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Recent advances in plant recombination

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Recombination is an essential cellular process and a source of genetic diversity. Recent studies have demonstrated the effects of various factors (e.g. DNA sequence similarity and activation of transposons) on rates of recombination and the distribution of recombination breakpoints in plants. These studies have also provided detailed characterizations of interchromatid and interhomolog recombination events. New approaches offer the promise of achieving the long-awaited goal of gene targeting in plants.

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Introduction

Recombination plays an important role in the repair of DNA damage and in chromosome segregation, and can create novel haplotypes (i.e. genetic diversity). To a large extent, the mechanisms and regulation of recombination pathways in plants have been inferred from extensive studies conducted in yeast and mammalian cells.

Homologous recombination events are initiated by double-stranded breaks (DSBs) [1] and can be classified as crossovers (COs) and noncrossovers (NCOs). Although it has been hypothesized that both types of events are resolved from double Holliday junctions (DHJs) [2], evidence from yeast shows that COs and NCOs are differentially regulated during meiosis [3,4]. The kinetics of COs and NCOs also differ in mitotic recombination, and mutations can affect the proportion of the two types of recombination events [5]. It has been suggested that most NCOs arise from synthesis-dependent strand annealing rather than from the resolution of DHJs [3–5].

There are two major pathways to repair DSBs: homologous recombination (HR) and non-homologous end-joining (NHEJ). The Rad51 protein plays a central role in HR,

whereas the Ku70/Ku80 heterodimer functions in NHEJ. In mammalian cells, defects in proteins that are involved in NHEJ (e.g. Ku70) can increase HR, indicating that the two pathways compete for the repair of DSBs [6,7]. In *Arabidopsis*, rates of HR decrease as plants age, whereas rates of point mutations increase. Coincidentally, the expression of *rad51* decreases while the expression of *ku70* increases as plants age. These data indicate that these two major recombination pathways are developmentally regulated [8]. The understanding that these two major pathways can compete with each other has been used to increase the efficiency of gene targeting in fungi [9,10].

This review summarizes recent progress in understanding genetic modifiers that affect recombination, interchromatid and interhomolog recombination, and genome-wide patterns of recombination in plant genomes. The resulting contributions to our mechanistic understanding have the potential to increase the efficiency with which HR-based tools can be applied to plants.

Cis-acting genetic modifier

Recombination does not occur randomly in a genome. Regions where recombination rates are significantly higher or lower than the genome average are termed recombination hot- and cold-spots, respectively [11–13]. A growing body of research has sought to understand the underlying mechanisms that are responsible for the differential distribution of recombination events. Two general classes of genetic modifiers, *cis* and *trans*, contribute to variation in the distribution of recombination events.

Cis genetic modifiers are closely linked to the intervals in which they modify rates of recombination. Although it has been shown previously that genic sequence polymorphisms in plants can influence recombination in *cis* [13], it is not clear how sequence divergence regulates either the rate of recombination or its effects on multi-genic intervals. In *Arabidopsis*, divergence levels of 0.16% [14] and 1.9% [15] can lead to three- and ten-fold reductions in rates of somatic recombination, respectively. In nearly isogenic maize lines, DNA sequence and structural polymorphisms from three haplotypes of a chromosomal interval, defined by the *a1* and *sh2* genes, have *cis*-effects on both meiotic recombination rates and the distribution of recombination breakpoints [16]. Inactivation of *AtMSH2* can increase rates of somatic homeologous recombination up to nine-fold, indicating that the mismatch repair system contributes to the decrease in recombination rates that is associated with DNA sequence polymorphisms [17].

Trans-acting genetic modifiers

In contrast to *cis* elements, *trans*-acting genetic modifiers are not closely linked to the interval in which they modify recombination. *Trans* genetic modifiers include chromatin remodelers, autonomous transposons, recombination machinery proteins, DNA-binding proteins, and other functional elements.

For example, the maize autonomous *MuDR* transposase can increase the rate of meiotic COs near a *Mu* insertion four-fold as compared with the rate of meiotic COs in plants that do not express *MuDR* transposase [18]. It has been hypothesized that the additional meiotic DSBs produced by the *MuDR* transposase are responsible for the increased rate of COs. To study the effect of *trans* genetic modifiers on meiotic recombination, a sequence identical *a1-sh2* interval was introgressed into three unique maize lines [19]. Molecular characterization of the recombinants demonstrated that *trans* modifiers affected both the rates of meiotic recombination and the distribution of breakpoints across the *a1-sh2* interval. It appears that these *trans* modifiers exert their effects in a region-specific manner because rates of recombination across a chromosomal interval in Chromosome 1S were not affected by these modifiers [19].

