Life Sciences

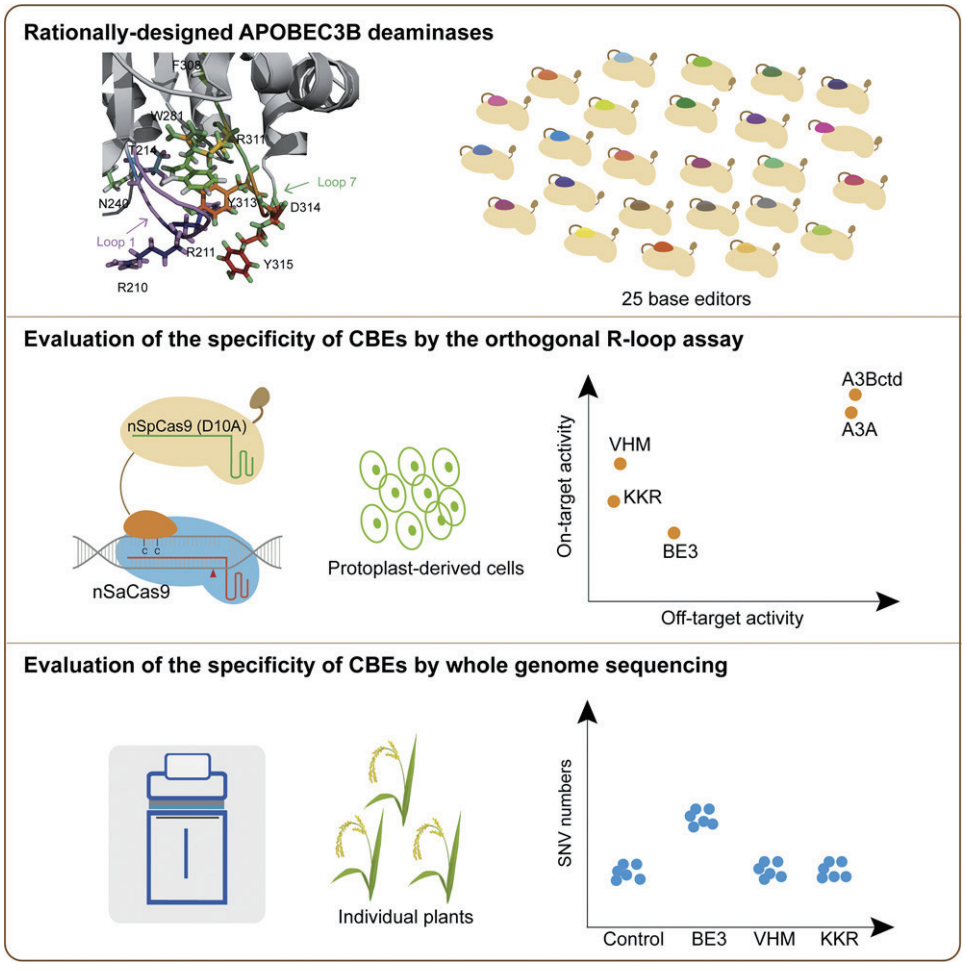
New Cytosine Base Editors with High Specificity and Precision

Base editors, which enable production of highly efficient targeted point mutations in genomic DNA without causing double-stranded DNA breaks, hold great promise for human gene therapy and crop trait improvement.

GAO Caixia's group from the Institute of Genetics and Developmental Biology (IGDB) of the Chinese Academy of Sciences has long been committed to developing plant base editing technologies. Their former studies showed that cytosine base editors (CBEs) induce unexpected genome-wide off-target mutations in rice (Science 2019, doi: 10.1126/*science*.aaw7166).

Recently, GAO's team created two new CBEs based on a truncated human APOBEC3 cytidine deaminase (A3Bctd) and developed a high-throughput assay for assessing sgRNA-independent deamination changes in plant CBEs.

They first developed a rapid, high-throughput and



Rationally-designed APOBEC3B deaminases improve the specificity of CBEs. (Image by IGDB).

inexpensive method for assessing CBEs in plants (nSaCas9-mediated orthogonal R-loop assay). In this assay, the orthogonal CRISPR system, nSaCas9, was used to create ssDNA regions in plant cells that acted as targets for sgRNA-independent deamination changes. To assess the nSaCas9-mediated orthogonal R-loop assay, they compared it with the whole-genome sequencing (WGS) assay.

The consistent results indicate that the nSaCas9-mediated orthogonal R-loop assay provides a logical, rapid and high-throughput method for assessing the sgRNA-independent off-target activities of CBEs.

Then they created 16 A3Bctd deaminase variants by rational design and evaluated their on-target efficiency and the sgRNA-independent off-target activities. They tested these A3Bctd-BE3 variants using the R-loop assay and selected seven mutations associated with efficient on-target editing activity and reduced off-target activity. After that, they combined these mutations and produced nine new A3Bctd-BE3 variants with double or triple amino acid substitutions.

In this way, they obtained two new CBE variants, A3Bctd-VHM-BE3 and A3Bctd-KKR-BE3, which exhibited efficient on-target activity and markedly reduced sgRNA-independent off-target activity.

In addition, these two new CBEs behaved more precisely at their target sites, and mainly produced single and double C edits. Also, they validated the high specificity of A3Bctd-VHM-BE3 and A3Bctd-KKR-BE3 by WGS assay.

The study, entitled "Rationally-designed APOBEC3B cytosine base editors with improved specificity," was published in *Molecular Cell* online on July 27.

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(IGDB)

Contact:
Dr. GAO Caixia
Institute of Genetics and Developmental Biology, CAS
Email: cxgao@genetics.ac.cn

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