Base editing and its implications in herbicide tolerance

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1. Need of herbicide tolerance: A case study
2. Basic mechanism and history of CRISPR/Cas9
3. Base editing : what and why?
4. Types of base editing and case studies: Cytosine base editing, adenine base editing and dual base editing
5. Shortcomings and conclusion

Why herbicide tolerance ? A story…

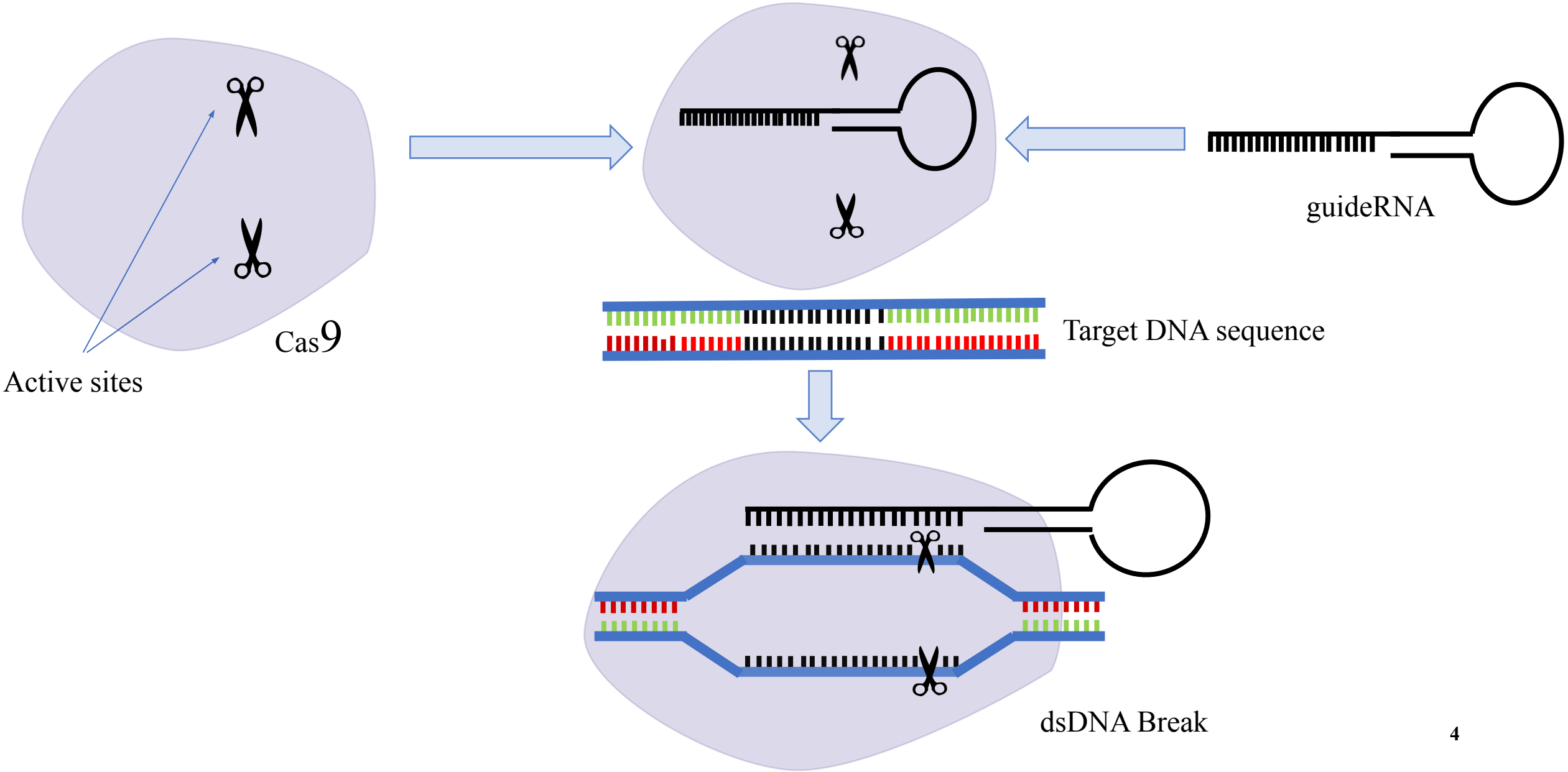
- For control of Jointed Goatgrass (Weed) Imidazolinone (IMI) is used
- IMI herbicide persist in soil causing heavy loss to crops such as sugarbeet, tomato and chilli
- Its persistence may vary for 90 to 360 days as per soil conditions due to this multi harvest from a same field in a same year is affected

