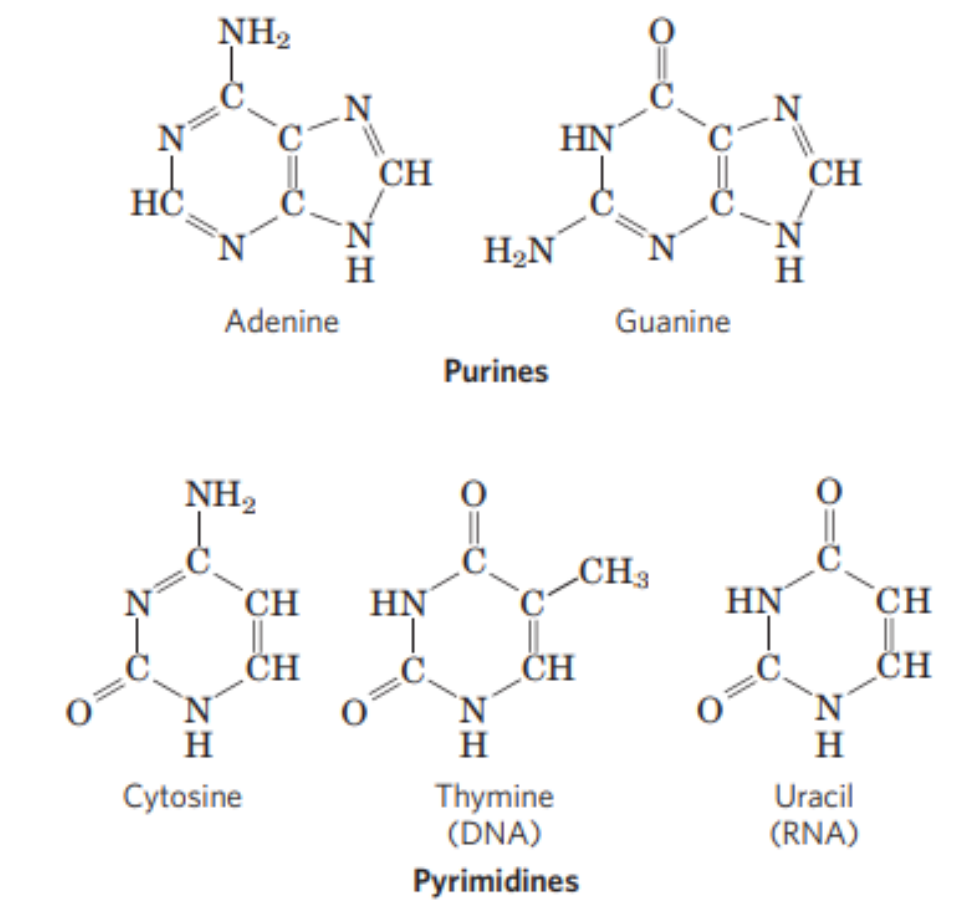
Phylogenic and Structural Analysis of the Cytosine Editing Protein APOBEC

