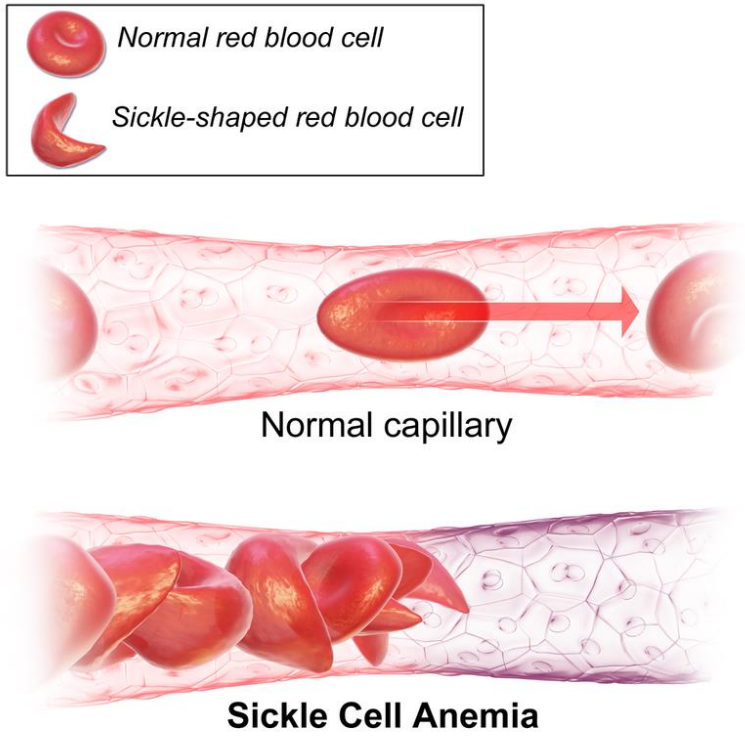
CRISPR技术的应用

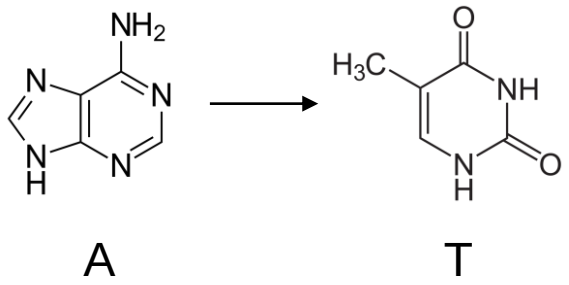
简要介绍DNA Base Editors的原理

张伟平
化生91

Point Mutation and Monogenetic Disorders

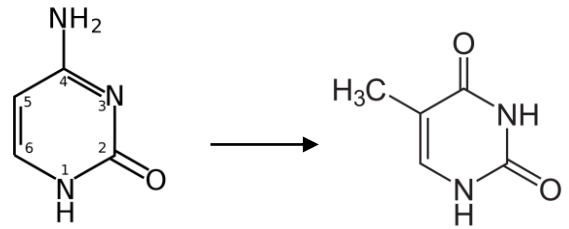


Sickle Cell Anemia



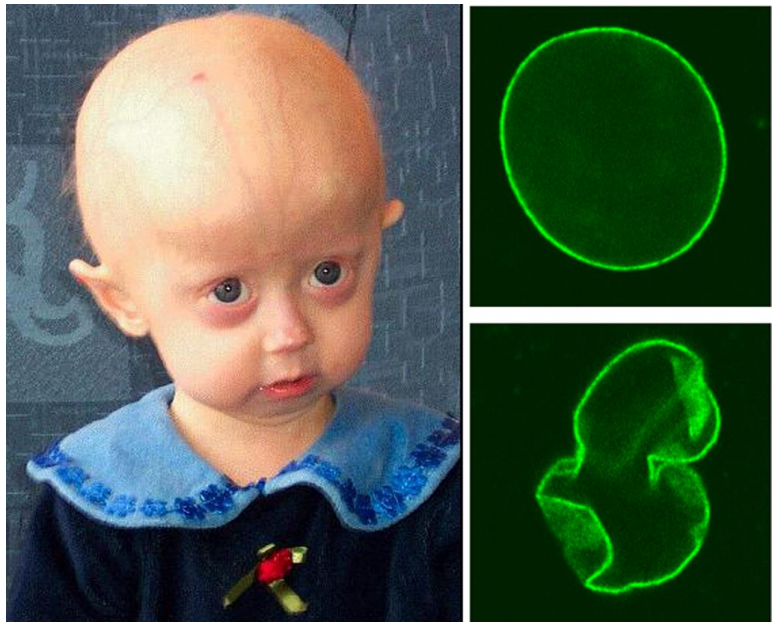
A T

Sickle Cell Anemia



C T

Progeria



Progeria

From Wikipedia

CRISPR/Cas9系统编辑DNA单碱基的缺点

- **NHEJ(Non-Homologous End Joining)**和**HDR(Homology Directed Recombination)**在DSB(Double Strains Break)形成之后相互竞争，使得同源模板插入/置换的效率降低（0.1%~5%）
- NHEJ易导致DNA断裂处碱基的插入或删除(indels)
- HDR需要引入额外的模板片段

Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A. & Liu, D. R. *Nature* 533, 420–424 (2016).

