Redirecting meiotic recombination by CRISPR–Cas-mediated chromosome engineering

A major bottleneck in plant breeding is the establishment or breakage of genetic linkages by random, naturally occurring meiotic recombination. This problem can be overcome by CRISPR–Cas-mediated chromosome engineering. By inverting ~17 Mb of chromosome 2 of *Arabidopsis thaliana*, we almost completely suppressed genetic crossovers in nearly the entire chromosome.

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The mission

Plant breeders rely on random crossovers between parental homologous chromosomes during meiotic recombination to break or create genetic linkages between genes to combine attractive traits[1]. Chromosomal rearrangements, such as inversions, are known to suppress crossovers in the rearranged area. Thus, the manipulation of meiotic recombination by chromosome engineering could be a useful tool for breeders to maintain favourable genetic linkages in a targeted manner. Previously, we have shown that it is possible to induce chromosomal inversions and translocations (with lengths in the Mb range) in *Arabidopsis thaliana* by CRISPR–Cas-mediated chromosome engineering[2,3]. By reverting a 1.1 Mb-sized inversion on chromosome 4 in *A. thaliana* that had occurred naturally, we demonstrated that the recombination landscape can be modulated by inducing crossovers in a previous recombination 'cold spot' using chromosome engineering[2]. In this study, we aimed to investigate whether we could exclude a substantial part of the *Arabidopsis* genome from meiotic recombination using chromosome engineering.

The observation

Utilizing our well-established chromosome engineering protocol[4], we induced two double strand breaks (DSBs), each in close proximity to one of the telomeric ends of chromosome 2, in the Columbia ecotype (Col-0) of *A. thaliana*, to invert the interjacent 17.1 Mb fragment (corresponding to approximately nine-tenths of the length of the chromosome). The inversion was detected in 1 hemizygous plant out of 1600 T2 (second generation of transgenic plants) plants by PCR-based screening, and it was confirmed by Sanger sequencing and fluorescence in situ hybridization (FISH) analysis in the homozygous and hemizygous mutants (Fig. 1). We performed a fertility assay and phenotypical analysis to assess the effects of the inversion. For analysis of crossover numbers and distribution, the homozygous inversion line was crossed with the ecotype (a plant variant that genetically adapted to a geographical location) Landsberg erecta (Ler-1). A single nucleotide polymorphism genotyping assay with TaqMan probes (for fluorophore-based detection of either the Col-0 or Ler-1 allele) was performed in 400 F2 (second filial generation) plants (obtained from crossing the homozygous inversion line with the Ler-1 line) and in control plants (obtained from crossing Col-0 with Ler-1) to detect marker changes between Col-0 and Ler-1 alleles. By analysing the patterns of marker changes in detail, we deduced the genetic state of the individual chromosomes from the diploid genomes.

Within the inversion borders, crossover numbers were reduced by 92% compared with the control. However, meiotic recombination was not completely suppressed. In 4% of the samples, we detected double crossovers within the inverted region, which indicates that meiotic pairing and recombination can, rarely, occur within the inverted region and lead to viable gametes. A single crossover within the inverted area leads to the formation of two chromosomes carrying two identical telomeric ends, as opposed to differing 5′ and 3′ telomeric ends, resulting in a massive loss of genetic information and non-viable progeny. However, a crossover in the telomeric ends or a double crossover within the inversion can result in viable gametes. The fertility assay revealed a seed number reduction per silique (seed pod) by a third in the hemizygous plants.

The implications

This study shows that it is possible to suppress recombination at the chromosomal level by chromosome engineering in a multicellular eukaryote. Recombination was suppressed in nearly the entire chromosome, which represents a substantial part (roughly an eighth) of the *Arabidopsis* genome. Our findings demonstrate that chromosome engineering can also be a valuable tool for plant breeders to exclude favourable allelic combinations of any size from recombination anywhere in the genome.

Although more time and effort are required to achieve chromosome engineering in crop plants, the successful induction of inversions has already been reported in maize[5].Thus, it is only a matter of time before we will see more applications of redirecting meiotic recombination in crop breeding.