Interchromatid and interhomolog recombination

Recombination can occur both between sister chromatids (interchromatid recombination) and between homologous chromosomes (interhomolog recombination). In yeast, interchromatid recombination is the major pathway during mitosis, whereas interhomolog recombination is the major pathway during meiosis [20,21].

In plants, tandemly arrayed duplicates have been utilized to study relationships between interchromatid and interhomolog recombination. Using synthetic direct repeats in *Arabidopsis* [22], frequencies of somatic recombination in homozygous plants exceeded those in hemizygous plants by a factor of two, suggesting that both interchromatid and interhomolog recombination occur during mitosis. Molecular characterization showed that most of these somatic events had resulted from gene conversion events as opposed to unequal COs.

Although meiotic unequal COs occur naturally in plants, detailed analyses of the distribution of the resulting recombination breakpoints were not previously available. Mapping of 25 independent meiotic unequal COs in *Arabidopsis* plants that were hemizygous for a synthetic RBCSB gene cluster established that recombination breakpoints clustered in regions that share high sequence homology between RBCS3B and Δ RBCS1B [23]. The *A1-b* allele of maize is a naturally occurring tandem duplication that shares the same genomic location as the single-copy *a1* gene. Meiotic unequal COs that

involve *A1-b* occur preferentially between homologous chromosomes rather than between sister chromatids. Pairing of the duplicated *A1-b* segments with the segments in the homolog does not occur randomly. The different pairing configurations lead to different outcomes: gene loss or duplication [24**]. Because of the high frequency of tandem genic duplications in maize [25,26] and other plant species, such as rice [27] and *Arabidopsis* [28], unequal COs might have greatly influenced genome structure throughout evolution [24**].

Genome-wide studies of recombination

Many earlier molecular genetic studies of recombination were focused on the characterization of recombination events in genes or small multi-genic intervals [12,13]. The availability of the *Arabidopsis* genome sequence (thanks to The *Arabidopsis* Genome Initiative 2000) has made it possible to study recombination on a genome-wide scale. Using 71 single nucleotide polymorphisms (SNPs) covering *Arabidopsis* chromosome 4, 1171 COs were detected in 702 F₂ plants. Further analyses showed that COs rates were significantly negatively correlated with G+C content and were not significantly correlated with the presence of genes or transposons [29]. Additionally, Singer *et al.* [30**] used a whole-genome exon array to map 16 000 single-feature polymorphisms in a collection of *Arabidopsis* recombinant inbred lines. Highly variable rates of recombination per physical distance were observed on all five chromosomes. Recombination hot spots had recombination rates as much as 70-fold greater than the genome average. The *Arabidopsis quartet* mutant has been used to show that the fractions of interference-insensitive COs on Chromosomes 2 and 4 are significantly smaller than those on the other three chromosomes. Lam *et al.* [31] hypothesized that the presence of nucleolus organizing regions (NORs) on these two chromosomes reduces the need for non-interfering COs.

Although the maize genome has not yet been fully sequenced, various methods have been used to study recombination in this species on a genome-wide scale. Single-copy *in situ* hybridization (FISH) analysis of maize chromosome 9, in combination with an analysis of genetic maps, showed a dramatic reduction in recombination rates in pericentromeric regions [32]. Consistently, cytogenetic mapping of recombination nodules (RNs) across the ten maize chromosomes showed that distal portions of chromosome arms have the highest average number of RNs and that the frequencies of RNs decrease towards the kinetochore [33]. The RN-cM map [34] was used to position 1195 expressed sequence tags (ESTs) onto the ten pachytene chromosomes. Strong correlations were observed between relative EST and RN frequencies for 2- μ m intervals, suggesting that recombination is closely associated with genes in maize [35]. Similarly, in wheat, gene densities are positively correlated with recombination rates along

chromosomes [36] and recombination rates are dramatically reduced in centromeric regions [37]. These results differ from the previously discussed findings from chromosome 4 of *Arabidopsis* [29]. One possible explanation for this difference is that the self-fertilizing mating behavior of *Arabidopsis* might decrease the influence of recombination on genome organization. By contrast, maize is a strongly cross-fertilizing species that exhibits high levels of DNA sequence polymorphism. Given the dense genetic map that is available for maize [38], the completion of the on-going maize genome-sequencing project will enhance our understanding of the distribution of recombination along maize chromosomes.