什么是Base Editors?

“Being a Chemist, I began working with my students to develop ways to **performing chemistry directly on an individual DNA base**”

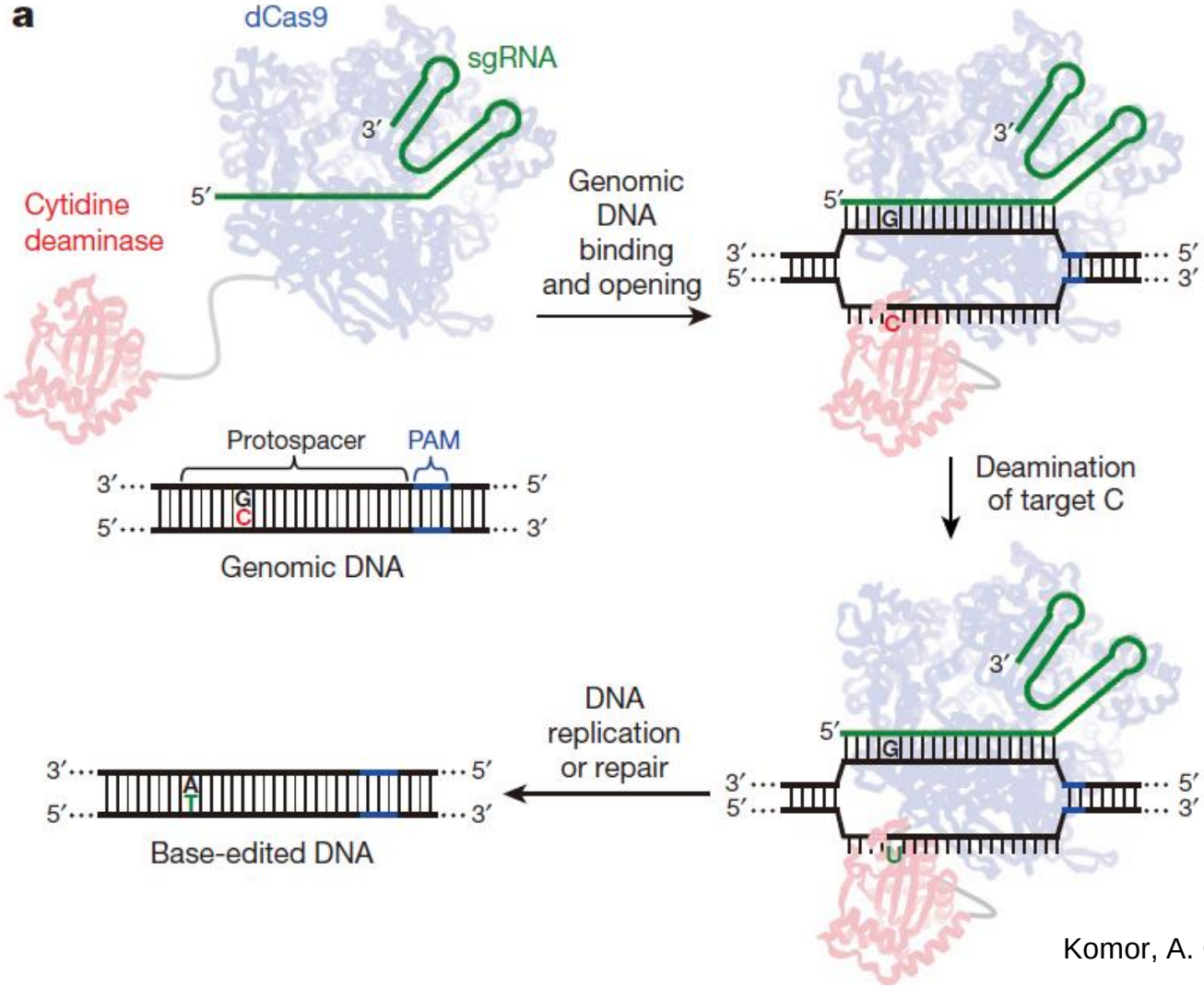
“CRISPR is like scissors, and base editors are like pencils”