Jointed Goatgrass

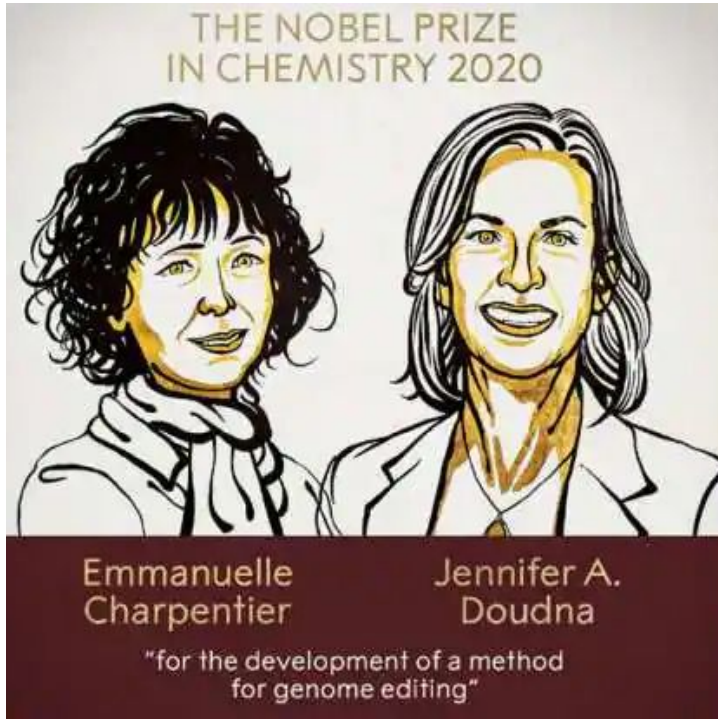
- New herbicides are required with less persistence in soil and crops are required to make resistant to new low persistence herbicide
- **METSULFURON**, a Sulfonyl urea also cause harm to wheat
- **What is next??**

Zhang et al (2019), *Nature plants*

Basic mechanism of CRISPR/Cas9



Contributors of the technology



Feng Zhang

Francisco Mojica

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Kira Makarova

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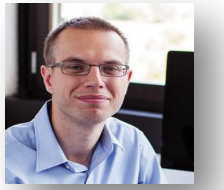
Philippe Horvath

Yoshizumi Ishino



Martin Jínek

Rodolphe Barrangou

Gilles Vergnaud

Sylvain Moineau

David Liu

Brief timeline

1993 — Discovery of CRISPR

2003 — CRISPR is an adaptive immune

2010 — 1. Cas9 is guided by crRNA
2. Discovery of tracrRNA

June,2012 — CRISPR can be programmed and customised, proved *invitro*

Jan,2013 — Genome editing in mammalian cells

2016 — Base editing

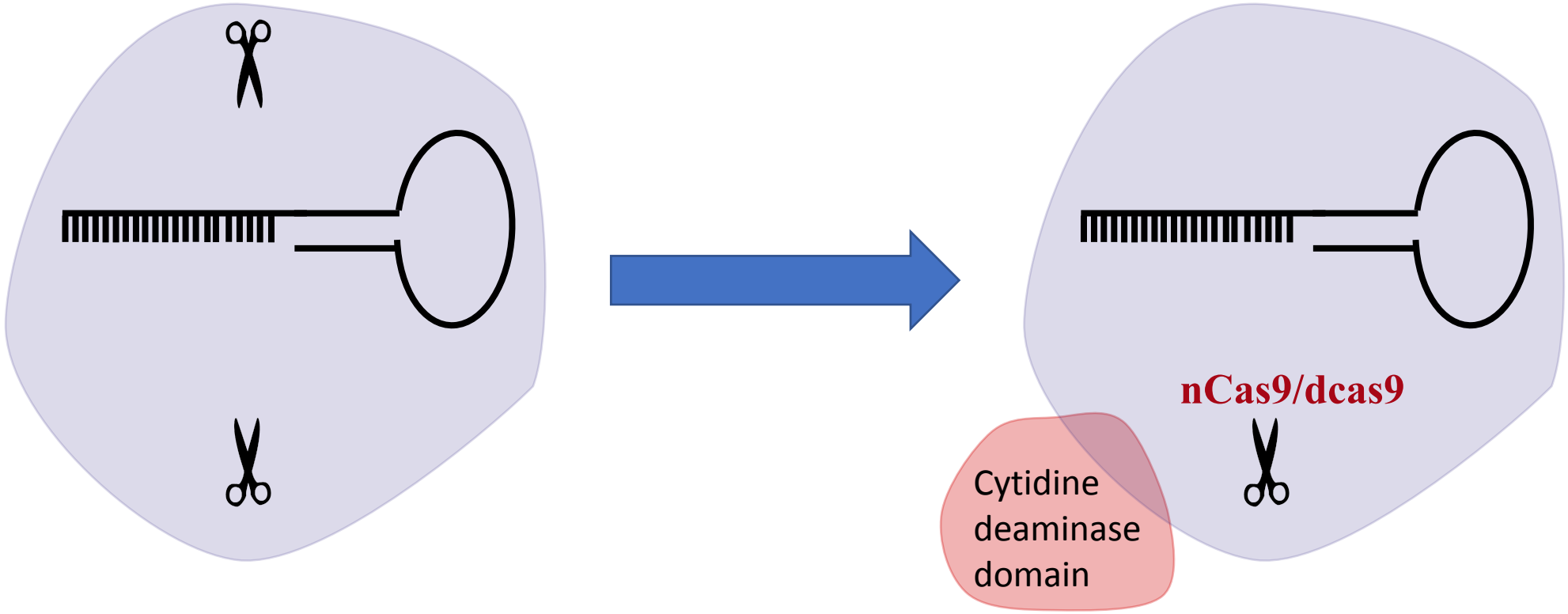
2019 — Prime editing

What and why base editing?