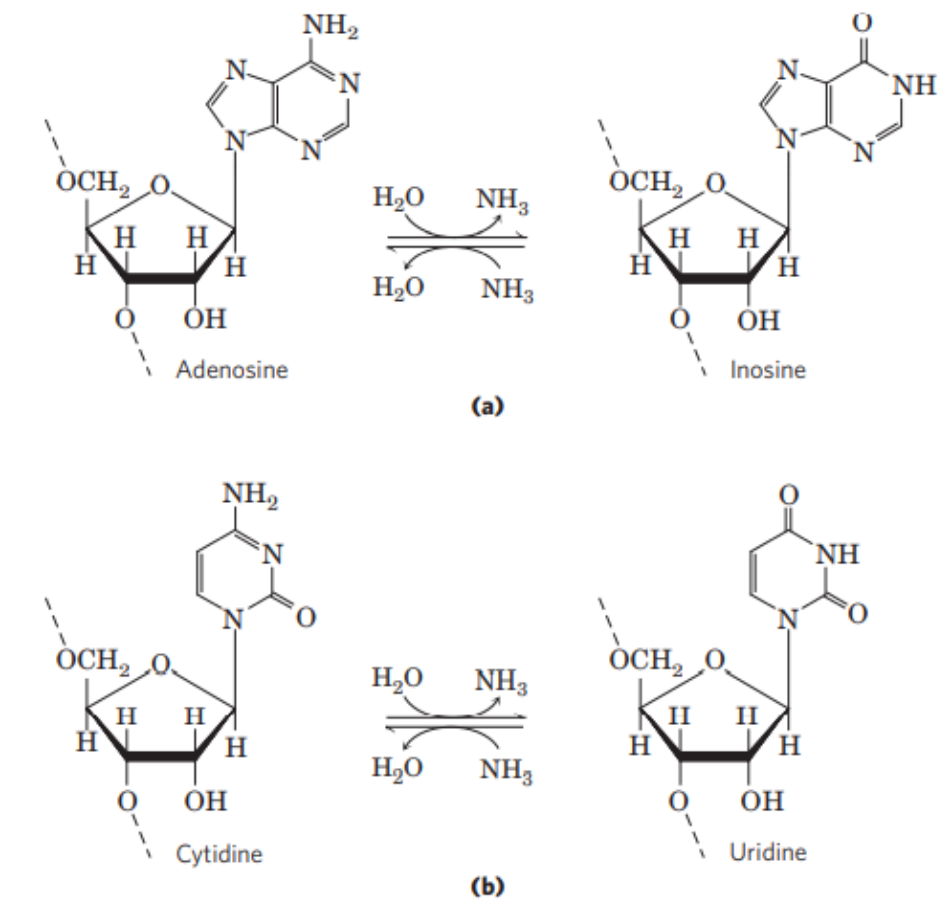
第十组：饶希晨 朱擎国 金一帆 王洪光

背景介绍

单碱基编辑器是什么？

主要的碱基及其脱氨反应





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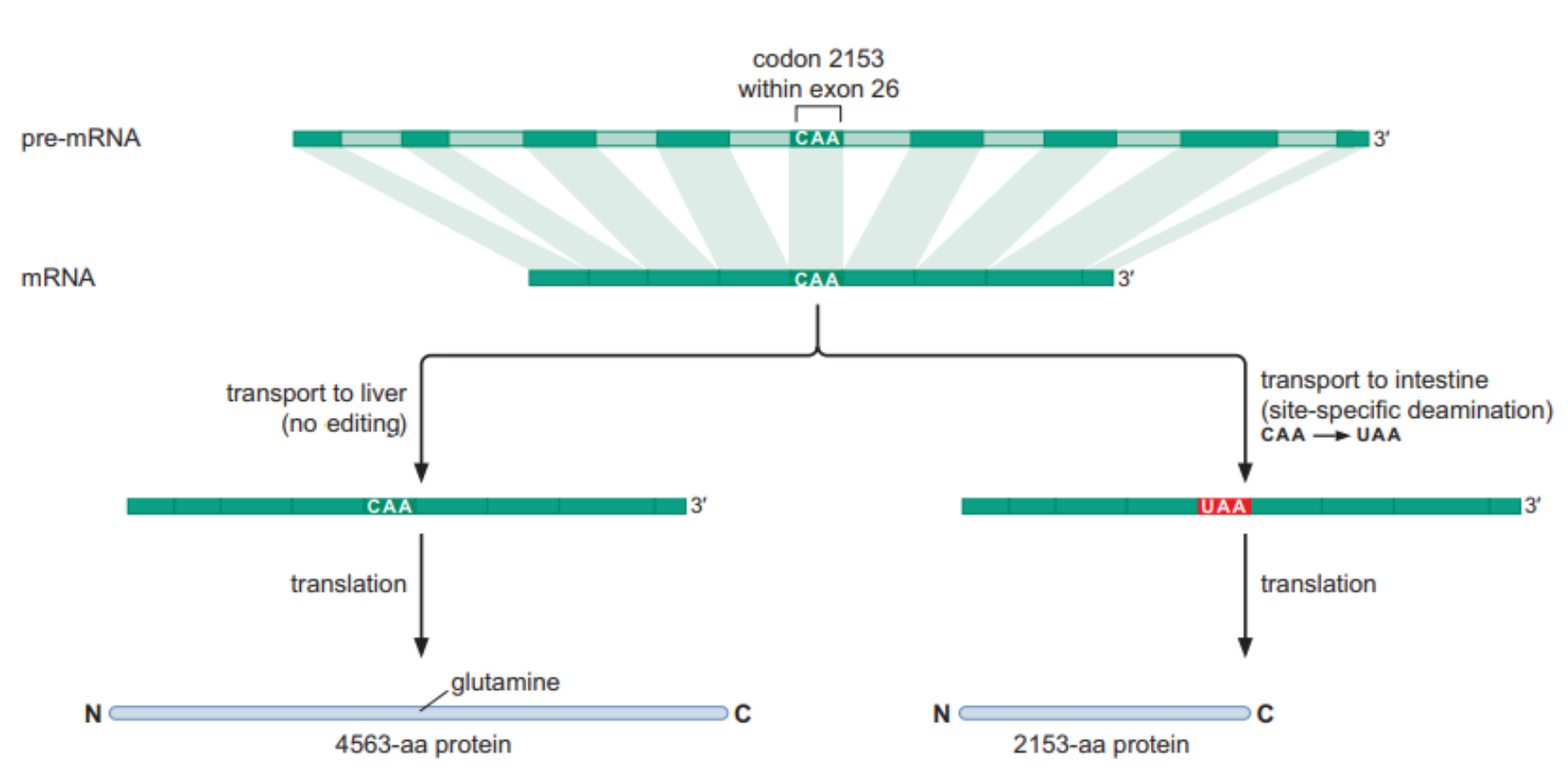
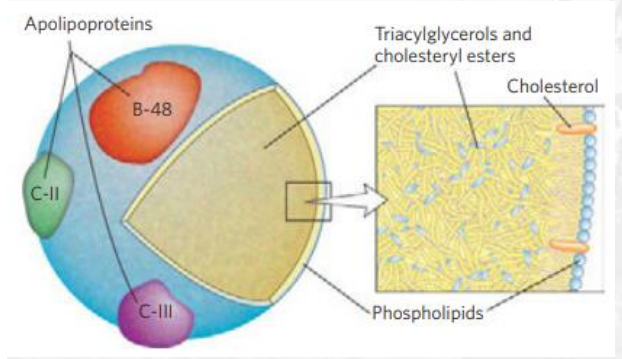
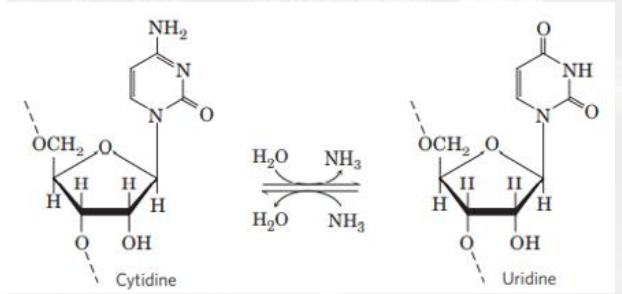
APOBEC蛋白的发现







APOBEC (apolipoprotein B mRNA editing catalytic polypeptide-like) Family 载脂蛋白

Molecular Biology of the Gene 7th Edition
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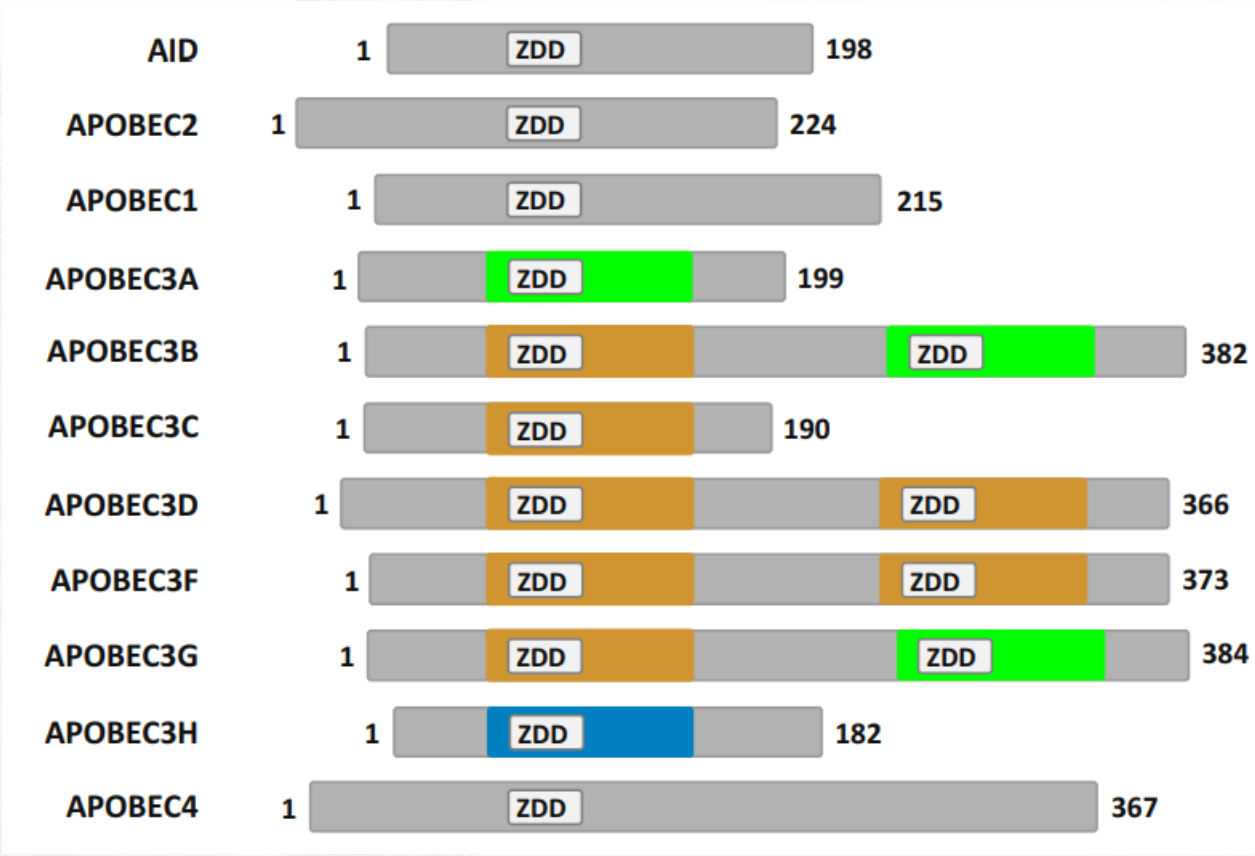
APOBEC蛋白家族及其功能