——By David Liu

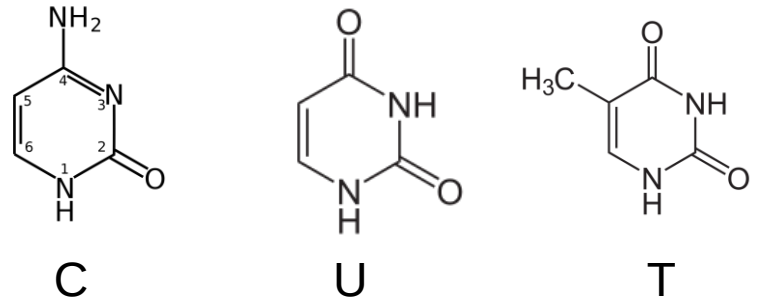
https://www.liugroup.us/

Prof. David R. Liu (刘如谦),
Chemical biologist

First C->T Base Editor (BE1)



- Cytidine deaminase (胞苷脱氨酶): rat APOBEC1, 催化mRNA的C—>U
- dCas9: 失去DNA断裂双键能力的Cas9



Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A. & Liu, D. R. *Nature* 533, 420–424 (2016).

第一代Base Editor的缺点

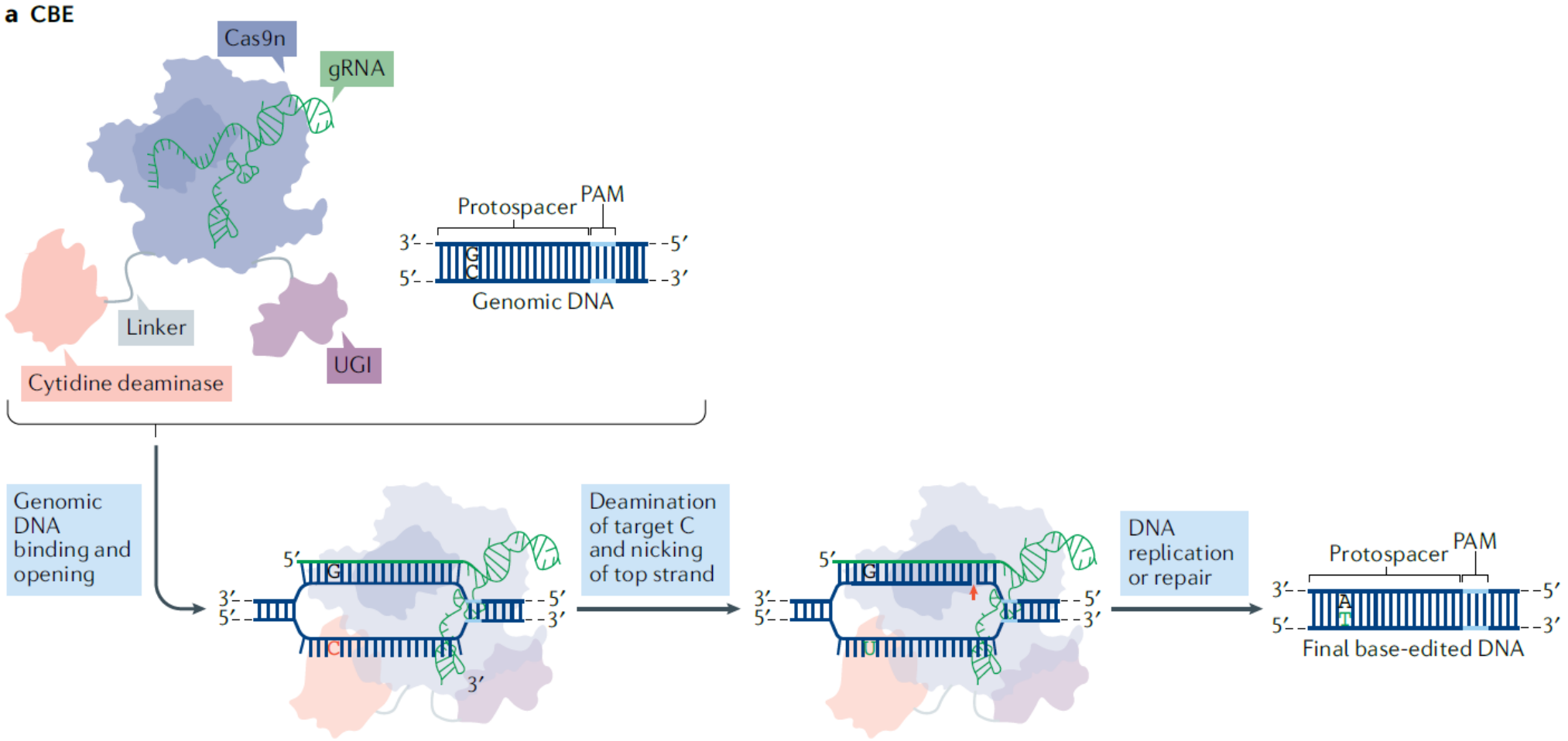
1. BE1在完成C·G>U·G后，依靠细胞自带的错配修复机制进行U·G>T·A，该过程效率低
2. 真核细胞DNA中含有U时，UNG(尿嘧啶DNA糖苷酶)会在U处切断N-糖苷键以移除U，从而激活DNA损伤修复机制，通常是将U·G修复成C·G，进一步降低U·G>T·A效率
3. UNG移除U后，AP lyase会切割DNA双链得到DSB，因此增大了indels的概率