- It is capable of introducing **high frequency of substitution mutation** which is in contrast to conventional genome editing
- Base editing involves **single strand breaks** whereas conventional genome editing involve double strand breaks which generally leads to undesirable insertion and deletions
- Base editing is **more precise and error free** as compared to conventional genome editing
- Base editing does not require **donor DNA template**

CYTOSINE BASE EDITING

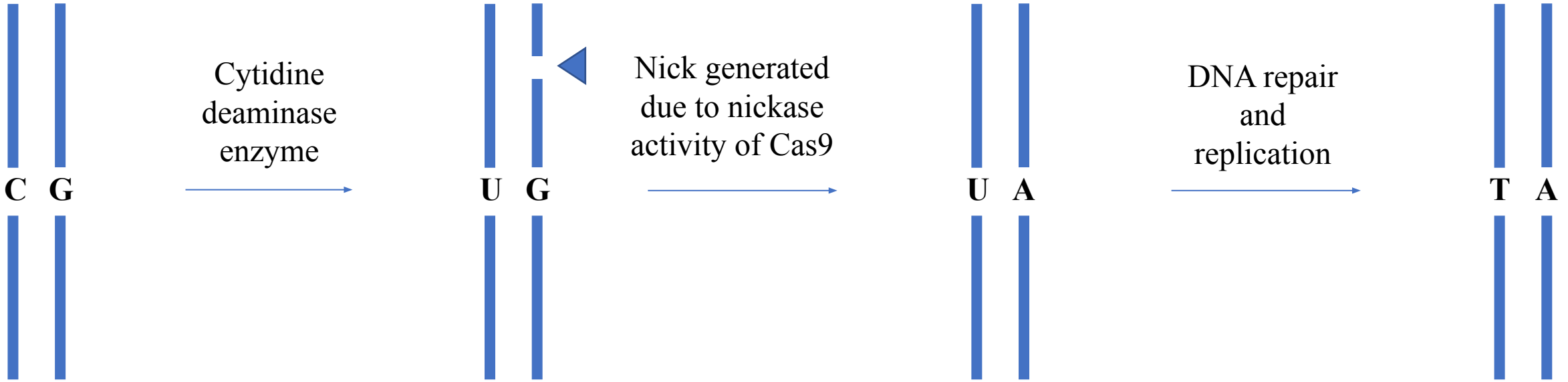
Difference between Cas9 for conventional genome editing and base editing