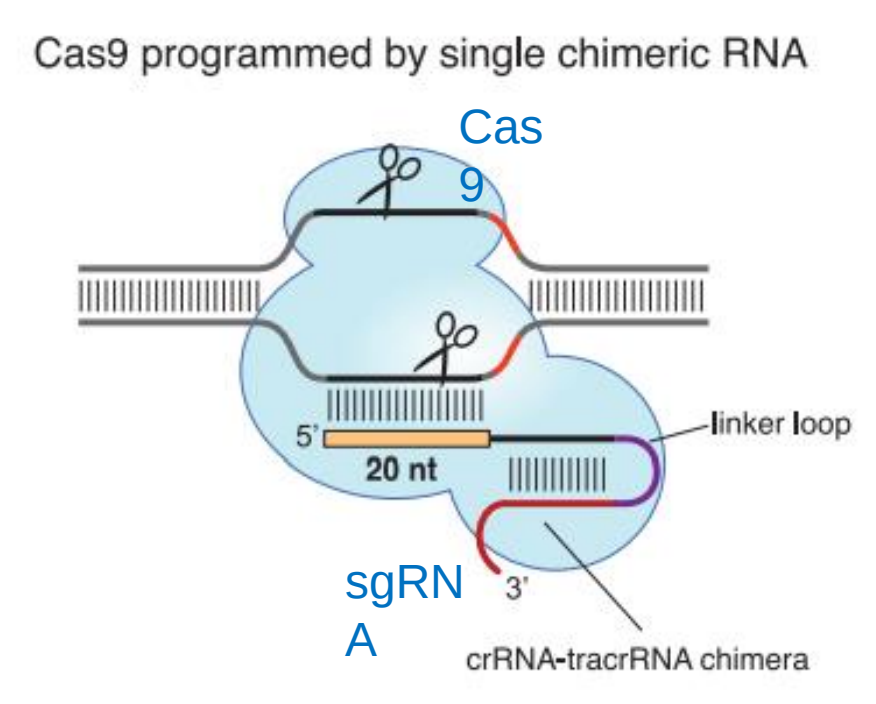
ZDD: zinc-dependent cytidine deaminase domain

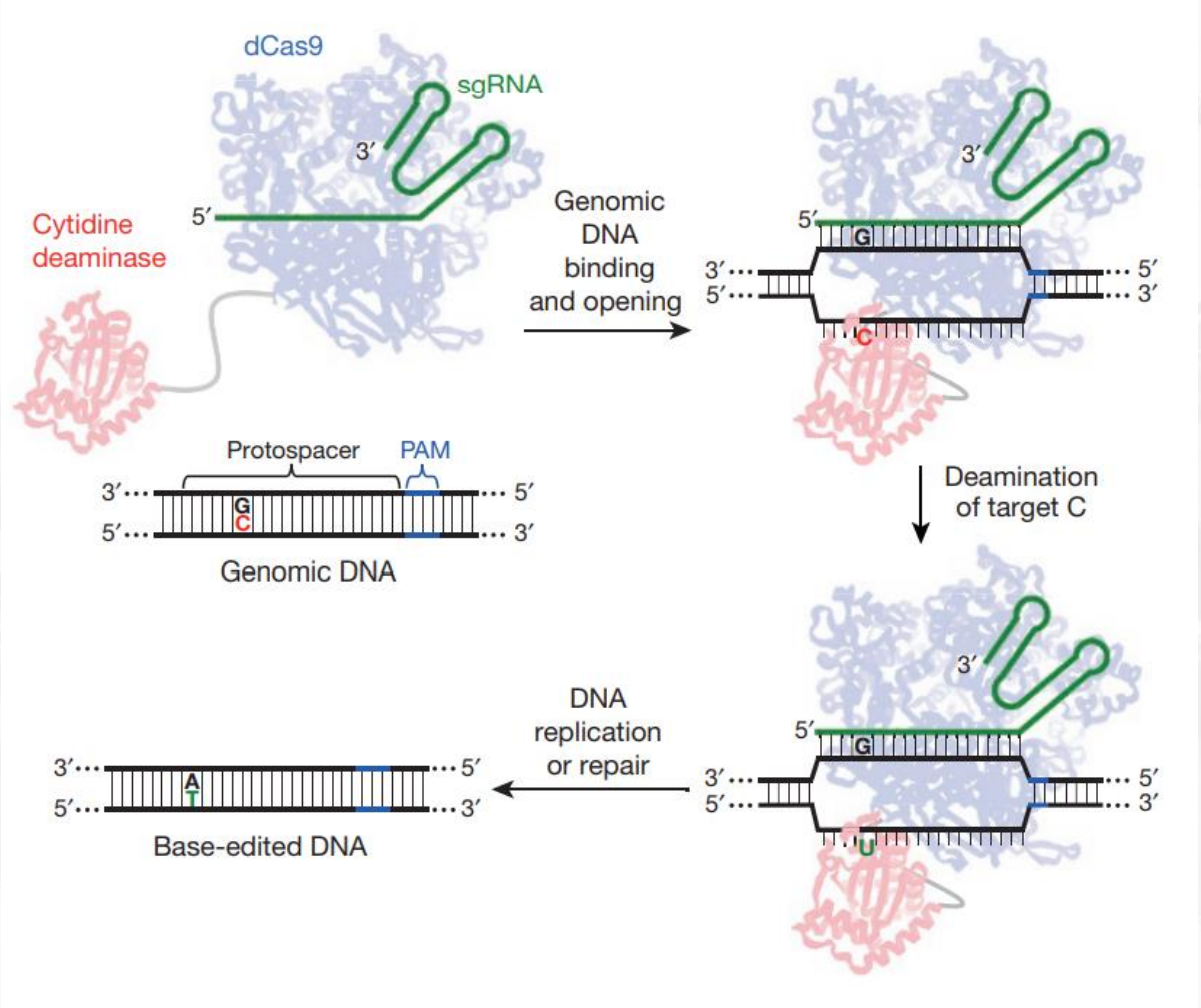
Table 1
Genomic structure, function and sequence specificity of APOBEC superfamily.

Gene	Chromosomal location	No of exons	Function	Tissue specific expression
AID	12p13	5	Immunoglobin diversification	Activated B cells
APOBEC1	12p13.1	5	Lipid metabolism and transport	Gastrointestinal tract
APOBEC2		3	Mitochondrial function	Differentiated skeletal and cardiac muscle
APOBEC3A	22q13.1	5	Viral restriction	Monocytes/ macrophages, non-progenitor cells
APOBEC3B	22q13.1	8	Viral restriction	IFN/-activated liver cells
APOBEC3C	22q13.1	4	Viral restriction	Immune centers, peripheral blood cells
APOBEC3D	22q13.1	7	Viral restriction	Immune centers, peripheral blood cells
APOBEC3F	22q13.1	8	Viral restriction	Immune centers, peripheral blood cells, IFN/-activated liver cells
APOBEC3G	22q13.1	8	Viral restriction	Immune centers, peripheral blood cells, IFN/-activated liver cells
APOBEC3H	22q13.1	5	Viral restriction	Immune centers, peripheral blood cells
APOBEC4	1q25.3	2	Unknown	

Salter JD, *et al*. Trends Biochem Sci. 2016 Jul;41(7):578-594.
Revathidevi S, *et al*. Cancer Lett. 2021 Jan 1;496:104-116.