Updated C->T Base Editor (BE2~BE4)

- APOBEC1: 一种胞苷脱氨酶
- Cas9n(Cas9 Nickase): 相较于dCas9，保留了HNH的活性，RuvC仍失活，从而切割非编辑链，引发细胞DNA损伤修复机制以移除G
- UGI(Uracil N-glycosylase Inhibitor): 尿嘧啶DNA糖苷酶(UNG)抑制蛋白，UNG可以切割DNA中的尿嘧啶处的糖苷键，UGI通过抑制UNG生物活性从而保护产物尿嘧啶
- Gam: μ噬菌体线性DNA末端保护蛋白，可以保护DSB末端，以减少indels

Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A. & Liu, D. R. *Nature* **533**, 420–424 (2016).
Komor, A. C. *et al. Sci. Adv.* **3**, 1–10 (2017).

Updated C->T Base Editor (BE2~BE4)

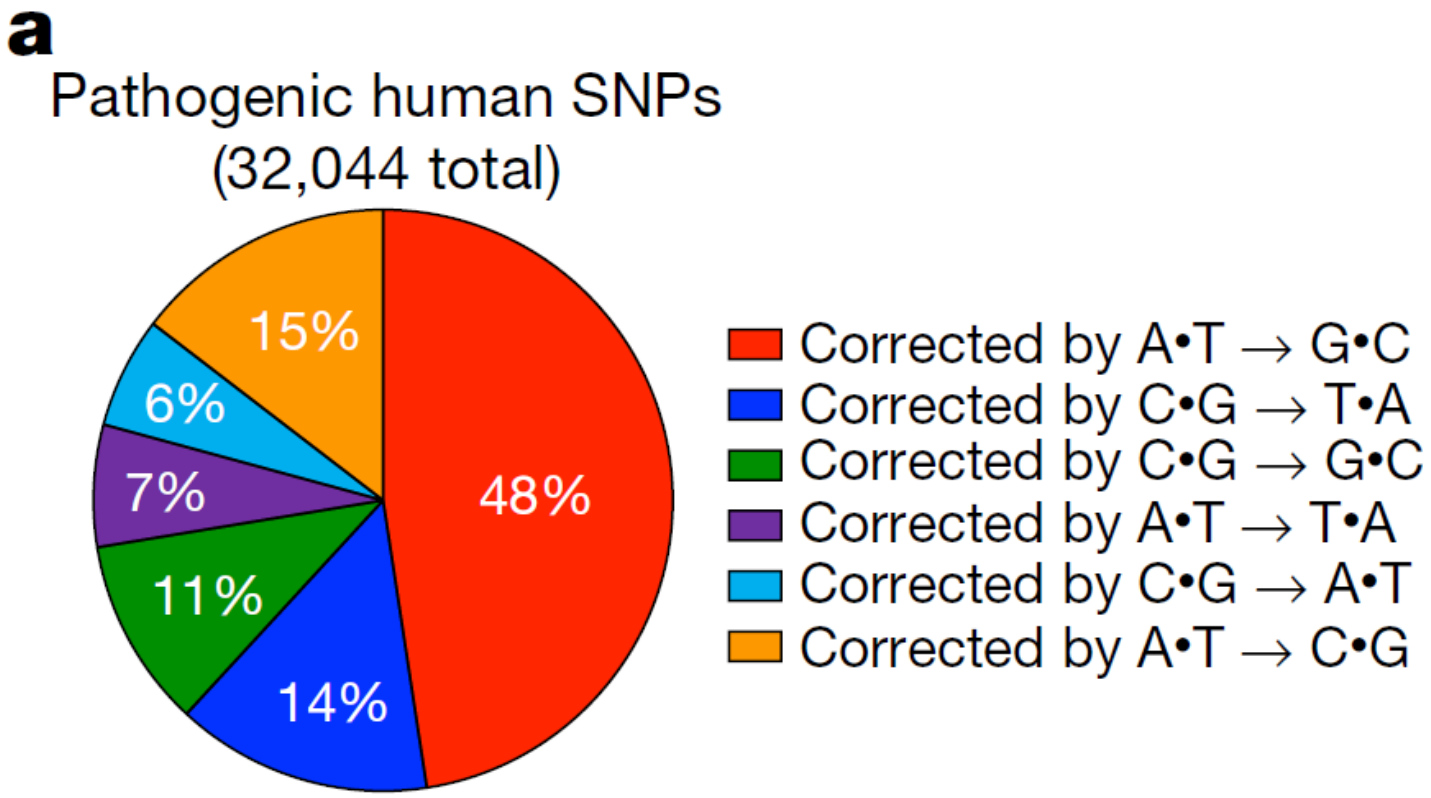


Porto, E. M., Komor, A. C., Slaymaker, I. M. & Yeo, G. W. *Nat. Rev. Drug Discov.* **19**, 839–859 (2020).