Conventional CRISPR protein

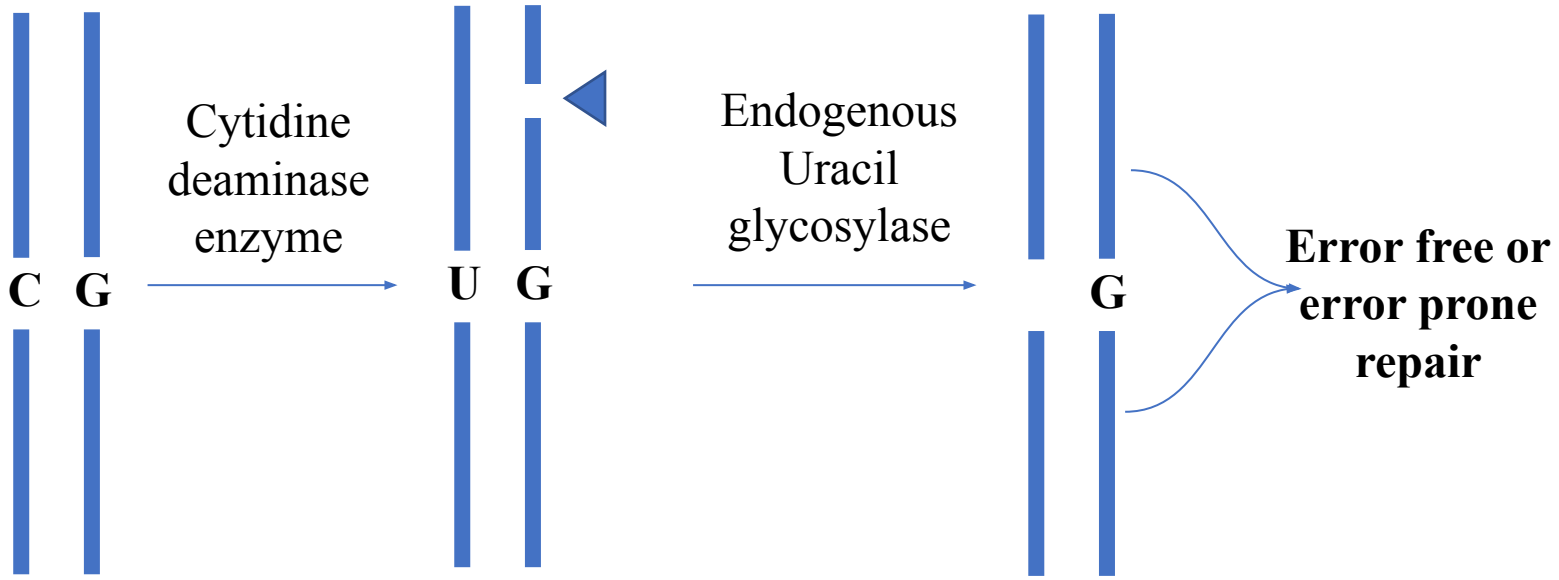
Chimeric protein

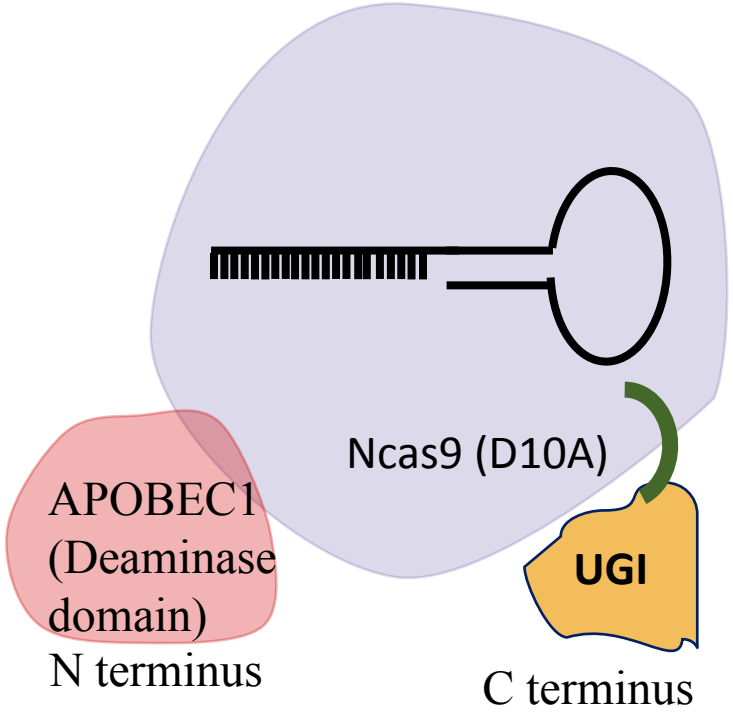
Mechanism of cytosine base editing



Komor et al (2016), *Nature*

Endogenous uracil glycosylase…a problem?





6 fold increase in editing efficiency

Komor et al (2016), *Nature*

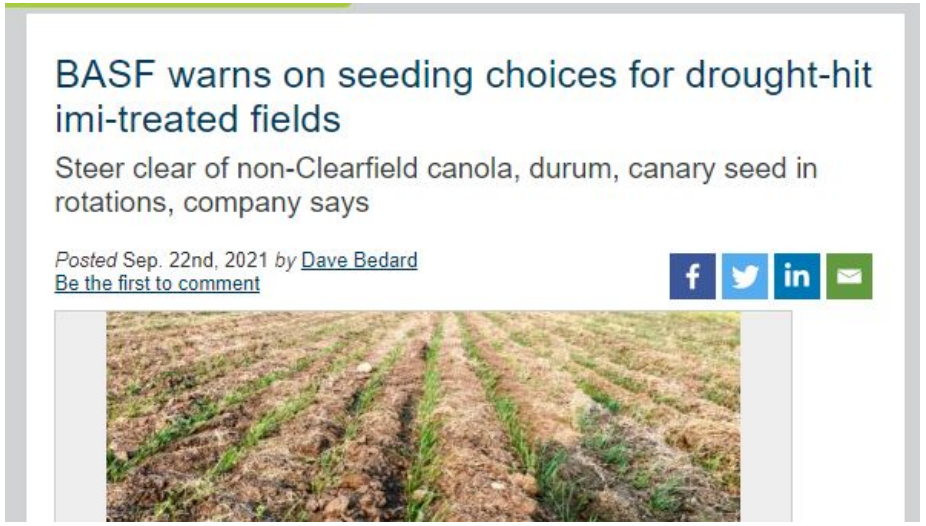
Back to story…

What is next??

- ✔ Finding a low persistent herbicide
- ✔ Making wheat resistant to that particular herbicide