从突变子变为编辑器





Cytidine base editor (CBE)

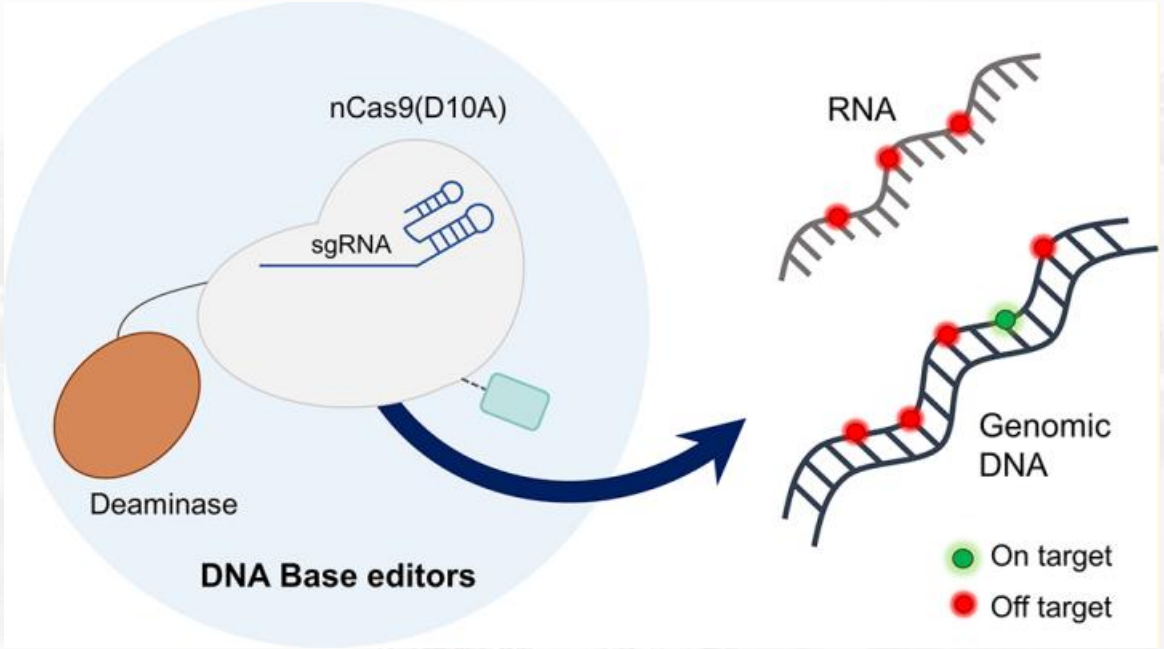
Jinek M, *et al*. Science. 2012 Aug 17;337(6096):816-21.
Komor AC, et al. Nature. 2016 May 19;533(7603):420-4.

胞嘧啶碱基编辑器（Cytidine base editor, CBE）的缺点

By-product in editing window

sequence	% of total reads
...CCCCCCCC...	62.4
...TTTTTTCC...	18.2
...TTTTTTTC...	13.4
...TTTTTTTT...	3.3
...TCCCCCCC...	0.8
...CCCCTTCC...	0.3
...CCCTTTCC...	0.3
...TTTTTCCC...	0.3
...CCCCTCCC...	0.3

Genome-wide and Transcriptome-wide Off-Target Editing



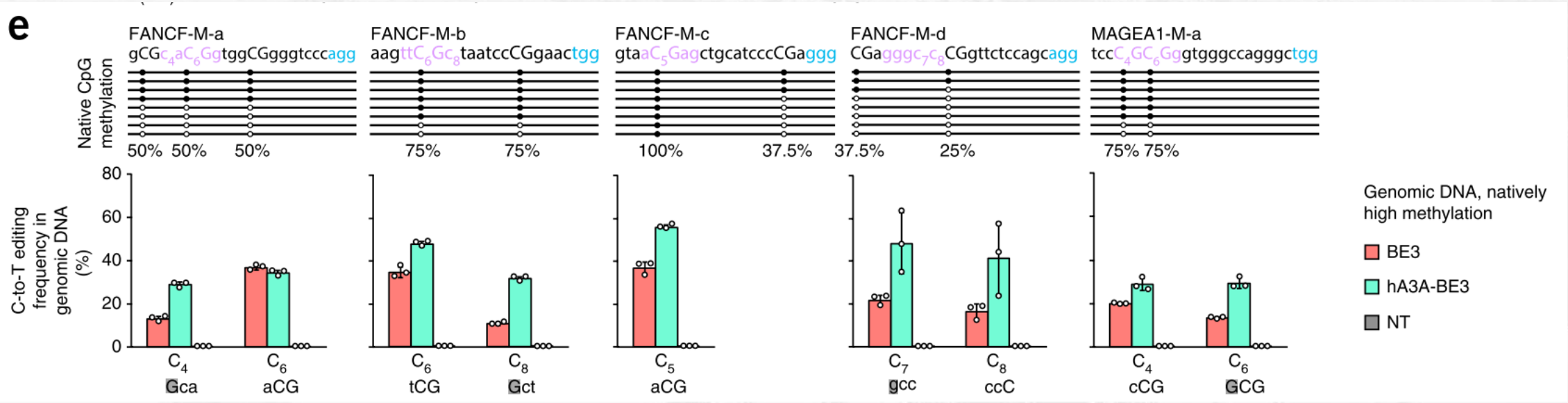
Komor AC, et al. Nature. 2016 May 19;533(7603):420-4.
Park S, *et al.* Biochemistry. 2019 Sep 10;58(36):3727-3734.

基于结构改进Human APOBEC3A (hAPOBEC3A)

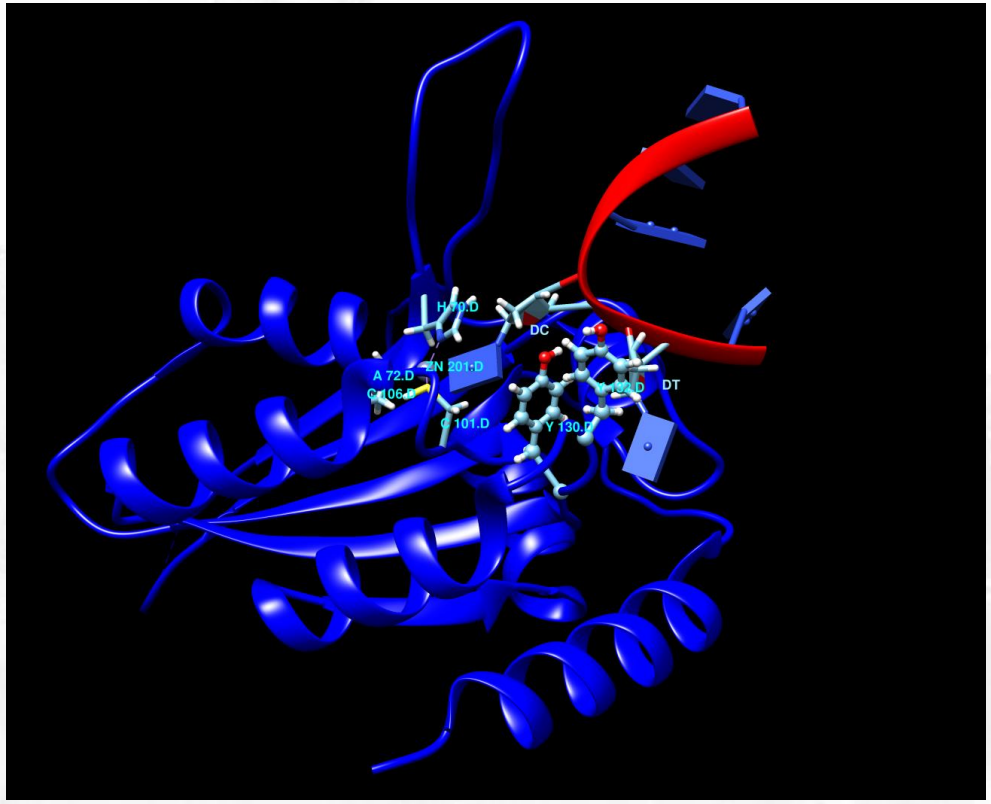
以晶体结构为基础，理性突变与DNA结合、脱氨酶活性相关的氨基酸，从而缩小编辑窗口或减少脱靶效应