Gene targeting and homologous recombination

Altering genes in their native environment is a powerful tool with which to study gene function and to modify organisms genetically. Although gene targeting has been widely applied in yeast and mice, its efficiency in plants is still not high enough for routine applications [39–41,42*]. Various methods have been tested to increase the efficiency of gene targeting in plants. DSBs that are generated by a rare cutting restriction enzyme, I-SceI, can enhance homologous integration frequency at the target site [43]. However, this strategy involves transgenic target sites that are inserted into the genome at random, and thus it is not likely to target endogenous genes [44].

Recently, two groups reported the use of zinc-finger nucleases (ZFN) to target specific genome sequences for mutagenesis [45**] and gene modification [46**]. A ZFN consists of a set of (usually three) zinc-finger motifs and a non-specific endonuclease, such as FokI [47*]. Each zinc-finger motif comprises about 30 amino acids and recognizes a DNA triplet. Dimerization of ZFNs is required for the efficient cleavage of double-strand DNA; two recognition sites in inverted orientation flanking a 6-base spacer promote cleavage [48,49]. A DSB is introduced into the target sequence when ZFNs bind to recognition sites, dimerize, and activate the endonuclease. Lloyd *et al.* [45**] separately transformed into *Arabidopsis* a construct containing a three-finger ZFN under the control of a heat-shock promoter and a synthetic recognition site for ZFN. Single-locus transformant lines were selected and subjected to heat shock to activate the ZFN and the resulting mutations analyzed. The mutation frequency at the ZFN recognition site was estimated to be as high as 0.2 (average 0.08) mutations per ZFN recognition site assayed by the disruption of an *EcoRI* site in the targeted sequence. These mutations range from simple deletions of 1–52 bp (78%), simple insertions of 1–4 bp (13%) and deletions/insertions (8%). Using tobacco, Wright *et al.* [46**] measured the rate of homologous recombination (HR) induced by a three-finger ZFN through the repair of a defective β -glucuronidase (GUS)::NPTII site transgene with a ZFN recognition site. Protoplasts from 10 transgenic

lines were electroporated with DNA that encoded ZFN and repair template donor DNA, and then screened for HR products. One of 50 transformants carried HR products without additional insertions or deletions. The high rate of targeting from both studies is encouraging. Because two inverted recognition sites are required, a three-finger ZFN actually has a recognition site of 18 bases. With a library of three-finger ZFN, it should be possible to target naturally occurring ZFN recognition sites in most of the genes in a plant genome [45**,46**].

Another approach for enhancing gene targeting efficiency relies on overexpressing endogenous plant recombination genes or heterologous HR pathway genes, but this has not typically been sufficient to alter recombination rates [50,51]. Recently, Shaked *et al.* [52] demonstrated that overexpressing the yeast Rad54 protein, a member of the SWI2/SNF2 chromatin remodeling gene family, greatly increased the rate of HR and at the same time seems to reduce NHEJ events. Expression of RAD54 increased the rate of gene targeting by an order of magnitude. In fungi, the disruption of Ku70 greatly increases the rate of gene targeting [9,10,53**,54]. *Arabidopsis* homologs of Ku70 and Ku80 [55] have been identified [56]. It is thus possible that inhibiting the NHEJ pathway could be used to increase rates of HR (and thus gene targeting) in plants.

Conclusions

The availability of additional genome sequences will enable global analyses of DSBs and recombination breakpoints, the identification of correlations between recombination breakpoints and genomic features such as DNA sequences or expression status, and the identification of homologous or novel genes that contribute to or regulate recombination pathways. We also anticipate that the combination of ZFN and the manipulation of endogenous recombination pathways will yield robust and efficient protocols for targeted gene replacement and mutagenesis in plants.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Paques F, Haber JE: **Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*.** *Microbiol Mol Biol Rev* 1999, **63**:349-404.
2. Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW: **The double-strand-break repair model for recombination.** *Cell* 1983, **33**:25-35.