Base Editor的特点

1. 保留DNA骨架，不造成DSBs
2. 通过化学修饰的方法直接对碱基进行化学修饰
3. 化学修饰得到的碱基往往不是目标碱基，需要细胞自身进一步处理

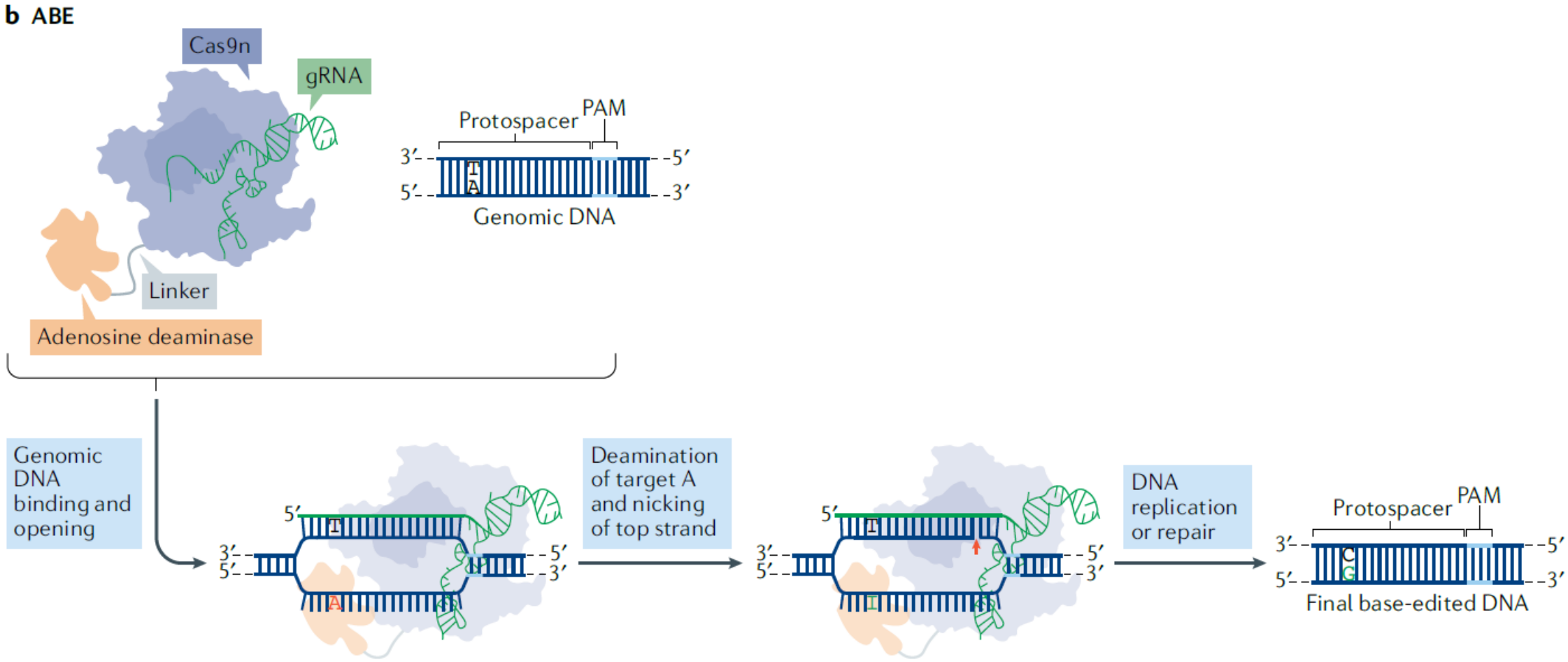
所有可能的Base Editors



根据SNP的所有种类，可以推断出一共需要6种Base Editor才可以解决所有碱基编辑问题

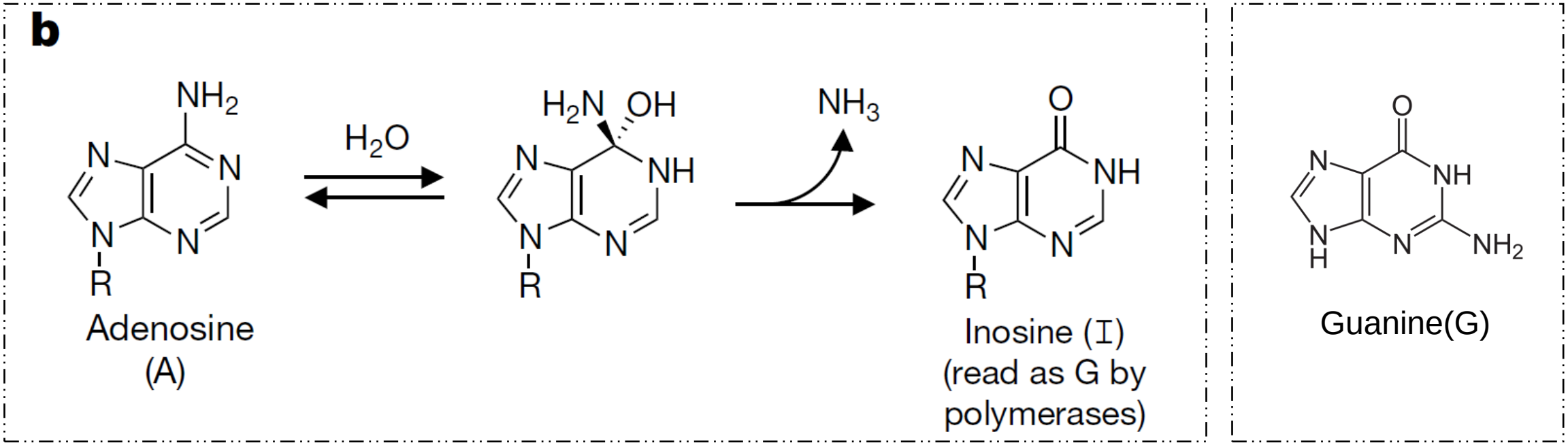