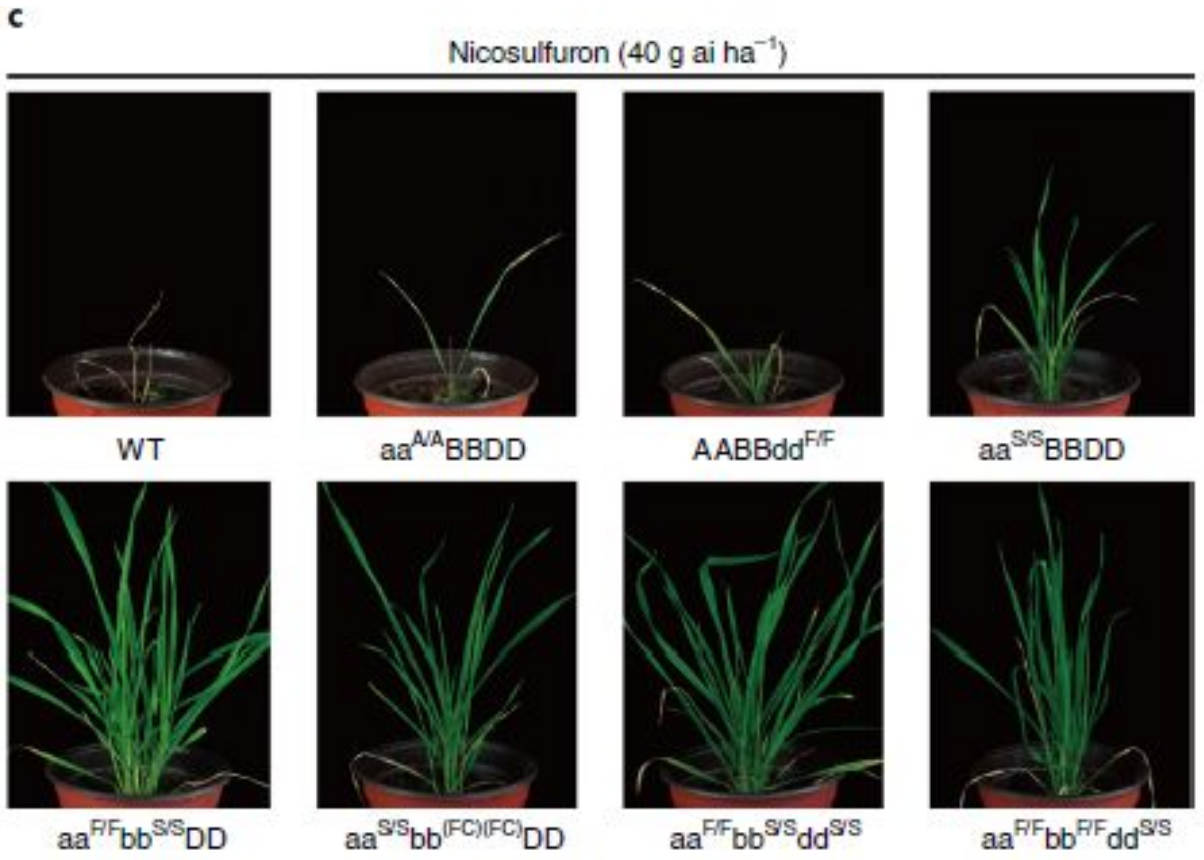
NICOSULFURON

Zhang et al (2019), *Nature plants*



- Acetolactate synthase (ALS), a key enzyme in the **biosynthesis of branched-chain amino acids**.
- ALS genes can harbor point mutations that confer sufficient **tolerance to herbicides**
- **TaALS-P174** site was edited using cytosine base editors
- Mutation efficiency was **2.5%**