3. Allers T, Lichten M: **Differential timing and control noncrossover and crossover recombination during meiosis.** *Cell* 2001, **106**:47-57.
 4. Hunter N, Kleckner N: **The single-end invasion: an asymmetric intermediate at the double-strand break to double-Holliday junction transition of meiotic recombination.** *Cell* 2001, **106**:59-70.
 5. Ira G, Malkova A, Liberi G, Foiani M, Haber JE: **Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast.** *Cell* 2003, **115**:401-411.
 6. Allen C, Kurimasa A, Brennemann MA, Chen DJ, Nickoloff JA: **DNA-dependent protein kinase suppresses double-strand break-induced and spontaneous homologous recombination.** *Proc Natl Acad Sci USA* 2002, **99**:3758-3763.
 7. Pierce AJ, Hu P, Han M, Ellis N, Jasin M: **Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells.** *Genes Dev* 2001, **15**:3237-3242.
 8. Boyko A, Zemp F, Filkowski J, Kovalchuk I: **Double-strand break repair in plants is developmentally regulated.** *Plant Physiol* 2006, **141**:488-497.
 9. Ninomiya Y, Suzuki K, Ishii C, Inoue H: **Highly efficient gene replacements in *Neurospora* strains deficient for nonhomologous end-joining.** *Proc Natl Acad Sci USA* 2004, **101**:12248-12253.
 10. Poggeler S, Kuck U: **Highly efficient generation of signal transduction knockout mutants using a fungal strain deficient in the mammalian ku70 ortholog.** *Gene* 2006, **378**:1-10.
 11. Lichten M, Goldman AS: **Meiotic recombination hotspots.** *Annu Rev Genet* 1995, **29**:423-444.
 12. Mezard C: **Meiotic recombination hotspots in plants.** *Biochem Soc Trans* 2006, **34**:531-534.
 13. Schnable PS, Hsia AP, Nikolau BJ: **Genetic recombination in plants.** *Curr Opin Plant Biol* 1998, **1**:123-129.
 14. Opperman R, Emmanuel E, Levy AA: **The effect of sequence divergence on recombination between direct repeats in *Arabidopsis*.** *Genetics* 2004, **168**:2207-2215.
 15. Li L, Santerre-Ayotte S, Boivin EB, Jean M, Belzile F: **A novel reporter for intrachromosomal homoologous recombination in *Arabidopsis thaliana*.** *Plant J* 2004, **40**:1007-1015.
 16. Yao H, Schnable PS: **Cis-effects on meiotic recombination across distinct *a1-sh2* intervals in a common *Zea* genetic background.** *Genetics* 2005, **170**:1929-1944.
 17. Li L, Jean M, Belzile F: **The impact of sequence divergence and DNA mismatch repair on homeologous recombination in *Arabidopsis*.** *Plant J* 2006, **45**:908-916.
 18. Yandea-Nelson MD, Zhou Q, Yao H, Xu X, Nikolau BJ, Schnable PS: ***MuDR* transposase increases the frequency of meiotic crossovers in the vicinity of a *Mu* insertion in the maize *a1* gene.** *Genetics* 2005, **169**:917-929.
 19. Yandea-Nelson MD, Nikolau BJ, Schnable PS: **Effects of trans-acting genetic modifiers on meiotic recombination across the *a1-sh2* interval of maize.** *Genetics* 2006, **174**:101-112.
 20. González-Barrera S, Cortés-Ledesma F, Wellinger RE, Aguilera A: **Equal sister chromatid exchange is a major mechanism of double-strand break repair in yeast.** *Mol Cell* 2003, **11**:1661-1671.
 21. Roeder GS: **Meiotic chromosomes: it takes two to tango.** *Genes Dev* 1997, **11**:2600-2621.
 22. Molinier J, Ries G, Bonhoeffler S, Hohn B: **Interchromatid and interhomolog recombination in *Arabidopsis thaliana*.** *Plant Cell* 2004, **16**:342-352.
 23. Jelesko JG, Carter K, Thompson W, Kinoshita Y, Grusis W: **Meiotic recombination between paralogous *RBCSB* genes on sister chromatids of *Arabidopsis thaliana*.** *Genetics* 2004, **166**:947-957.
