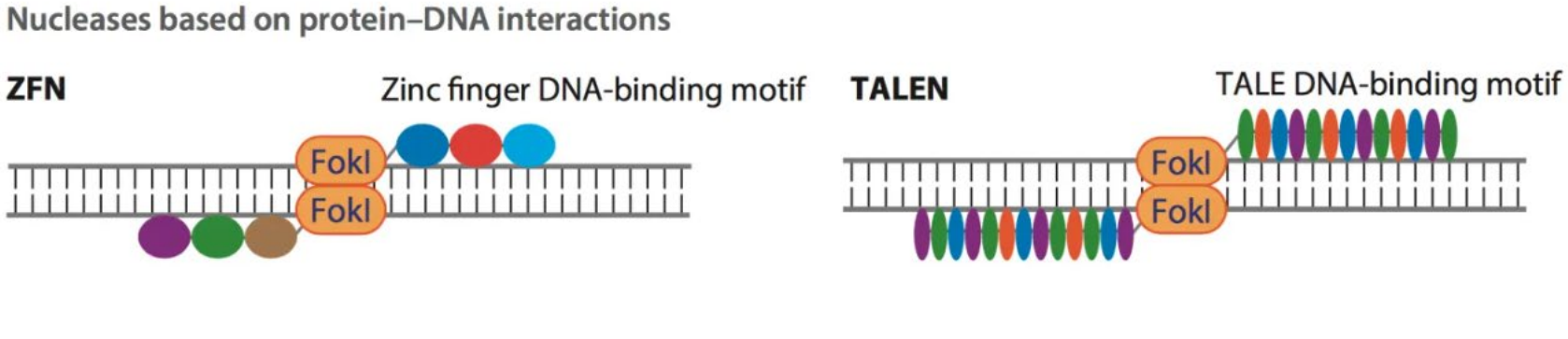
线粒体碱基编辑工具DdCBEs的优化

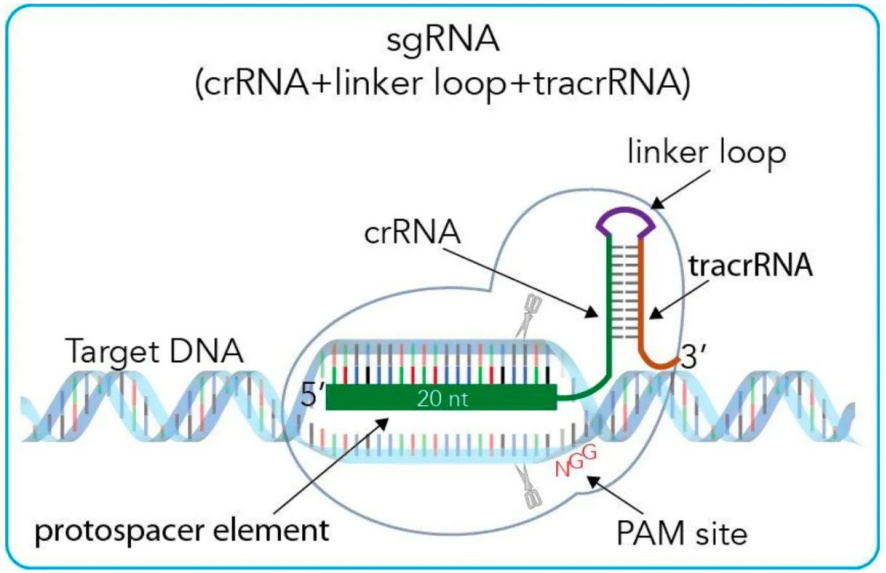
汇报人：魏晓旭
组员：叶远志、薛雨洲、翁永佳

基因编辑的发展历史

ZFN-TALEN 蛋白定位

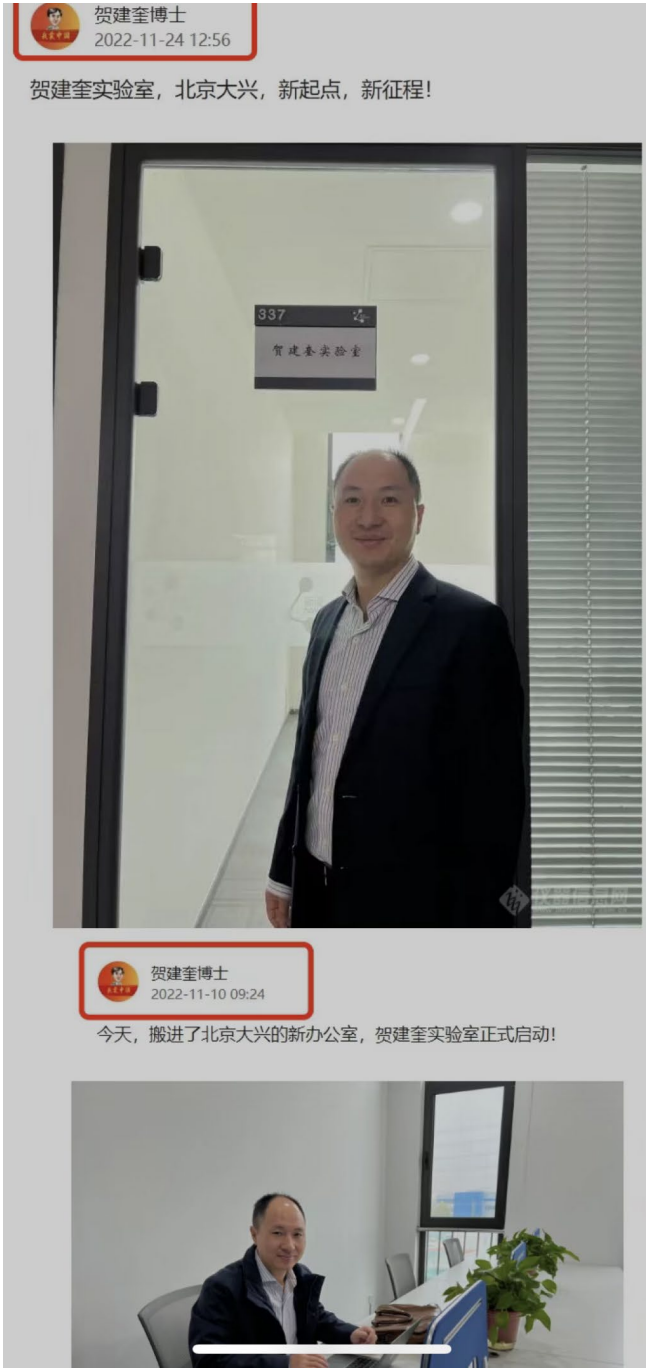


CRISPR RNA定位