Gaudelli, N. M. *et al. Nature* **551**, 464–471 (2017).

A->G Base Editor (ABE)



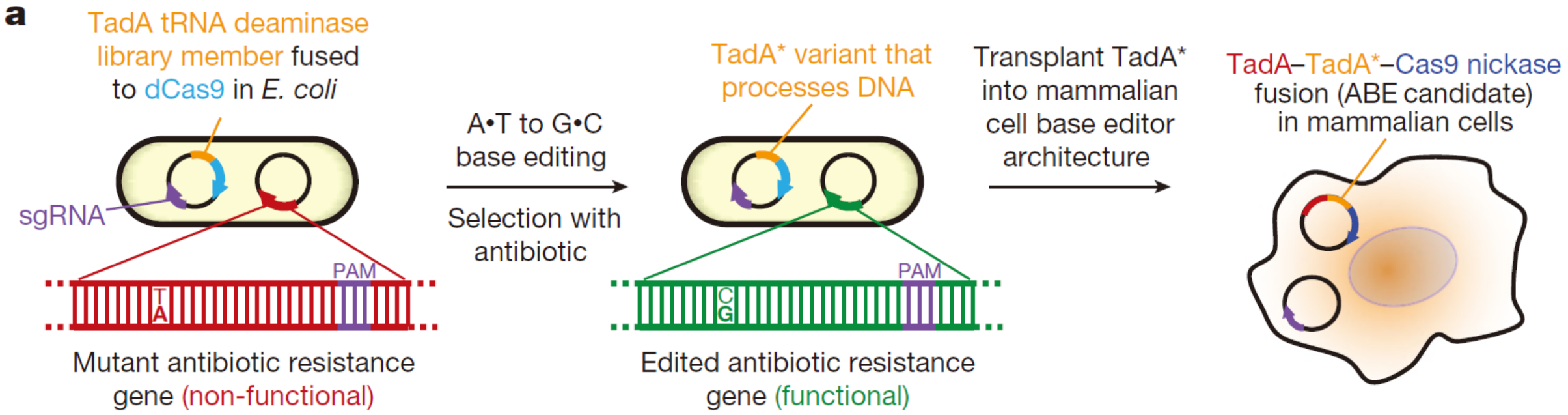
Porto, E. M., Komor, A. C., Slaymaker, I. M. & Yeo, G. W. *Nat. Rev. Drug Discov.* **19**, 839–859 (2020).

