Virus-induced genome editing using a miniature CRISPR system

The tobacco rattle virus was engineered to express the RNA-guided TnpB enzyme ISYmu1 and guide RNA. This miniature CRISPR system enabled transgene-free germline genome editing in *Arabidopsis thaliana* without the need for tissue culture or plant transformation.

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The problem

Plant breeding relies on genetic diversity to generate novel phenotypic traits. With the advent of genome editing technologies, the ability to rationally design and create genetic diversity is a reality. However, current genome editing systems (for example, CRISPR–Cas9) are encoded within transgenes, and tissue culture and/or plant transformation are used to make transgenic plants, after which genetic crosses remove the transgenic material but retain the edits. As this process is time-consuming and costly, a transient gene editing system that generates heritable edits without the need for tissue culture or transgenics would allow more effective plant genome editing. Zinc finger nucleases and meganucleases can be delivered to plants via tobacco rattle virus (TRV) to trigger heritable genome editing[1,2], but they rely on protein–DNA interactions to make edits. Utilizing programmable RNA-guided endonucleases that can edit plant DNA is advantageous, although viral delivery of the entire CRISPR–Cas9 gene editing system (that is, Cas9 and guide RNA (gRNA)) is challenging owing to the large size of Cas9; so far TRV has only been used to deliver the gRNA to a Cas9-expressing transgenic plant for heritable genome editing[3,4].

The solution

We developed an approach to deliver a complete, miniature RNA-guided TnpB editing system, utilizing the TnpB DNA endonuclease ISYmu1, for transgene-free germline editing in *Arabidopsis thaliana*.

We first tested the activities of the very small TnpB RNA-guided DNA endonucleases ISDra2, ISYmu1 and ISAam1 using a single transcript design in which one promoter was used to drive expression of the TnpB and the gRNA. Using an *A. thaliana* protoplast system, we observed that all three TnpBs were capable of genome editing; ISYmu1 showed the highest efficiency in editing *AtPDS3*, which was chosen because homozygous mutations cause an easy-to-score phenotype of white tissue.

Focusing on ISYmu1, we next tested its activity in transgenic T1 plants using different gRNAs, which revealed highly active (gRNA12; targeting the promoter) and lowly active (gRNA2; targeting the coding region) gRNAs targeting *AtPDS3*. We next delivered ISYmu1 with either of these two gRNAs to *A. thaliana* seedlings via TRV infection. We observed editing of somatic cells of infected plants for both *AtPDS3* gRNA2 and gRNA12, with ISYmu1–gRNA12 displaying the highest editing activity. For gRNA2, which targeted the *AtPDS3* coding region, we observed white sectors that indicated biallelic edits in these regions of the plants. Genotypic analysis of the progeny from *A. thaliana* plants that showed high levels of somatic editing revealed inheritance of biallelic edits for both of the *AtPDS3* target sites.

We next tested whether TRV infection could deliver ISYmu1–gRNA to additional loci by targeting the *AtCHLI1* gene, mutation of which causes an easy-to-score yellow phenotype. We observed ISYmu1-mediated somatic editing for three of the six gRNAs tested (Fig. 1a); edited alleles were transmitted to the next generation of plants, with biallelic mutations displaying an obvious yellow phenotype (Fig. 1b).

The implications

Our goal was to develop an easy and rapid one-step technique for creating transgene-free edited plants. We used *A. thaliana* as a proof of concept owing to the wealth of protocols and resources available for studying this plant. However, given that TRV has a host range of more than 400 species, we anticipate that this approach will translate to additional plants, including economically valuable crops and ornamental species. Furthermore, many other plant viruses have a similar capacity to carry genetic material and are probably amenable to carrying miniature RNA-guided editing systems.

Although our approach presents an important advance in plant genome editing, additional work is necessary to realize its full potential. Three primary bottlenecks remain: the proportion of infected plants displaying high somatic editing needs to be increased; the frequency of germline transmission needs to be increased; and ISYmu1-mediated editing is not yet as consistently robust as editing using other genome editing reagents, such as CRISPR–Cas9.