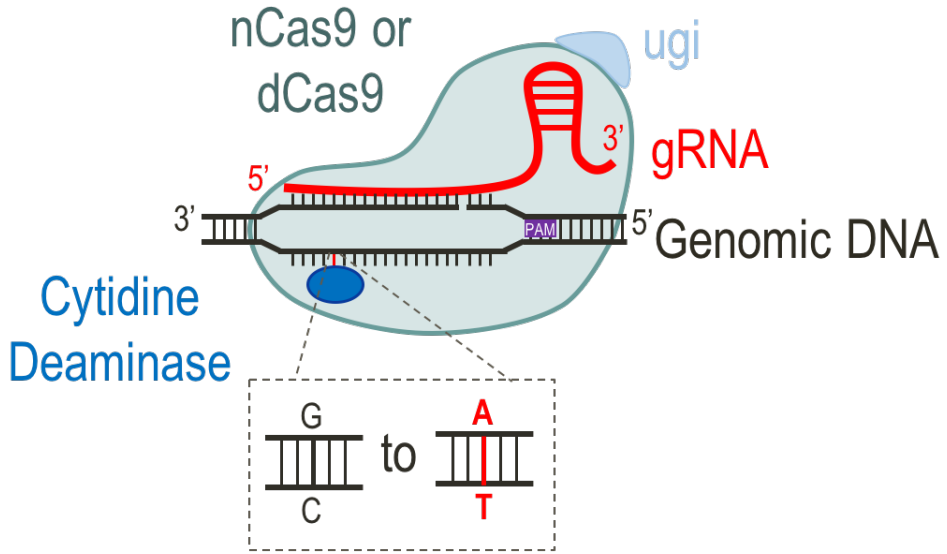
贺建奎事件

事实上，我感到很自豪。"贺建奎周三出席于香港举行的第二届人类基因组编辑国际峰会，在其声称编辑了人类胚
台并让婴儿出生后，首次回应外界的批评与质疑。会议上，有人质疑该事件的真实性，有人称其越过了伦理道德底
线，诺贝尔奖得主巴尔的摩(David Baltimore)批评说"科学界自我监管失败"。贺建奎并未透露实验细节，但称另一
名妇女也植入了经过基因编辑的胚胎。