Number of plants	Genomes mutated
8	3
2	2
3	1
3	Silent mutations

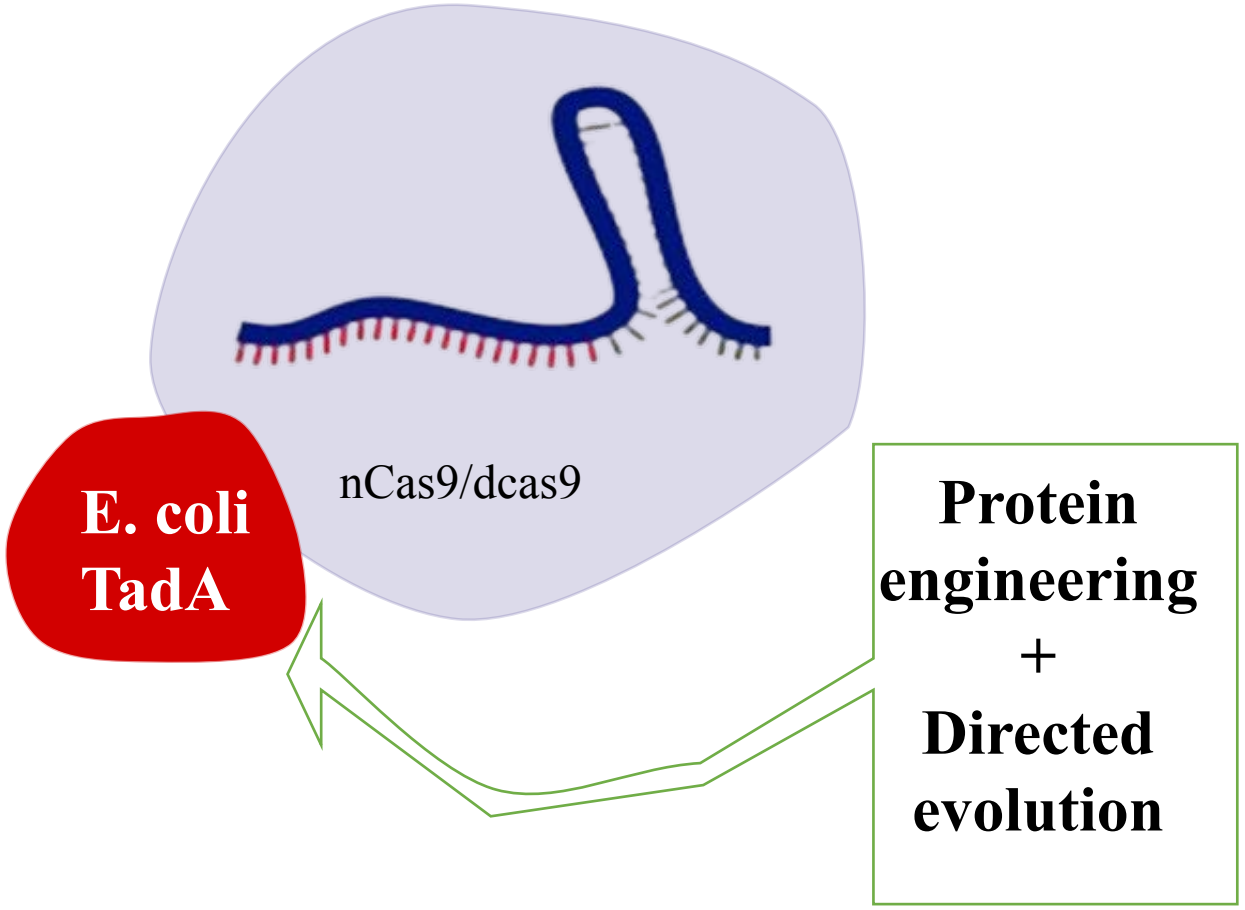


* Plant having more edited alleles were more resistant

Zhang et al (2019), ***Nature plants***

ADENINE BASE EDITING

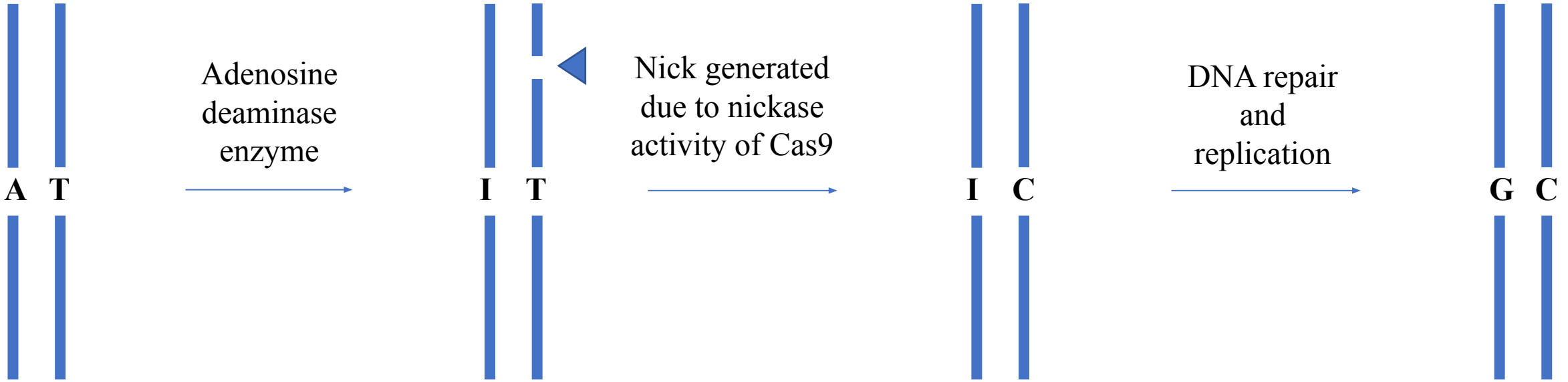
Adenine base editing



- Unlike cytidine deaminases, adenine DNA deaminases do not occur in nature.

***E. coli* TadA is a tRNA ADENINE DEAMINASE that converts adenine to inosine in the single stranded anticodon loop of tRNA Arg.**

Mechanism of adenine base editing



Gaudelli et al (2017), *Nature*

Lolium rigidum
A Forage Crop

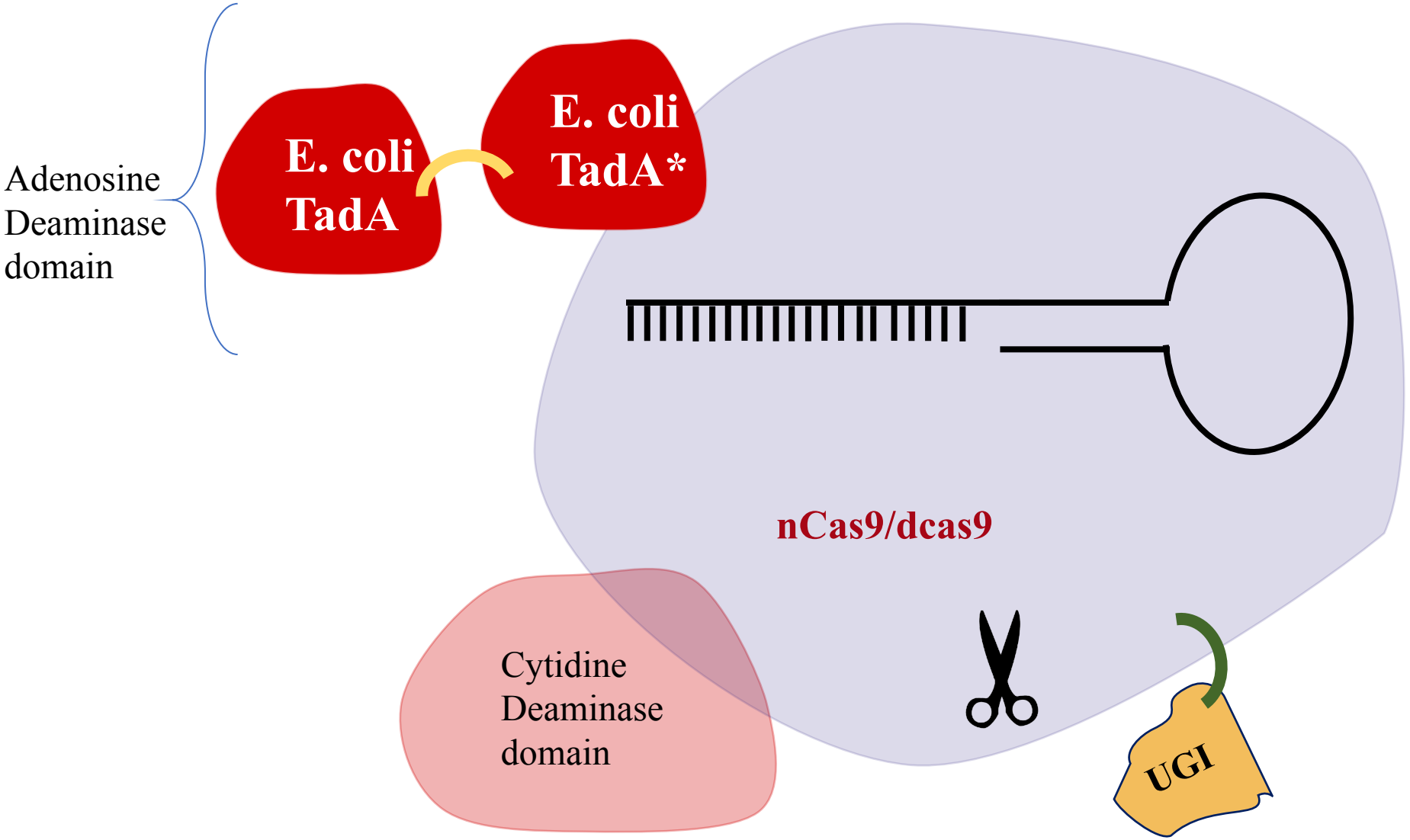
medium supplemented with haloxyfop-R-methyl