In the future, we plan to generate further chromosomal rearrangements, such as the repositioning of centromeres or chromosome fusions, using CRISPR–Cas. We expect that these rearrangements will drastically redirect the genome-wide patterns of recombination, further widening the tool box of chromosome engineering for plant breeding.

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EXPERT OPINION

"This work builds from recent achievements by the authors, in which Cas9 was used to induce chromosome rearrangements. In chromosome 2 of Col-0 *Arabidopsis*, the authors induce a 17 Mb inversion that encompasses the majority of the chromosome. The manuscript represents a significant advance in chromosome engineering, and the recombination insights are fascinating". **Ian Henderson, University of Cambridge, Cambridge, UK.**

FIGURE

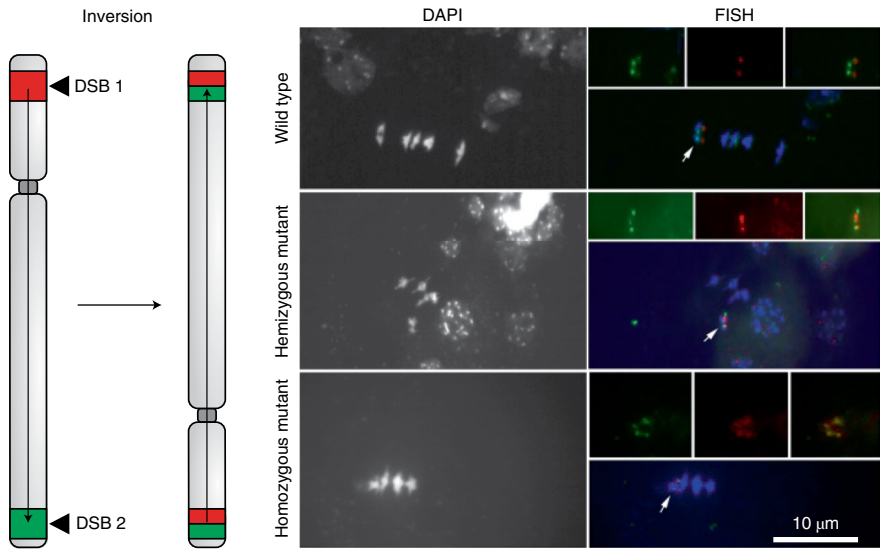

Fig. 1 | FISH analysis of the inversion. Left: Schematic overview of the chromosome regions detected with fluorescently labelled bacterial artificial chromosome (BAC) clones spanning the 5′ (red) and 3′ (green) cleavage sites. Black triangles indicate the location of the DSB. Right: FISH results of metaphase I meiocytes. Arrows indicate meiocytes in which the location of both fluorescently labelled BAC clones is visible. The induction of the inversion leads to the presence of both fluorescence signals at both ends of the chromosome. DAPI, 4′,6-diamidino-2-phenylindole. © 2022, Rönspies, M. et al.

BEHIND THE PAPER

When I started to work with the first available molecular scissors (the intron-encoded endonuclease I-SceI) in plants 30 years ago, one of my long-term goals was to redirect meiotic recombination. In the end, reaching this goal has taken us more than 25 years. This achievement was, on one hand, due to the fact that the CRISPR–Cas revolution had finally given us a tool at hand that was cheap and efficient enough to ensure a high cutting efficiency. On the other hand, we realized that the induction of homologous recombination to manipulate meiotic recombination was not the solution: although we were able to induce gene targeting efficiently in somatic cells early on, for 20 years all our efforts to redirect recombination during meiosis failed. Finally, a strategy switch—the induction of chromosomal restructuring by DSB-induced, non-homologous end joining in somatic cells—brought the breakthrough. **H.P.**

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FROM THE EDITOR

"Using CRISPR for chromosome engineering has been possible for a couple of years, since the publication of targeted inversions of large chromosomal regions in maize and rice. But here in *Arabidopsis*, a 17 Mb region of chromosome 2 (nine-tenths of this chromosome) was reversed. This reversion almost completely suppressed crossovers in this chromosome, which is an advantage in crop breeding". **Editorial Team, *Nature Plants*.**