碱基编辑器

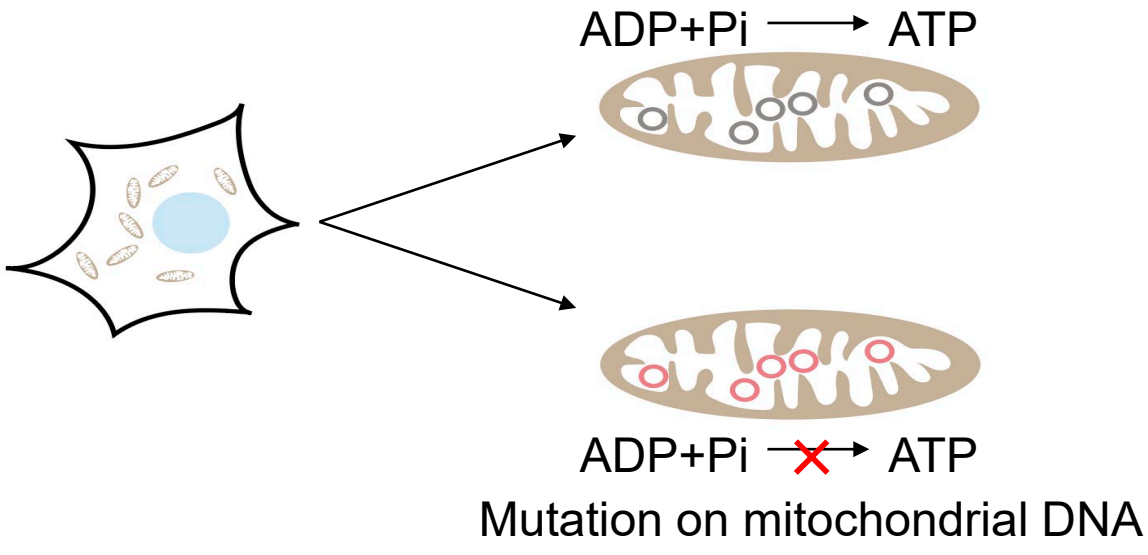
David R. Liu



传统的CRISPR/Cas9技术通过在靶点处**产生DNA双链断裂（DSB）**，从而诱发细胞内的同源重组（HDR）和非同源末端连接（NHEJ）**修复**途径，进而实现对基因组DNA的定点敲除、替换、插入等修饰。然而，DSB引发的DNA修复很难实现高效稳定的单碱基突变。

胞嘧啶脱氨酶，它的作用是将**胞嘧啶 (Cytosine, C)脱氨基变为尿嘧啶 (Uracil，U)**，在 DNA 复制过程中则变为胸腺嘧啶 (Thymine，T)，那么产生的结果就是碱基C变成了碱基T；同时，互补链上原来与C互补的鸟嘌呤(Guanine,G)将会替换为腺嘌呤(Adenine,A)，最终实现了在一定的活性窗口内C到T的编辑

线粒体相关疾病



- 16569bp，双链闭合环状DNA分子
- 编码呼吸链中的一些关键酶，影响细胞的能量代谢
- 母系遗传、多拷贝、高异质性及高变异率

Leber遗传性视神经病(LHON)：致病基因主要有MT-ND1, MT-ND4, MT-ND6，最常见的致病变异是位于MT-ND1的m.3460G>A,位于MT-ND4的m.11778G>A和位于MT-ND6的m.14484T>C。该病表现为青少年或成人无痛性视力丧失，相继影响两眼。极少的患者还可表现出运动障碍、震颤、心脏传导缺陷、肌肉无力、麻木、协调性差等症状。

Rahman, S. *J Intern Med* **287**, 609–633 (2020).

