PLANT & ANIMAL SCIENCE

Genome editing in crops: from bench to field

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Conventional plant breeding can accelerate crop improvement by crossing superior plants with other compatible plants, or randomly induced variants generated by chemical- or radiation-induced mutagenesis. However, its contribution to crop improvement may be limited by a declining genetic base that depends on existing natural allelic variations. Moreover, conventional mutation is time consuming and requires expensive screening of large populations. During the past 20 years, transgenesis has been used for crop improvement. For example, in the USA, more than 90% of cultivated soybeans and corn contain transgenes that confer traits such as resistance to insects or herbicides. Unlike conventional breeding, the production of transgenic plants can overcome natural barriers to breeding, and thereby increase the available genetic variation. Transgenesis, however, has its limitations. Transgenic crops generally carry foreign genes inserted randomly in the genome, and their commercialization is frequently prevented by public concern over health and environmental safety issues. Hence, the needs of an ever-increasing human population call for new and publically acceptable breeding techniques that can rapidly, efficiently, and accurately produce innovative varieties.

GENOME EDITING HARNESSES DNA REPAIR PATHWAYS

Editing genomes in living cells has recently become possible through the discovery of sequence-specific nucleases (SSNs) that can be engineered to target genomic sites [1]. SSNs enable precise genome editing by introducing DNA double-strand breaks (DSBs) that subsequently trigger DNA repair by either non-homologous end joining (NHEJ) or homologous recombination (HR) (Fig. 1) [2]. The NHEJ is error-prone and frequently introduces small deletions and insertions at the junction of the newly rejoined chromosome, some of which cause gene knockouts by generating frameshift mutations. In genome editing by HR, DNA templates bearing sequence similarity to the break site are used to introduce sequence changes at the target locus. HR can be used to change single amino acids or small stretches of amino acids in proteins, or single base pairs or groups of base pairs in control elements. Thus, DNA repair by HR is a precise gene-targeting method.

SSN-BASED TARGETED GENOME EDITING TECHNOLOGIES

There are currently three major types of SSNs: zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system (Fig. 2). ZFNs were the first method used to edit genes. They bind DNA via arrays of engineered zinc-finger proteins fused to the catalytic domain of the FokI endonuclease (Fig. 2a) [3]. TALENs are also chimeric enzymes, and combine FokI and a DNA-binding domain derived from transcription activator-like (TAL)

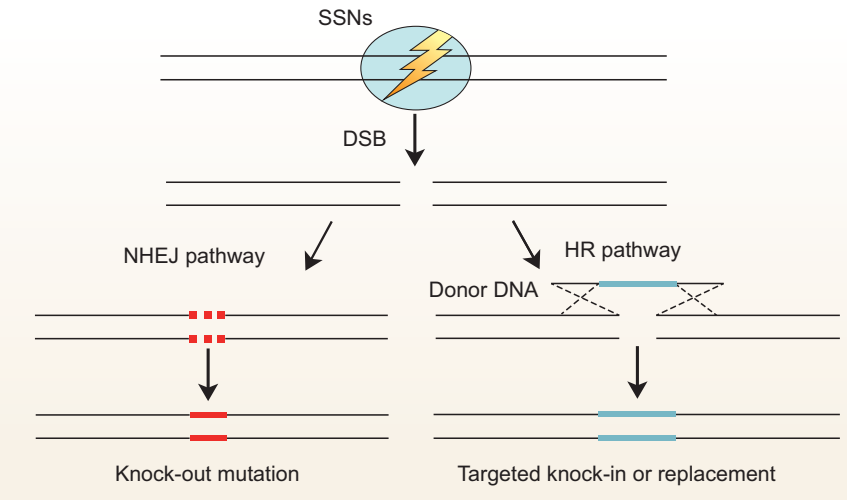

Figure 1. Genome editing harnesses DNA repair pathways. DSBs induced by SSNs trigger the NHEJ and HR DNA repair pathways. The NHEJ pathway is often imprecise, and frequently introduces small deletions and insertions at the junction of the newly rejoined chromosome. This can cause a frameshift or premature stop codon, and generate gene knockout mutations. Alternatively, in the presence of a homologous donor DNA template spanning the DSB, the HR repair pathway can be activated and a targeted gene knock-in or replacement can be generated.