Li et al (

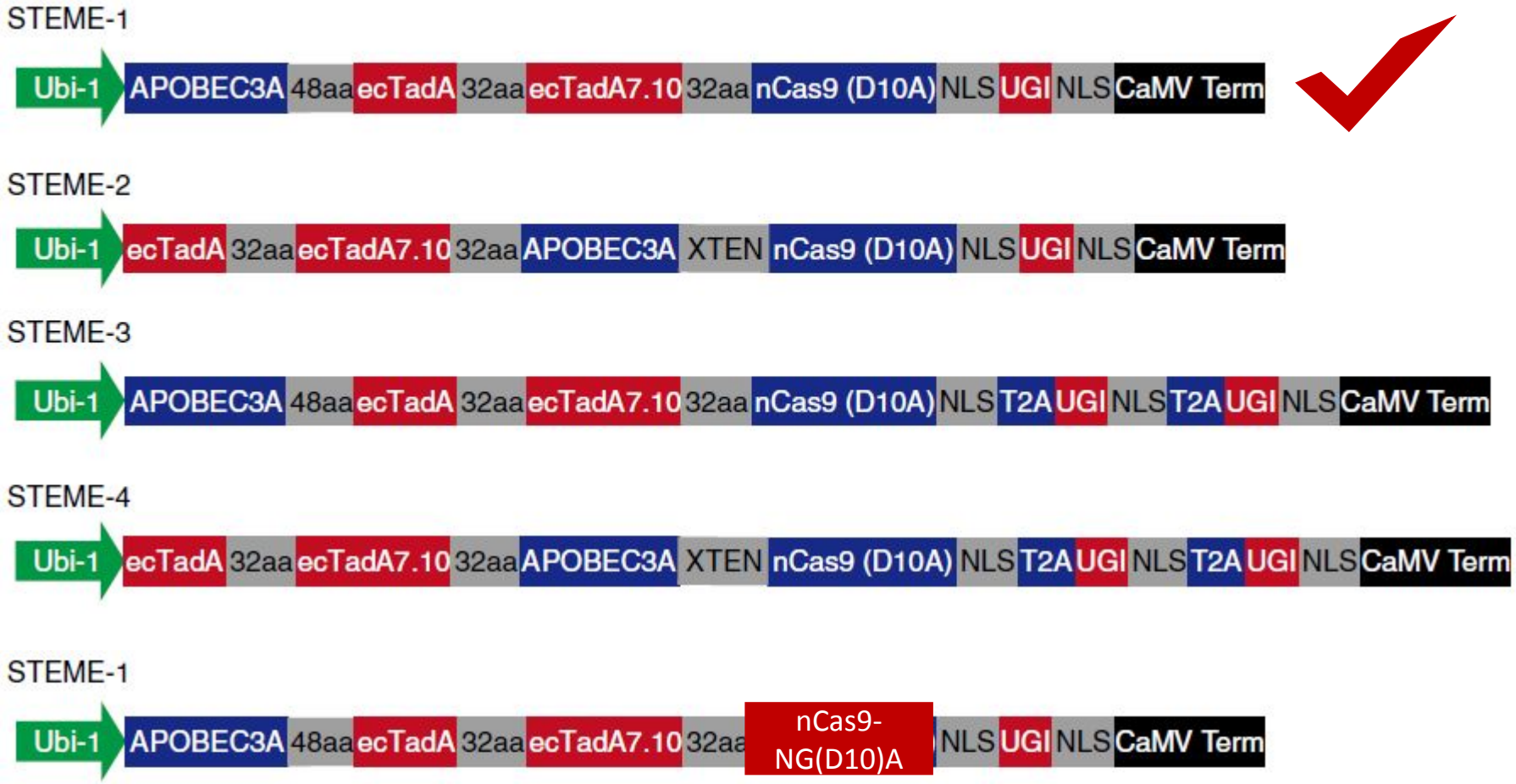
- The half-life of haloxyfop in the rhizosphere soil **is 2.6–4.9 days** (Liang et al, 2020)
- **Acetyl-coenzyme A carboxylase (ACC)** is an important target for herbicide tolerance
- T to C replacement (C2088R) **in *Lolium rigidium*** could endow plants with resistance to various herbicides
- The point mutation C2088R in *Lolium rigidum* corresponds to **C2186R in rice** (*Oryza sativa*), which is the target site ***OsACC*-T1 in** this study
- The mutant plant survived haloxyfop-R-methyl with normal phenotypes and no symptoms of damage whereas wild-type plants displayed severe stunting and withered leaves