线粒体缺乏sgRNA的转运机制，不能使用CRISPR系统

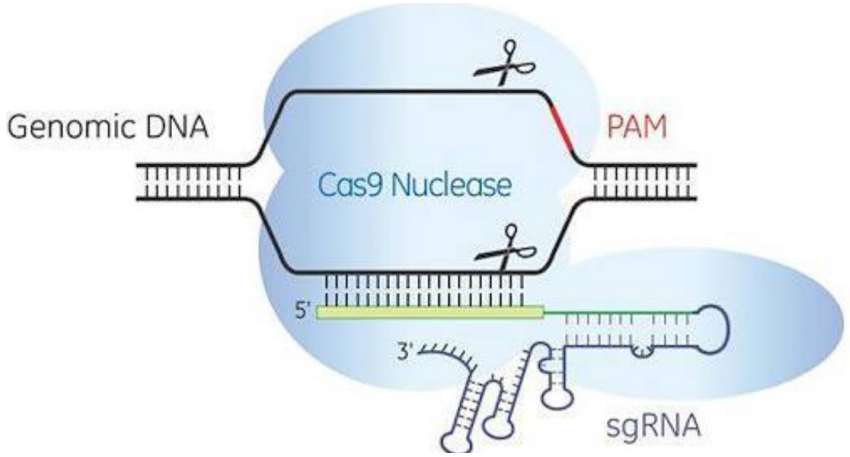
Trends in Genetics

CellPress
REVIEWS

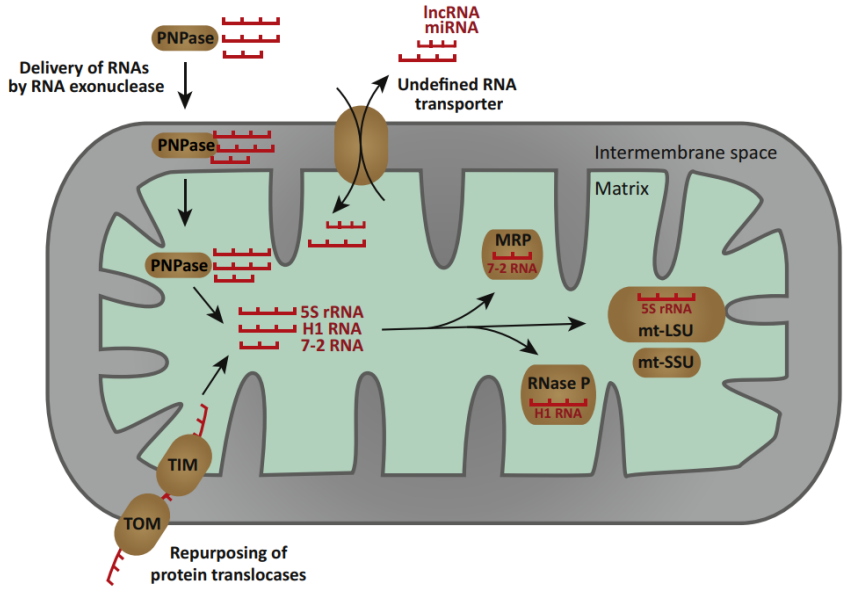
Opinion

Mitochondrial Genome Engineering: The Revolution May Not Be CRISPR-Ized

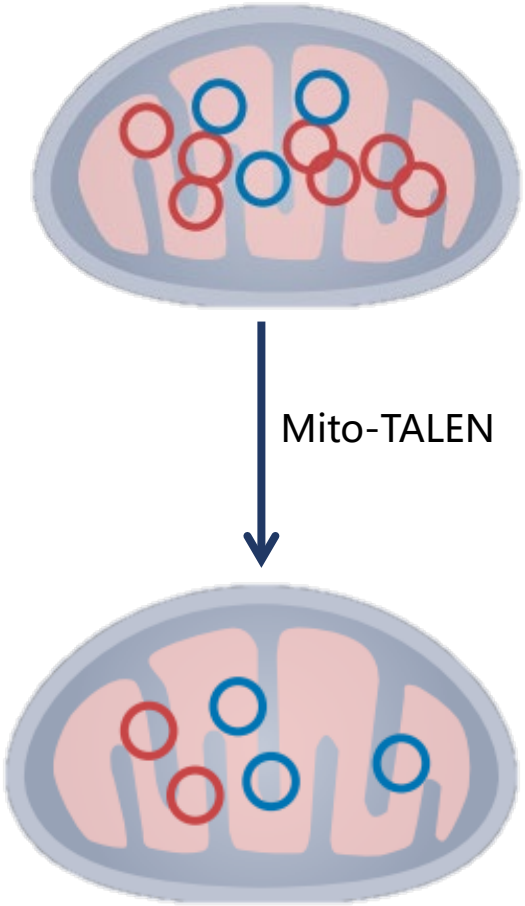
Payam A. Gammage,[1,*] Carlos T. Moraes,[2,*] and Michal Minczuk[1,*]

