Development and application of genome-editing technologies for plant pathogenic fungi

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Abstract

Genome editing technologies have become a powerful tool for advanced genome manipulation in basic and applied research fields because of the development of programmable nucleases such as TALEN (transcription activator-like effector nuclease) and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeat/CRISPR-associated protein9). These nucleases were developed from molecular machineries established during “arms race” coevolution between hosts and parasites. Various molecular interactions (battles) for survival have also been observed between plants and pathogenic fungi, and one of the unique traits (survival strategies) of some plant pathogenic filamentous fungi is to abdicate sexual reproduction (loss of sexual reproduction). We were interested in how sterile plant pathogenic fungi can change their own genomes during coevolution with plants.

The rice blast fungus *Pyricularia oryzae* is a model plant pathogenic fungus for studying plant–pathogen interactions. Field isolates of *P. oryzae* have shown chromosomal diversifications, including deletions, duplications, and multiple translocations of pathogenicity-related genes (*Avr-Pita*) (PLoS Pathog 7:e1002147, 2011). We hypothesized that somatic homologous recombination (HR) drives asexual genome evolution in *P. oryzae* and created an HR detection/selection marker system. Using this system, we revealed that somatic HR commonly occurs during asexual reproduction and is induced by various stress conditions. Similar reactions were also detected at the *Avr-Pita* locus, suggesting that somatic HR may contribute to pathogenic changes. Basically, somatic HR had the function of DNA repair, but the properties were different from those of other eukaryotes.

Because genome editing generally employs the induction of DNA repair mechanisms using programmable nucleases, we applied the findings from studies on pathogenic fungal genome evolution to developing original genome editing. We have successfully developed programmable nucleases optimized for filamentous fungi, such as ZFN (zinc finger nuclease), TALEN, CRISPR/Cas9, CRISPR/Cas12a, and CRISPR/Cas13b, and have also successfully developed original technologies such as efficient high-throughput gene disruption, mRNA editing, non-cutting base editing, HR-mediated knock-in/base editing, and chromosome editing. Thus, the developed genome editing strategies could enable free editing of fungal genomes (J Gen Plant Pathol 86:523–525, 2020).

Some pathogenic filamentous fungi can only reproduce asexually, but the mechanisms and biological significance have been a long-standing mystery. By combining the developed genome editing with a backcrossing strategy, we showed that functional mutations of Pro1, a global transcriptional regulator of mating-related genes, is one cause of loss of female fertility of *P. oryzae* in nature. Various types of mutations in *PRO1* were detected worldwide in *P. oryzae*, including pandemic isolates of wheat blast fungus. Because the *PRO1* mutant increases conidial release and mycovirus elimination, loss of sexual reproduction might be an important event in the pandemic evolution (iScience 26:107020, 2023).