DUAL BASE EDITORS

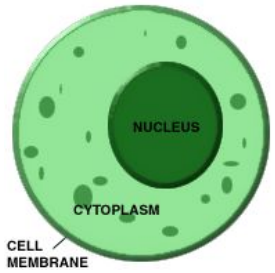
DUAL BASE EDITORS



Saturated targeted endogenous mutagenesis editors (STEME) vectors



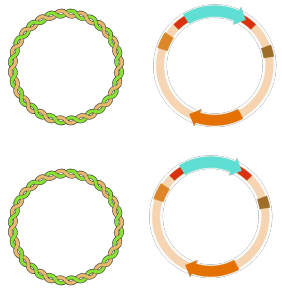
Li et al (2020), *Nature biotechnology*



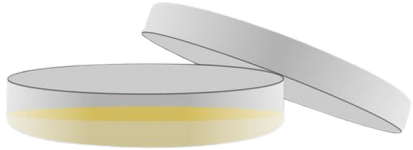
PROTOPLAST

PLANT

Vector preparation



Protoplast culture

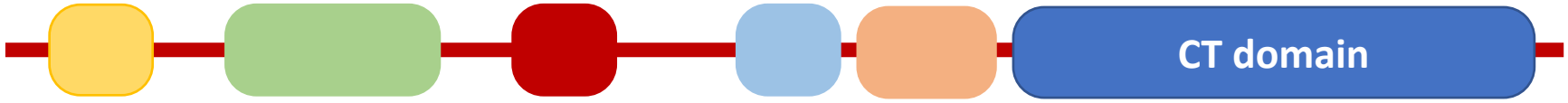


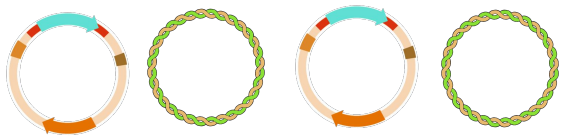
Deep sequencing

STEME-1 and STEME1-NG

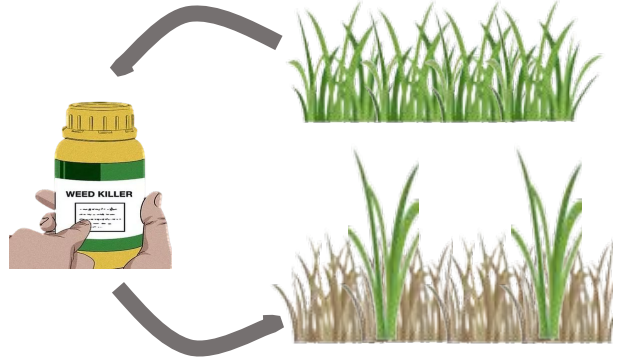
1. *OsACC* gene of rice was targeted for imparting resistance against haloxyfop
2. Simultaneous C > T and A > G conversions **(0.49– 15.10%)** in rice protoplasts
3. Expanded the targeting scope of STEME-1 to increase its utility by using STEME-NG
4. Simultaneous C:G>T:A and A:T> G:C events contributed to **18.4%** of the observed STEME-NG diversity

Li et al (2020), *Nature biotechnology*





Description	Number
No. of sgRNAs designed	200
STEME-1	102
STEME-NG	98



Efficiency of simultaneous editing of cytosine and adenine – 3.84%

Li et al (2020), *Nature biotechnology*

SHORTCOMINGS

Limited PAM sites

- Use of base editors are limited by presence of NGG PAM sites.
- New types of Cas protein and engineered Cas9 variants with altered PAM sequences have been introduced into base editors e.g., LbCpf1-BE0

Wide editing window

- CBEs can edit any sequence present in its catalytic window (4-5 nucleotides)
- It will pose a problem in case of continuous stretch of "C" (CCCCC).
- Base editors with narrow editing window (1-2 nts) are desirable

Off target activity

- Increased level of deaminase and UGI increases off target activity
- Sequence independent deamination was also found
- ABE also find out to cause off target activity at RNA level in mammalian cells

CONCLUSION

- Due to **increasing resistance of weeds to already available herbicides**, there is dire need of identifying new herbicides and making crops resistant to them.
- Base editing can be effectively used to generate **wide array of mutations in ACC and ALS** type of genes to impart herbicide tolerance to plants.
- Base editing is **better than conventional breeding in terms of target specificity**, increased precision and reduced unwanted variation
- Base editing can also be used to enhance **molecular diversity and directed evolution** by inducing different mutations
- Base editing cannot be used to insert specific sequences. Moreover, **prime editing** can be used for that purpose

Thanks