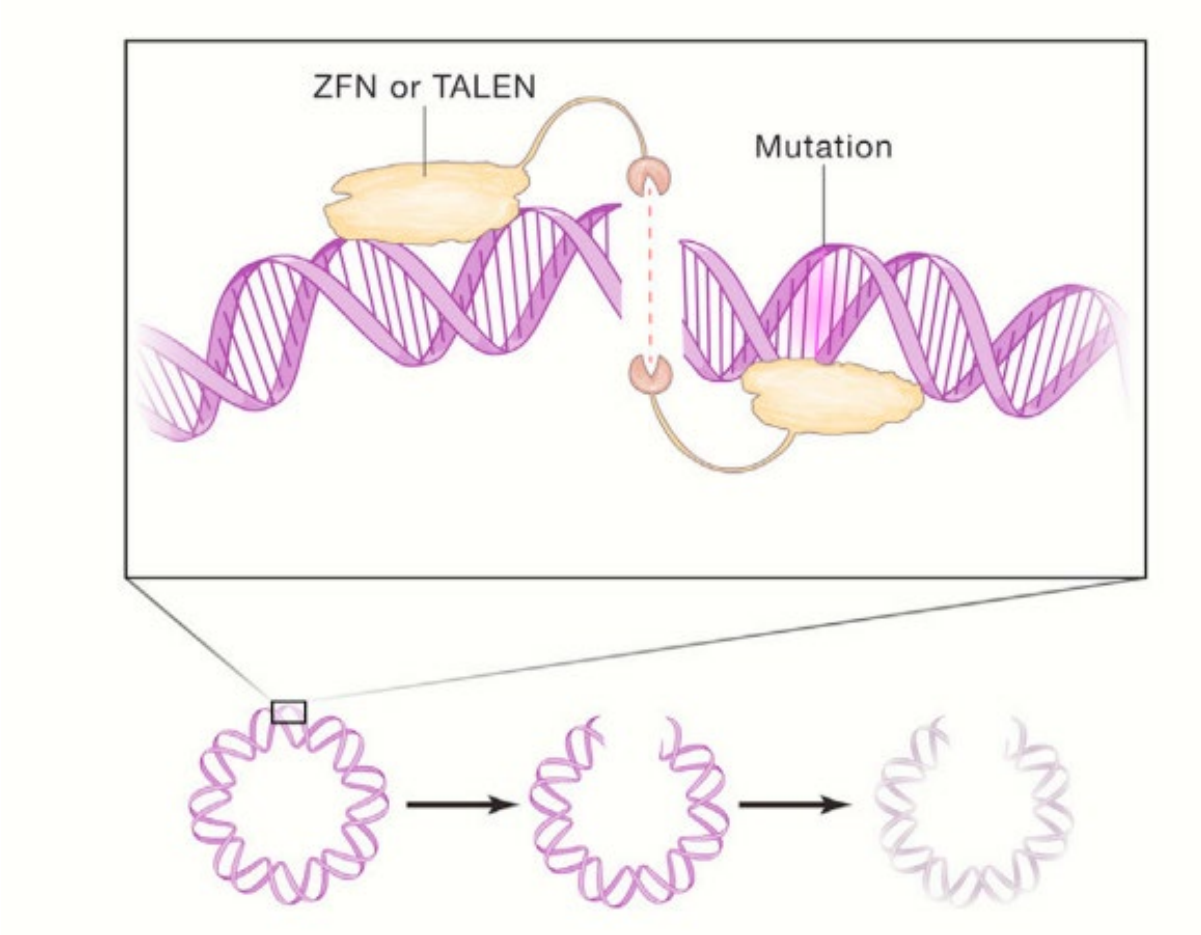


缺乏进入线粒体机制



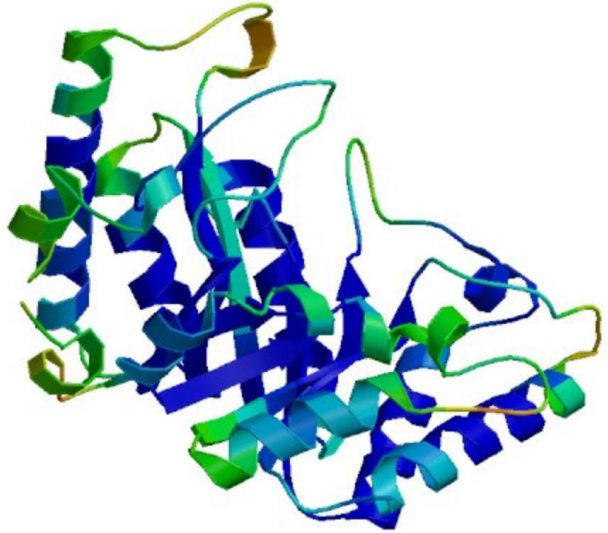
Mito-TALEN编辑会造成线粒体基因拷贝数减少



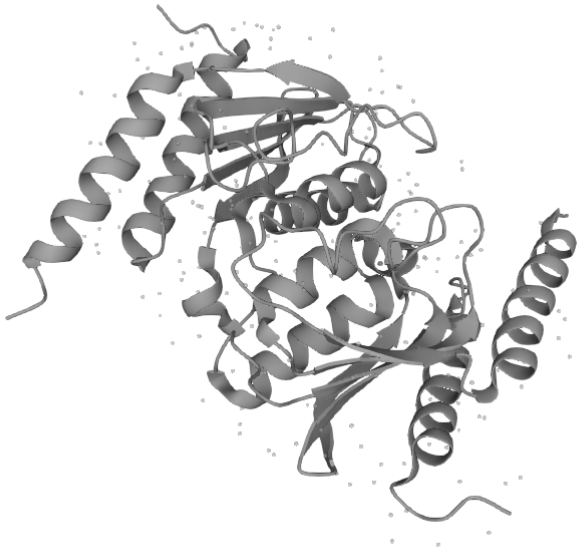


Russell, O.M., et al., Mitochondrial Diseases: Hope for the Future. Cell, 2020..