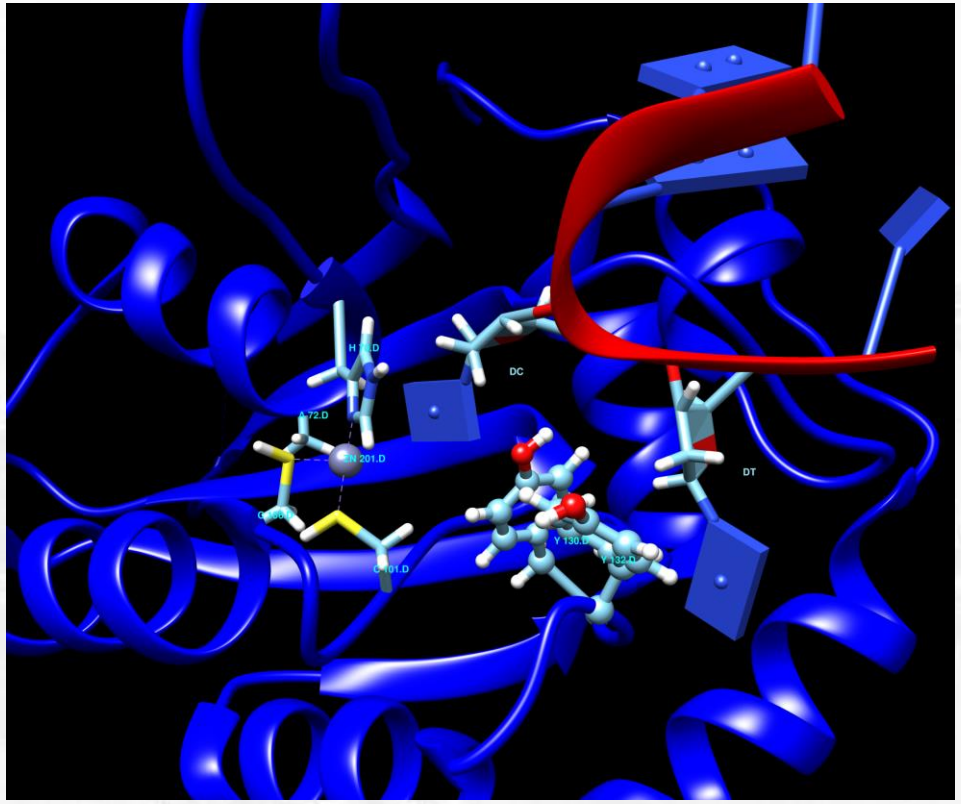
hAPOBEC3A

- 由于基因组上存在CpG甲基化位点，rAPOBEC1的脱氨酶活性会有所降低；在这些位点上，hAPOBEC3A比rAPOBEC1拥有更高的C-to-T编辑活性
- 但是新的hAPOBEC3A-BE3的编辑窗口过大，需要缩小



hAPOBEC3A-ssDNA结构

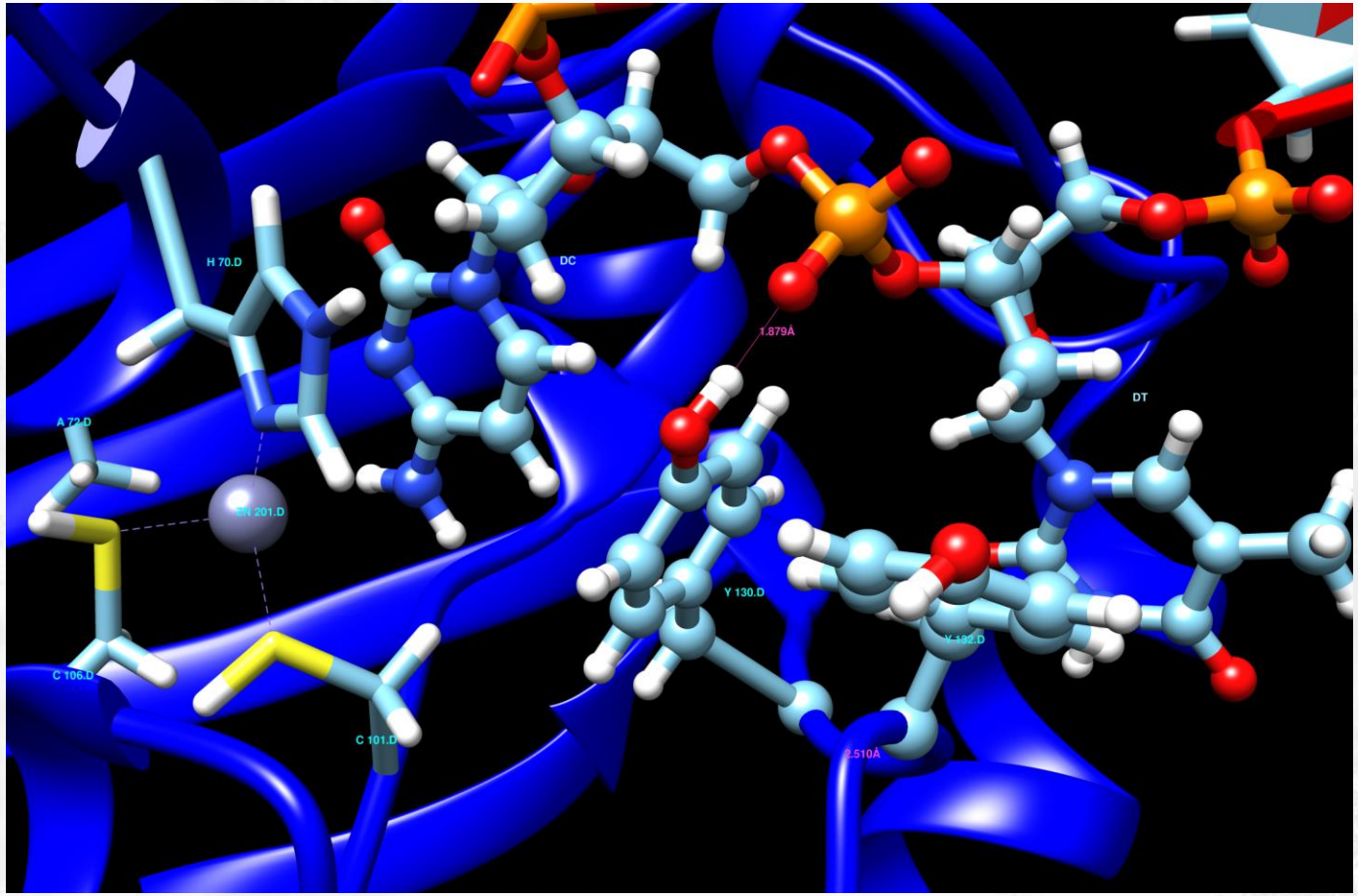




脱氨酶活性口袋：Tyr70, Ala72, Cys191, Cys106
与单链DNA上的TC motif接触的氨基酸：Tyr130和Tyr132
DNA序列：5′-ATCGGG-3′