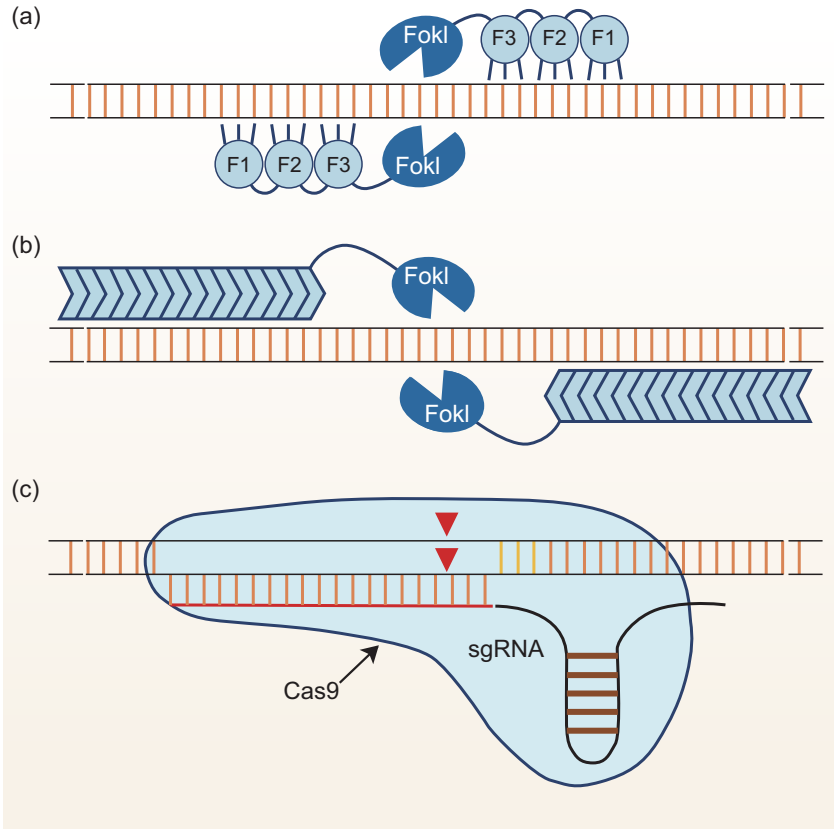

Figure 2. Schematic of the three major types of SSNs. (a) Two ZFNs bound to their target site. The zinc-finger arrays (light blue) fused to the catalytic domain of the FokI endonucleases (dark blue) are separated by a 5–7-bp spacer sequence. Each zinc finger typically recognizes three nucleotides. (b) Two TALENs bound to their target site. The TAL effector arrays (light blue) fused to FokI nucleases are separated by a 15–20-bp spacer sequence. Each TAL effector module specifically binds one nucleotide. (c) The CRISPR/Cas system targets DNA through base pairing between DNA sequences at the target site and a CRISPR-based sgRNA. Cas9 has two nuclease domains (shown by red arrowheads) that cleave one strand of double-stranded DNA each.

effectors. DNA binding by TALENs is achieved through arrays of the TAL DNA-binding motif (Fig. 2b) [4]. The modular nature of these DNA-binding motifs allows them to target DNA sequences efficiently, making them easier to engineer than ZFNs. Both approaches have proved effective, but their use is limited by the need to engineer a specific protein pair for each new target. The CRISPR/Cas system provides bacteria and archaea with adaptive immunity against viruses and plasmids [5]. In this system, a single-guide RNA (sgRNA) directs the Cas9 nuclease to make double-stranded cuts in a matching target DNA sequence to which it binds via Watson–Crick base pairing (Fig. 2c) [6]. The simplicity of the cloning strategy, the feasibility of multiplex engineering, and fewer limitations on potential target sites make the CRISPR/Cas system efficient and versatile. TALEN technology was selected by the journal *Science* as one of the 10 breakthroughs of 2012, and the CRISPR system received the same accolade in 2013.

APPLICATIONS OF GENOME EDITING IN CROP IMPROVEMENT

SSNs have been used to make gene knockouts in several important crops, including barley, soybean, maize, rice, and wheat [7]. One of the first successful targets for SSN mutagenesis was maize *IPK1*, which encodes the enzyme that catalyzes the final step in phytate biosynthesis. This mutation abolishes IPK1 expression, which permits the production of high levels of phytate and low levels of inorganic phosphate [8]. Further, targeted gene knockout via TALEN-induced NHEJ-mediated repair has been achieved in rice [9]. The bacterial pathogen *Xanthomonas oryzae* causes a blight that leads to significant annual losses in rice production. TALENs were used to generate a mutation in the effector-binding site in the promoter of rice *OsSWEET14*, thereby eliminating effector-induced transcription and reducing the pathogen's virulence. More recently, they were used to generate soybean varieties that produce nearly four times as much oleic acid as the parent [10].

FUTURE DIRECTIONS

Although the delivery of these nucleases into plant cells and the relatively specific nature of each nuclease platform remain challenges, the ability to efficiently alter genes using SSNs promises to profoundly improve our understanding of gene function and the creation of crop plants with desirable traits. Genome editing based on SSNs is one of the most promising novel plant breeding technologies for crop improvement. Gene knockouts are valuable for generating new genetic variants, and genome editing can be used to make knockout collections for agronomically important crop plants such as rice and maize. The plants created by SSN mutagenesis do not appear to have any foreign DNA in their genomes, and are often indistinguishable from natural variants or those produced by conventional mutagenesis. They may therefore fall outside the existing regulations affecting genetically modified crops. Additionally, because SSNs can be used to introduce single nucleotides or long stretches of DNA at predefined genomic sites, the types of insertion they produce may avoid the position effects associated with random insertion by traditional transgenesis. Further, if multiple transgenes are inserted at the same site, such a gene stack

will be inherited as a single Mendelian locus, allowing introduction of several different transgenes into the genome. We believe that progress in genome editing in plants promises to open exciting new avenues for crop improvement.

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