目前的脱氨酶底物是单链的DNA



APOBEC1（胞嘧啶脱氨酶）



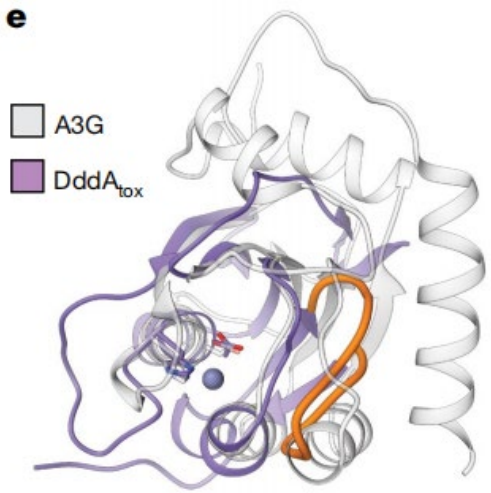
TadA(腺嘌呤脱氨酶)

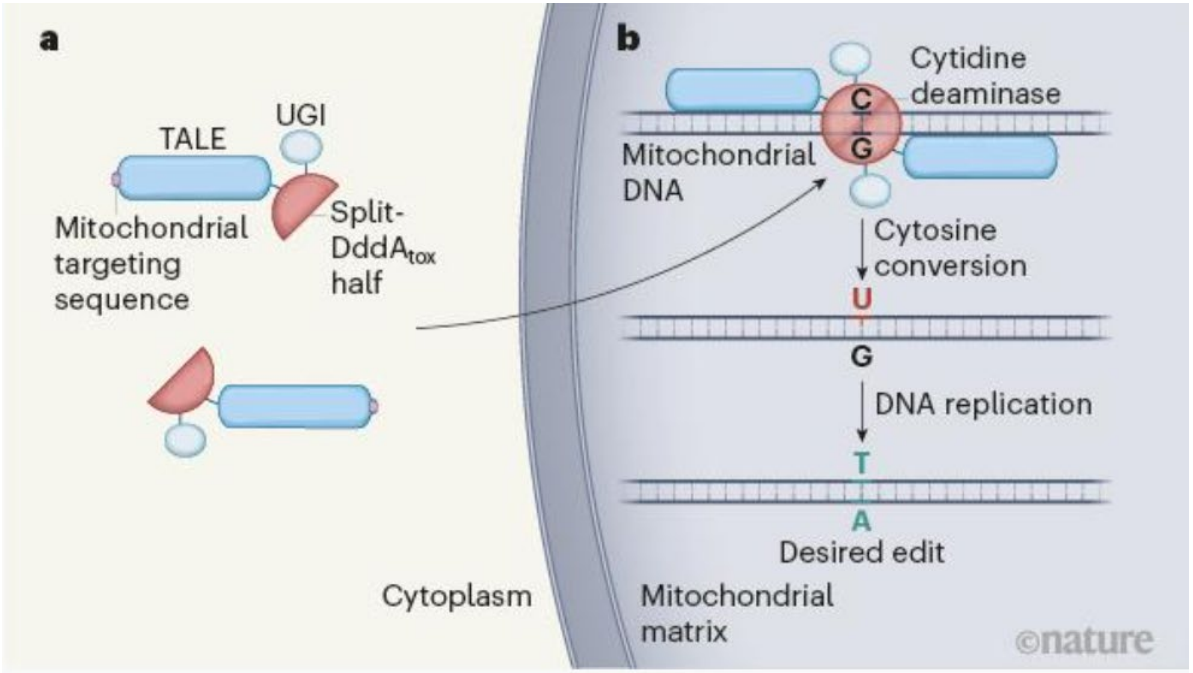
线粒体碱基编辑器DdCBE

A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing

Beverly Y. Mok, Marcos H. de Moraes, Jun Zeng, Dustin E. Bosch, Anna V. Kotrys, Aditya Raguram, FoSheng Hsu, Matthew C. Radey, S. Brook Peterson, Vamsi K. Mootha, Joseph D. Mougous ✉ & David R. Liu ✉

Nature **583**, 631–637 (2020) | Cite this article





Uniprot : DddA蛋白

P0DUH5 · DDDA_BURC1

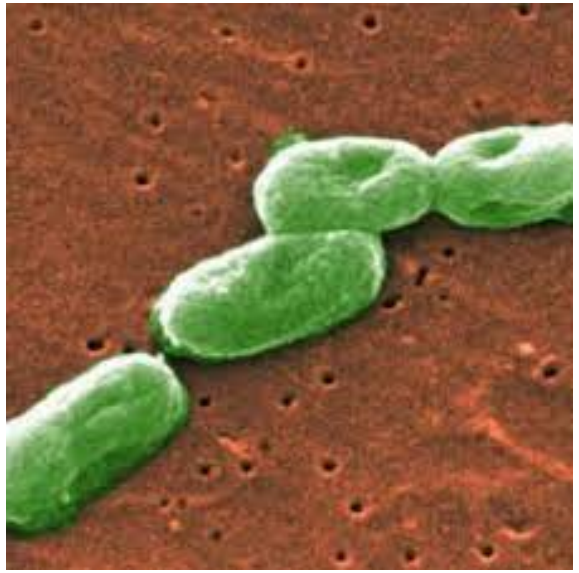
Protein[i]	Double-stranded DNA deaminase toxin A	Amino acids	1427
Gene[i]	dddA	Protein existence[i]	Evidence at protein level
Status[i]	UniProtKB reviewed (Swiss-Prot)	Annotation score[i]	5/5
Organism[i]	Burkholderia cenocepacia (strain H111)		