Future experiments in our laboratory will aim to improve the efficiency of TRV infection and spread to enhance the editing of somatic and germ cells, as well as to increase ISYmu1's enzymatic activity through protein and gRNA engineering. We also aim to identify novel TnpBs that recognize unique target-adjacent motif sequences to allow for editing in more genetic contexts.

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EXPERT OPINION

"Transgene-free, tissue culture-free gene editing has been the holy grail in plant biotechnology. Weiss et al. demonstrate one approach for creating heritable edits using plant RNA viruses to deliver both a Cas nuclease and a guide RNA through infection. This gene editing approach has the potential to be enabled in many different plant species." **Daniel Voytas, University of Minnesota, St. Paul, MN, USA.**

FIGURE

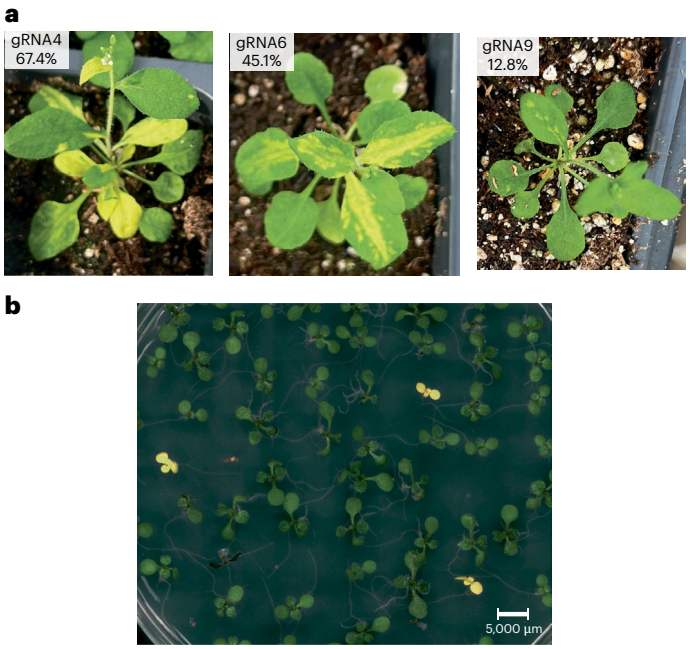

Fig. 1 | Transmission of biallelic mutations in the *AtCHLI1* gene. a, Representative pictures of TRV-infected *A. thaliana* plants displaying yellow sectors (the phenotype of biallelic mutations) about two weeks after TRV-mediated delivery of ISYmu1–gRNA. The gRNA used and somatic editing efficiency achieved is indicated in the upper left corner of each picture. **b**, Progeny seedlings from the plant showing 67.4% ISYmu1–gRNA4-mediated somatic editing of *AtCHLI1* from panel **a**. The yellow seedlings harbour ISYmu1-targeted biallelic mutations.

BEHIND THE PAPER

This work was funded by a National Science Foundation (NSF) Plant Genome Research Project grant (thank you, NSF!) that is supporting a really fun collaboration between the Jacobsen laboratory at UCLA and the laboratories of Jennifer Doudna and Jill Banfield at UC Berkeley. The project was inspired by the recent discovery that miniature RNA-guided TnpB endonucleases could make targeted edits in mammalian cells, and our realization that TnpBs were small enough to be encoded in many plant RNA viruses. Seeing the expected phenotype of biallelic *AtPDS3* and *AtCHLI1* genome edits was incredibly exciting, especially when we first saw that the edits were heritable. Moving forward, we are excited to work further with the Doudna and Banfield laboratories to improve our system so that it might be useful for *A. thaliana* CRISPR screens. We are also starting projects aimed at using these tools for crop engineering. **T.W & S.E.J.**

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FROM THE EDITOR

"This study stands out because it demonstrates the simultaneous delivery of both gRNAs and a Cas nuclease by viral vector to plants to enable transgene-free germline editing, a key step forward for in planta gene editing that potentially circumvents the challenging and time-consuming tissue culture step." **Jun Lyu, Senior Editor, *Nature Plants*.**