Shi, K., Carpenter, M.A., et al, 2017 Nat. Struct. Mol. Biol

Y130F和Y132D突变

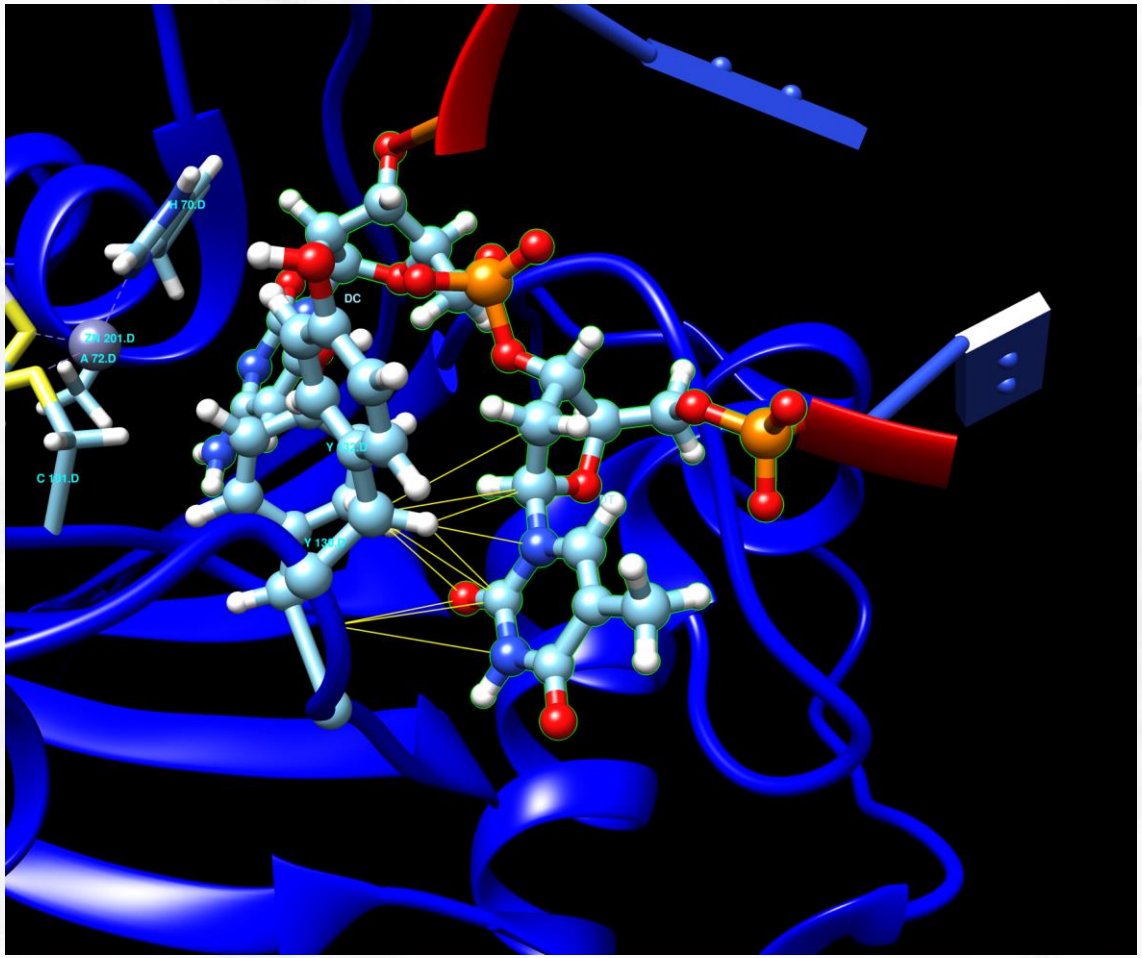


- Tyr130的羟基与DNA的backbone有氢键相互作用（图中粉红色直线显示，1.879Å）
- Tyr130突变为Phe130，氢键相互作用消失，DNA与活性口袋的结合减弱

Wang, X., Li, J., Wang, Y., Yang, B., Wei, J., Wu, J., et al, 2018, Nat. Biotechnol