Entry | Feature viewer | Publications | External links | History

BLAST | Download | Add | Add a publication | Entry feedback

DddA : Double-stranded DNA deaminase toxin A　双链DNA脱氨酶(胞嘧啶脱氨酶)

DddA的来源



Burkholderia cenocepacia
伯克霍尔德菌：棒状革兰氏阴性菌

T6SS-delivered antibacterial toxin：六型细菌毒素分泌系统分泌的细菌毒素：VI型分泌系统是一种高度保守的系统，是把效应蛋白直接一步注射到目的细胞的蛋白分泌系统，广泛存在于革兰氏阴性菌中（约1/4），T6SS是膜内的一个完整的分泌装置，它以接触依赖的方式将将毒性效应物质传递给真核细胞和原核细胞,其效应子细胞壁降解酶、细胞膜靶向蛋白（磷脂酶和成孔蛋白）以及核酸酶

DddA结构与功能

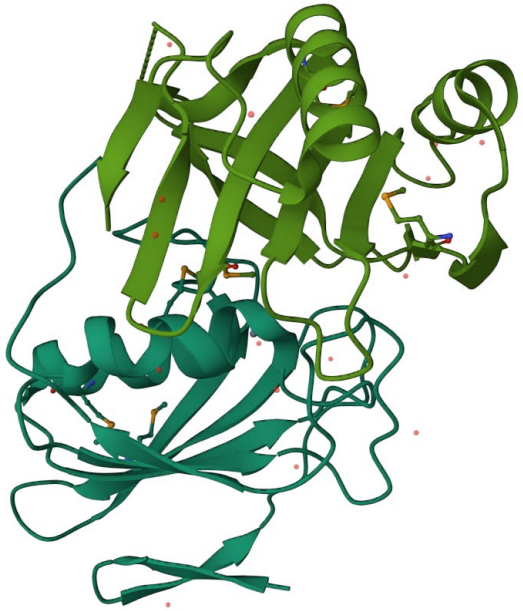
Uniprot中DddA的结构信息

PDB	6U08	X-ray	2.49 Å	A/C/E/G	1261-1427	PDBe · RCSB-PDB · PDBj · PDBsum
AlphaFold	AF-P0DUH5-F1	Predicted			1-1427	AlphaFold

3D structure databases

AlphaFoldDB	P0DUH5	ModBase	Search...
SMR	P0DUH5	PDBe-KB	Search...

DddA结构与功能



Display Files ▾ Download Files ▾

6U08

Double-stranded DNA-specific cytidine deaminase type VI secretion system effector and cognate immunity complex from Burkholderia cenocepacia

PDB DOI: 10.2210/pdb6U08/pdb

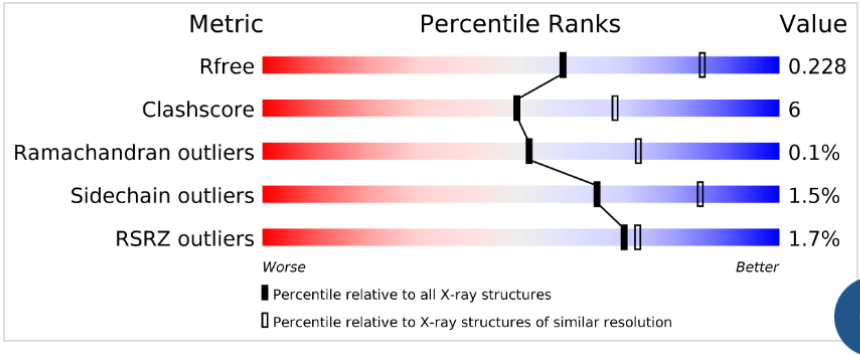
Classification: TOXIN
Organism(s): Burkholderia cenocepacia
Expression System: Escherichia coli
Mutation(s): No

Deposited: 2019-08-13 **Released:** 2020-07-15
Deposition Author(s): Bosch, D.E., de Moraes, M.M.H., Mougous, J.D.
Funding Organization(s): National Institutes of Health/National Institute Of Allergy and Infectious Diseases (NIH/NIAID)