A->G Base Editor(ABE)



问题：自然中缺少直接将DNA的A变成G或者I的酶，已有的文献(截至2017)只报道了催化**游离的腺嘌呤(Adenine)**、**RNA的腺苷(Adenosine)**、**RNA-DNA双链中的腺苷(Adenosine)**水解脱氨的酶

定向进化获得催化DNA中A->I的酶

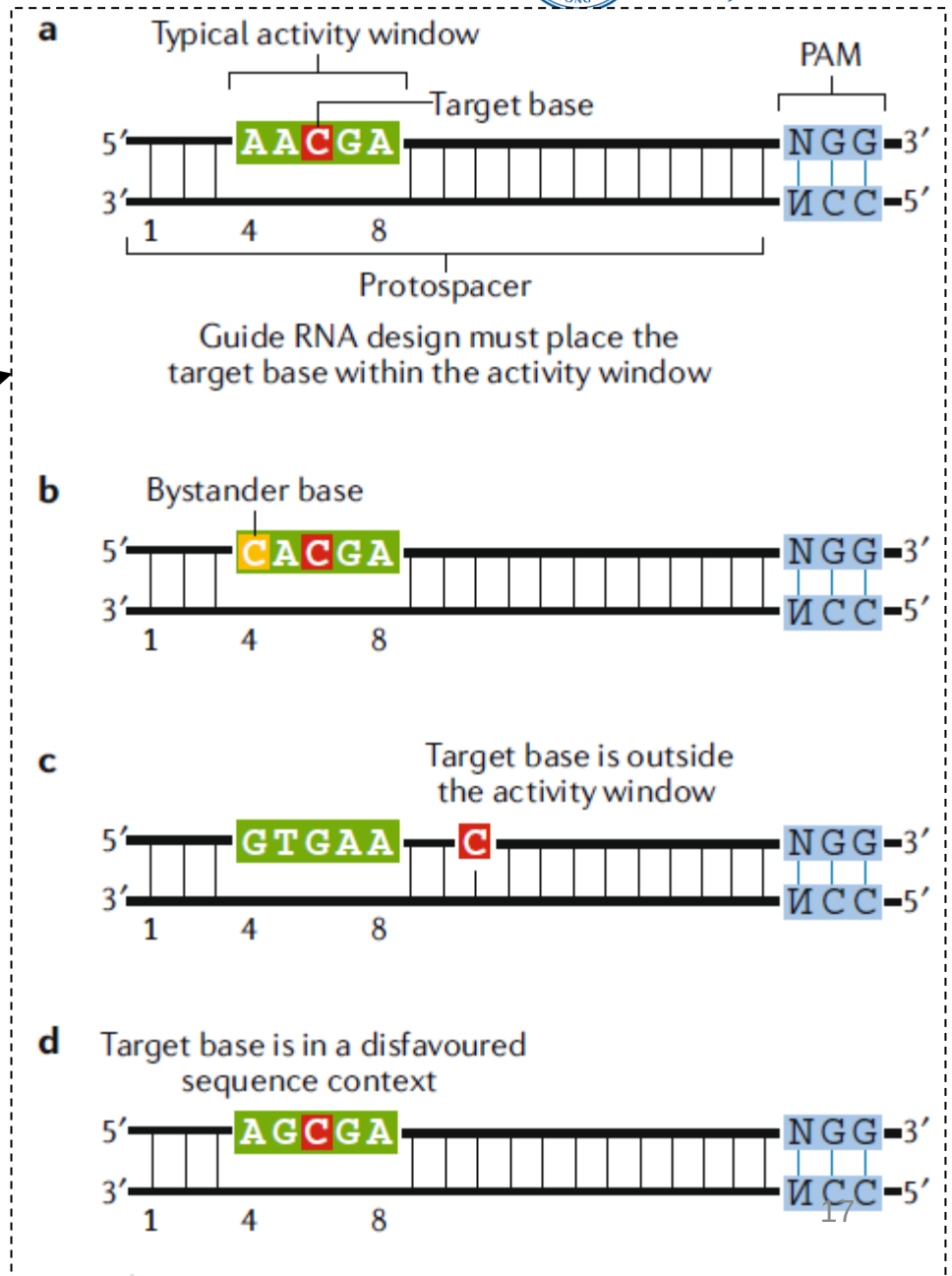


TadA：一种tRNA腺嘌呤脱氨酶，催化$tRNA^{Arg}$反密码子区的A->I

Gaudelli, N. M. *et al. Nature* **551**, 464–471 (2017).