Shi, K., Carpenter, M.A., et al, 2017, Nat. Struct. Mol. Biol

Y130F和Y132D突变

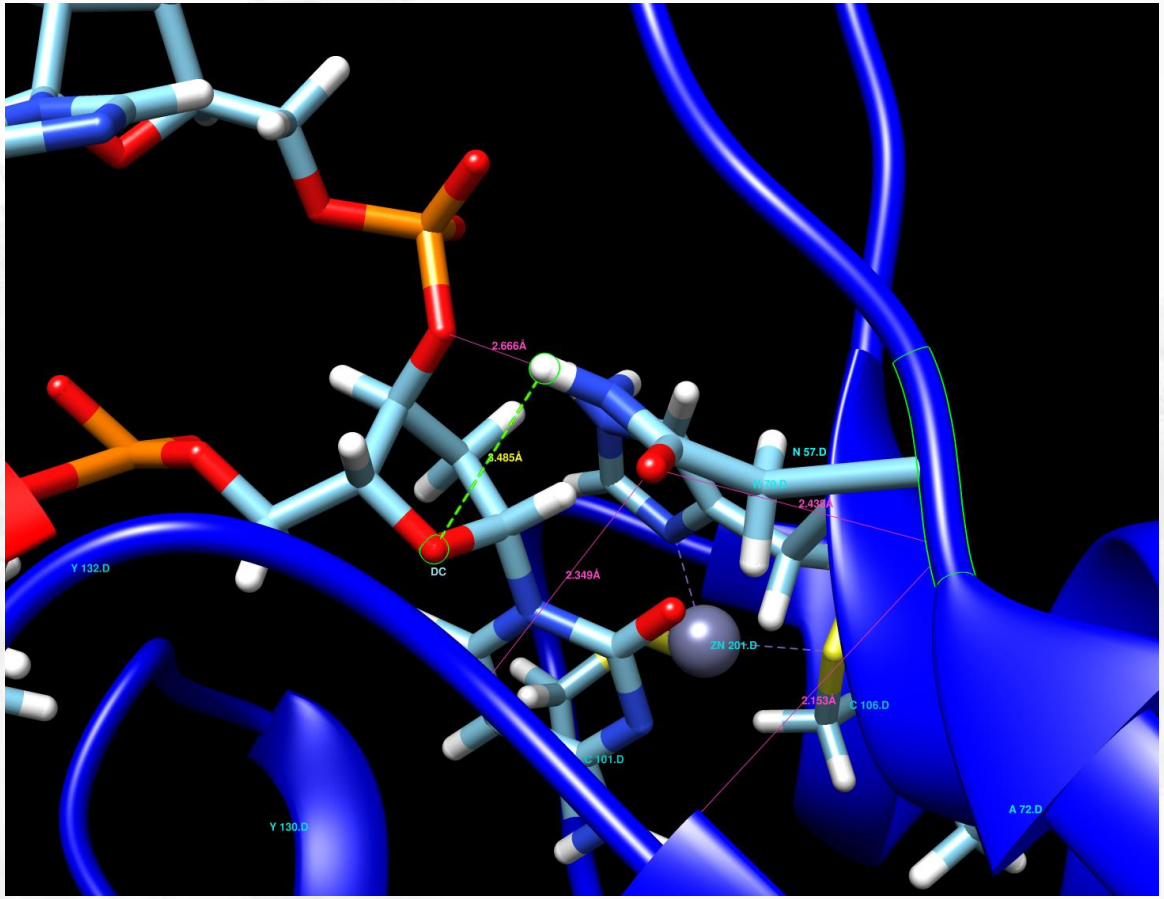


- Tyr132与DNA上的TC motif上的T有很多疏水相互作用
- Tyr132的侧链芳环对这种疏水作用很重要，因为将其突变为Ala，hAPOBEC3A的活性会下降很多，而突变为Phe则与野生型有着相似的活性
- Tyr132突变为Asp后，由于Asp具有强烈的负电性，疏水口袋被破坏，hAPOBEC3A的活性减弱

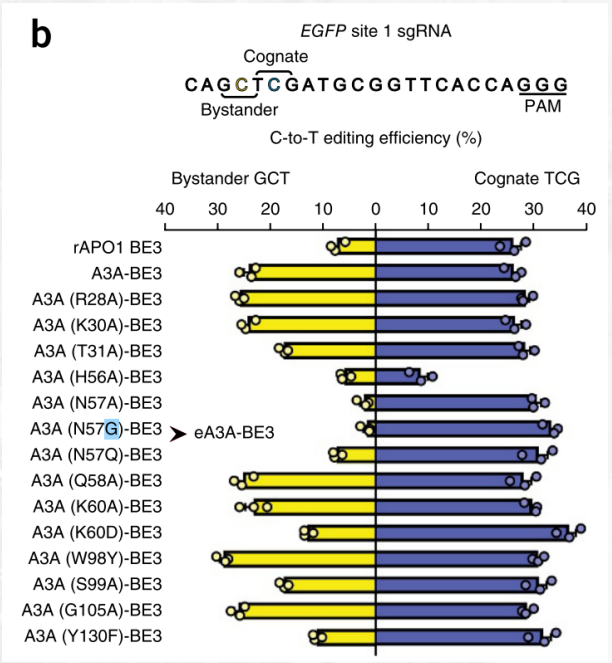
Wang, X., Li, J., Wang, Y., Yang, B., Wei, J., Wu, J., et al, 2018. Nat. Biotechnol

Shi, K., Carpenter, M.A., et al, 2017, Nat. Struct. Mol. Biol

增强对TC motif的依赖性



- Asn57与TC motif上的C的糖环上的氧原子、以及磷酸骨架上的氧原子有氢键相互作用
- Asn57突变为Gly或者Ala（两者均为非极性氨基酸），减弱了这种相互作用
- 这使得hAPOBEC3A更依赖Tyr132与T的相互作用
- 增强了对TC motif的依赖