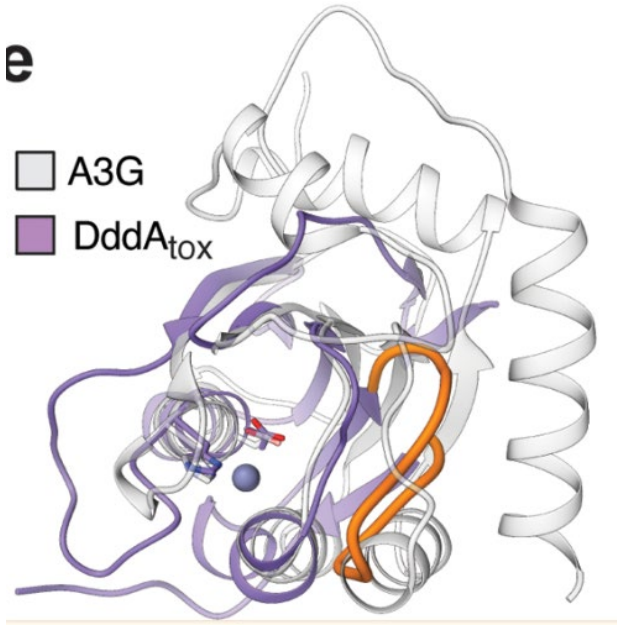
Experimental Data Snapshot

Method: X-RAY DIFFRACTION
Resolution: 2.49 Å
R-Value Free: 0.228
R-Value Work: 0.172
R-Value Observed: 0.174

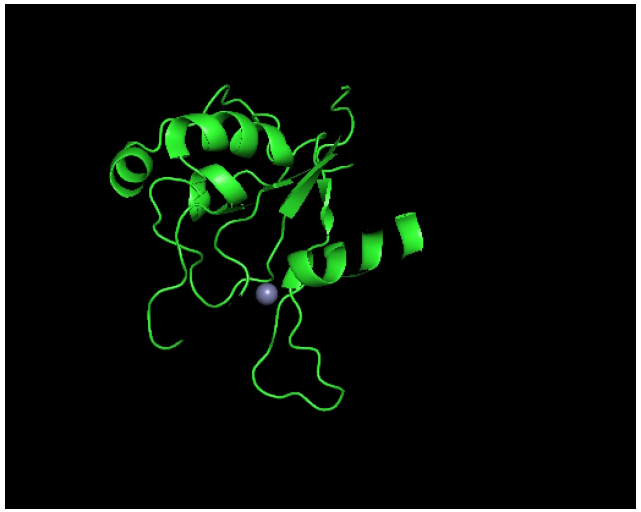
wwPDB Validation 3D Report | Full Report

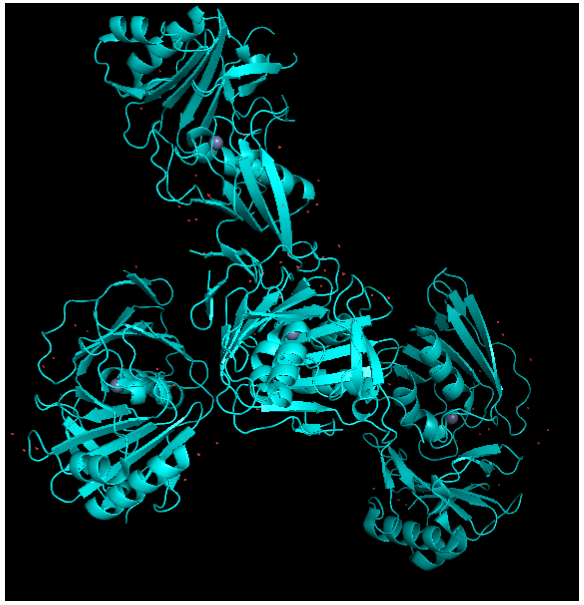


DddA 与APOBEC1相似程度对比

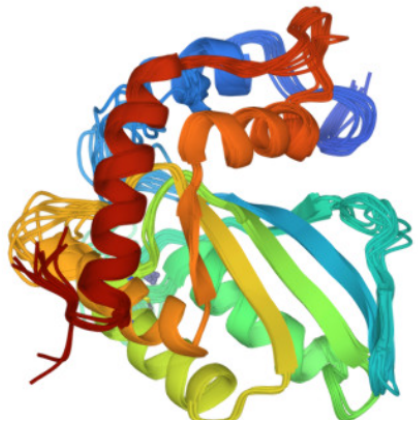


pymol





NMR Ensemble



Display Files | Download Files

2KEM

Extended structure of citidine deaminase domain of APOBEC3G

PDB DOI: 10.2210/pdb2KEM/pdb

Classification: HYDROLASE
Organism(s): Homo sapiens
Expression System: Escherichia coli
Mutation(s): Yes

Deposited: 2009-01-30 **Released:** 2009-06-02
Deposition Author(s): Harjes, E., Gross, P.J., Chen, K., Lu, Y., Shindo, K., Nowarski, R., Gross, J.D., Kotler, M., Harris, R.S., Matsuo, H.

谢谢！