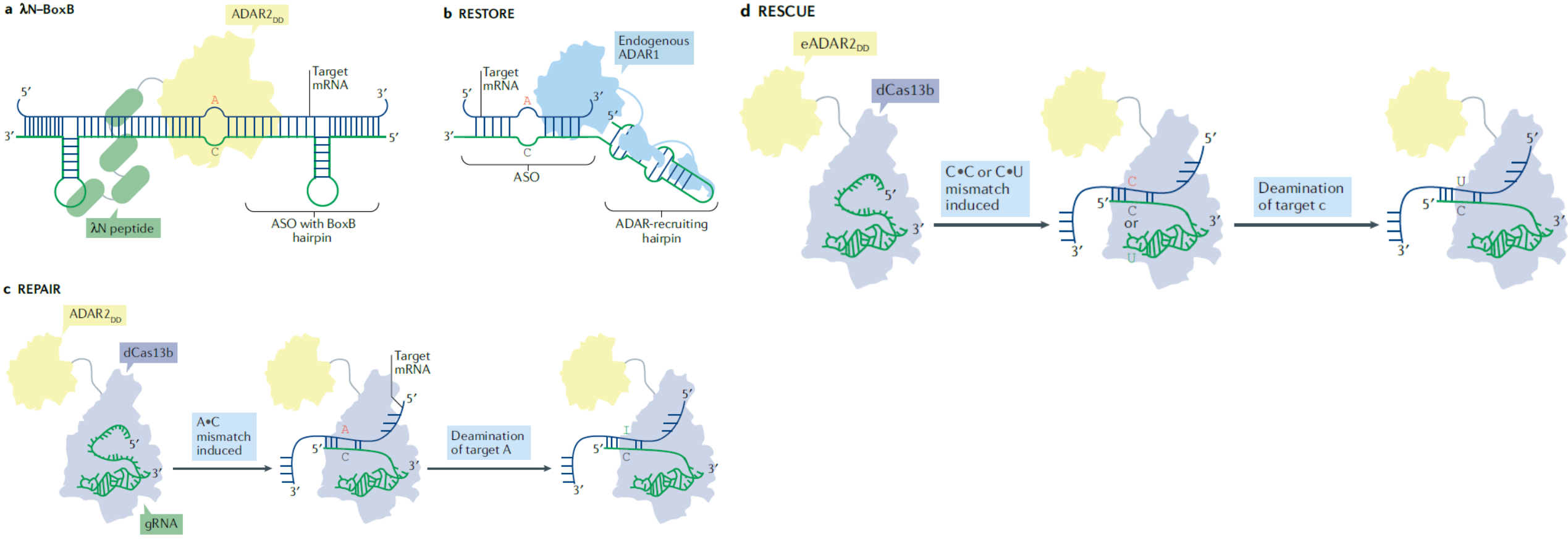
Base Editor的不足与提升方向

1. 编辑效率与产物纯度
2. Indels的产生
3. Base Editor的脱靶
4. PAM位点的识别
5. 编辑窗(Editing Window)和旁观者编辑(Bystander Edits)
6. ……



Rees, H. A. & Liu, D. R. *Nat. Rev. Genet.* **19**, 770–788 (2018).

RNA Base Editors



Porto, E. M., Komor, A. C., Slaymaker, I. M. & Yeo, G. W. *Nat. Rev. Drug Discov.* **19**, 839–859 (2020).

参考/推荐资料

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1. Rees, H. A. & Liu, D. R. Base editing: precision chemistry on the genome and transcriptome of living cells. *Nat. Rev. Genet.* **19**, 770–788 (2018).
2. Porto, E. M., Komor, A. C., Slaymaker, I. M. & Yeo, G. W. Base editing: advances and therapeutic opportunities. *Nat. Rev. Drug Discov.* **19**, 839–859 (2020).

BE1~3

3. Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A. & Liu, D. R. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* **533**, 420–424 (2016).

BE4

4. Komor, A. C. *et al.* 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**, 1–10 (2017).

ABE

5. Gaudelli, N. M. *et al.* Programmable base editing of T to G C in genomic DNA without DNA cleavage. *Nature* **551**, 464–471 (2017).

提高Cas9对PAM的兼容性

6. Hu, J. H. *et al.* Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. *Nature* **556**, 57–63 (2018).

Protocols

7. Huang, T. P., Newby, G. A. & Liu, D. R. Precision genome editing using cytosine and adenine base editors in mammalian cells. *Nat. Protoc.* **16**, 1089–1128 (2021).

David Liu TED Talk

8. David R. Liu: Can we cure genetic diseases by rewriting DNA? | TED Talk