Gehrke, J.M., et al, 2018, Nat. Biotechnol

Shi, K., Carpenter, M.A., et al, 2017, Nat. Struct. Mol. Biol

APOBEC同源蛋白的系统发生树

由于并不是所有的需要被编辑的C，都位于TC motif中，仍然需要其他方法来缩小APOBEC的编辑窗口

测试其他的APOBEC同源蛋白，是开发新的、编辑窗口更小、脱靶更低的APOBEC的方法之一

人类APOBEC家族系统发生树的构建

数据来源：UniProt
使用ClustalW比对
使用最大似然法建树
软件：MEGA7

Pairwise Alignment

Gap Opening Penalty	15.00
Gap Extension Penalty	5.00

Multiple Alignment

Gap Opening Penalty	15.00
Gap Extension Penalty	5.00

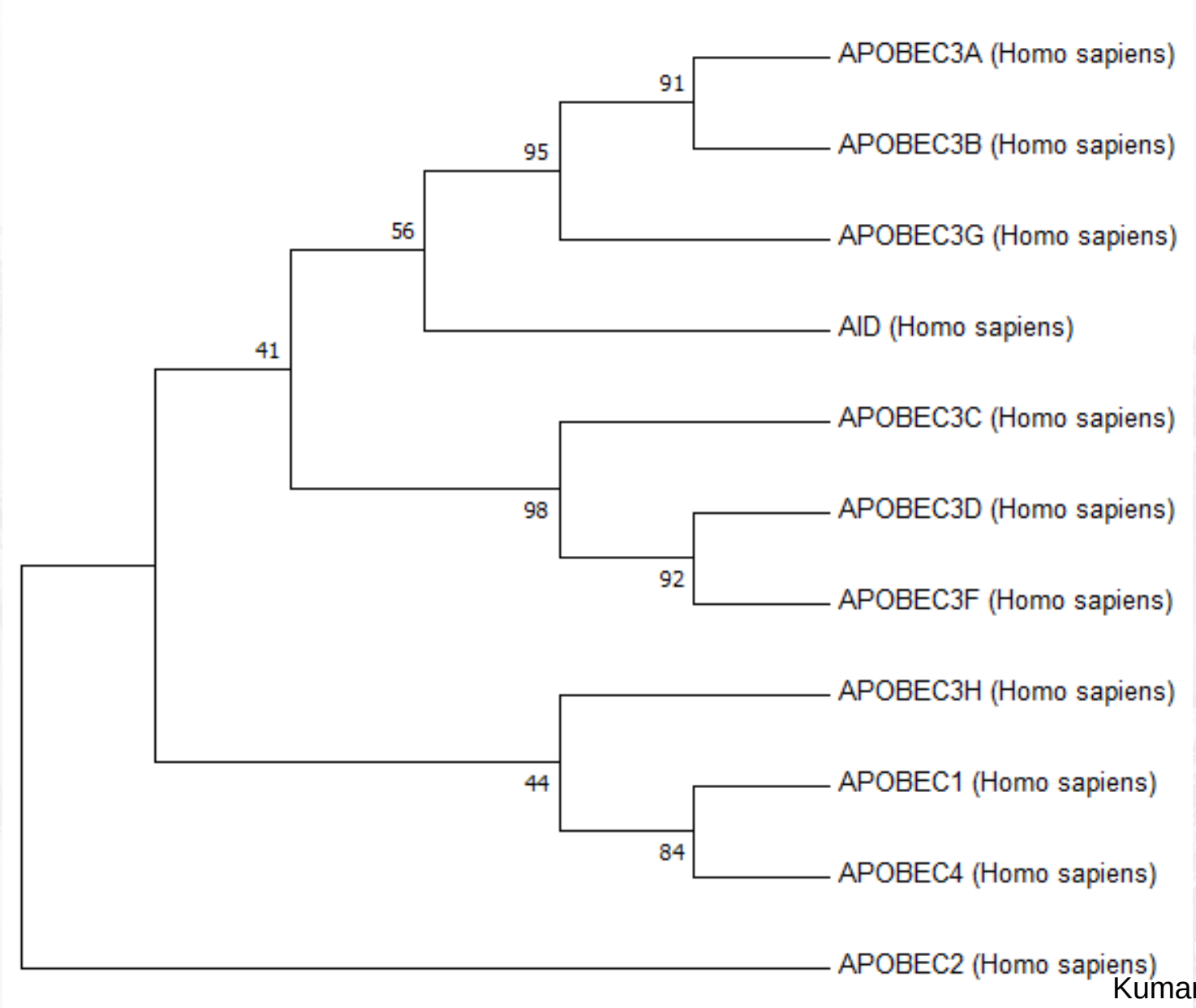
▸ Weight

Use Negative Matrix	OFF
Delay Divergent Cutoff (%)	30

Option		Setting
ANALYSIS		
Statistical Method	→	*Maximum Likelihood*
PHYLOGENY TEST		
Test of Phylogeny	→	*Bootstrap method*
No. of Bootstrap Replications	→	*200*
SUBSTITUTION MODEL		
Substitutions Type	→	*Amino acid*
Model/Method	→	*Jones-Taylor-Thornton (JTT) model*
RATES AND PATTERNS		
Rates among Sites	→	*Uniform Rates*
No of Discrete Gamma Categories	→	Not Applicable
DATA SUBSET TO USE		
Gaps/Missing Data Treatment	→	*Use all sites*
Site Coverage Cutoff (%)	→	Not Applicable
TREE INFERENCE OPTIONS		
ML Heuristic Method	→	*Nearest-Neighbor-Interchange (NNI)*
Initial Tree for ML	→	*Make initial tree automatically (Default - NJ/BioNJ*
Initial Tree File	→	Not Applicable
Branch Swap Filter	→	*None*
SYSTEM RESOURCE USAGE		
Number of Threads	→	*7*

Kumar, S., Stecher, G., and Tamura, K, 2016, Mol. Biol. Evol.

人类APOBEC家族系统发生树的构建



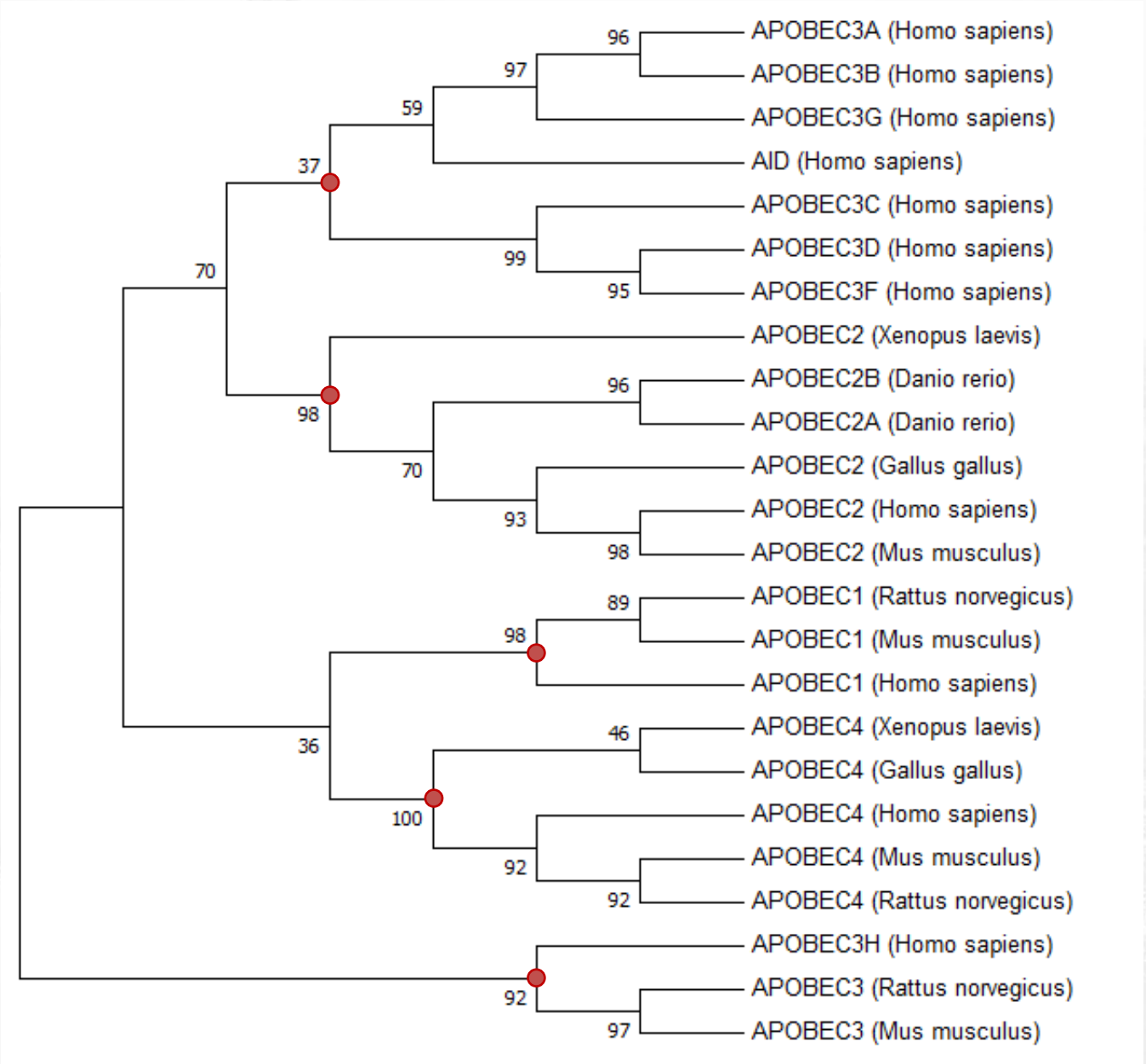
- 假设：亲缘关系越近的旁系同源体，功能和性质越相似，反之差异性越大
- APOBEC4和APOBEC1与APOBEC3A的亲缘关系最远，可以作为一个候选蛋白进行测试

Kumar, S., Stecher, G., and Tamura, K, 2016, Mol. Biol. Evol.

代表性物种间APOBEC家族系统发生树的构建

Species	Protein	Count	Source
斑马鱼（*Danio rerio*）	APOBEC2a APOBEC2b	2	UniProt
非洲爪蟾（*Xenopus laevis*）	APOBEC2 APOBEC4	2	
鸡（*Gallus gallus*）	APOBEC2 APOBEC4	2	
小鼠（*Mus musculus*）	APOBEC1 APOBEC2 APOBEC3 APOBEC4	4	
大鼠（*Rattus norvegicus*）	APOBEC1 APOBEC3 APOBEC4	3	

代表性物种间APOBEC家族系统发生树的构建



- 旁系同源蛋白基本上聚类在一起
- 来自大鼠的rAPOBEC1与人源的hAPOBEC3A也有着较远的亲缘关系，两者在甲基化序列上表现出的活性显著不同
- 可以尝试人源的APOBEC2或者小鼠来源的APOBEC2，因为这两者与rAPOBEC1和hAPOBEC3A有着最远的亲缘关系

Kumar, S., Stecher, G., and Tamura, K, 2016, Mol. Biol. Evol.

感谢聆听！

祝寒假快乐！