 24. Yandea-Nelson MD, Xia Y, Li J, Neuffer MG, Schnable PS: **Unequal sister chromatid and homolog recombination at a tandem duplication of the *A1* locus in maize.** *Genetics* 2006, **173**:2211-2226.
- A recent paper comparing unequal recombination between sister chromatids and homolog chromosomes at a naturally occurring tandem duplication.
25. Messing J, Bharti AK, Karlowski WM, Gundlach H, Kim HR, Yu Y, Wei F, Fuks G, Soderlund CA, Mayer KF, Wing RA: **Sequence composition and genome organization of maize.** *Proc Natl Acad Sci USA* 2004, **101**:14349-14354.
 26. Emrich SJ, Li L, Wen T-J, Yandea-Nelson MD, Fu Y, Guo L, Chou H-H, Aluru S, Ashlock DA *et al.*: **Nearly identical paralogs (NIPs) and implications for maize (*Zea mays* L.) genome evolution.** *Genetics* 2006, in press.
 27. Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H *et al.*: **A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*).** *Science* 2002, **296**:92-100.
 28. Zhang L, Gaut BS: **Does recombination shape the distribution and evolution of tandemly arrayed genes (TAGs) in the *Arabidopsis thaliana* genome?** *Genome Res* 2003, **13**:2533-2540.
 29. Drouaud J, Camilleri C, Bourguignon PY, Canaguier A, Berard A, Vezon D, Giancola S, Brunel D, Colot V, Prum B *et al.*: **Variation in crossing-over rates across chromosome 4 of *Arabidopsis thaliana* reveals the presence of meiotic recombination 'hot spots'.** *Genome Res* 2006, **16**:106-114.
 30. Singer T, Fan Y, Chang HS, Zhu T, Hazen SP, Brigg SP: **A high-resolution map of *Arabidopsis* recombinant inbred lines by whole-genome exon array hybridization.** *PLoS Genet* 2006, **2**:e144.
- An exciting technology for studying recombination on a genome-wide scale.
31. Lam SY, Horn SR, Radford SJ, Housworth EA, Stahl FW, Copenhaver GP: **Crossover interference on nucleolus organizing region-bearing chromosomes in *Arabidopsis*.** *Genetics* 2005, **170**:807-812.
 32. Wang CJ, Harper L, Cande WZ: **High-resolution single-copy gene fluorescence *in situ* hybridization and its use in the construction of a cytogenetic map of maize chromosome 9.** *Plant Cell* 2006, **18**:529-544.
 33. Anderson LK, Doyle GG, Brigham B, Carter J, Hooker KD, Lai A, Rice M, Stack SM: **High-resolution crossover maps for each bivalent of *Zea mays* using recombination nodules.** *Genetics* 2003, **165**:849-865.
 34. Anderson LK, Salameh N, Bass HW, Harper LC, Cande WZ, Weber G, Stack SM: **Integrating genetic linkage maps with pachytene chromosome structure in maize.** *Genetics* 2004, **166**:1923-1933.
 35. Anderson LK, Lai A, Stack SM, Rizzon C, Gaut BS: **Uneven distribution of expressed sequence tag loci on maize pachytene chromosomes.** *Genome Res* 2006, **16**:115-122.
 36. Akhunov ED, Goodyear AW, Geng S, Qi LL, Echalié B, Gill BS, Miftahudin, Gustafson JP, Lazo G, Chao S *et al.*: **The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms.** *Genome Res* 2003, **13**:753-763.
 37. Peng JH, Zadeh H, Lazo GR, Gustafson JP, Chao S, Anderson OD, Qi LL, Echalié B, Gill BS, Dilbirli M *et al.*: **Chromosome bin map of expressed sequence tags in homoeologous group 1 of hexaploid wheat and homoeology with rice and *Arabidopsis*.** *Genetics* 2004, **168**:609-623.
 38. Fu Y, Wen TJ, Ronin YI, Chen HD, Guo L, Mester DI, Yang Y, Lee M, Korol AB, Ashlock DA, Schnable PS: **Genetic dissection of intermated recombinant inbred lines using a new genetic map of maize.** *Genetics* 2006, **174**:1671-1683.
 39. Puchta H: **Gene replacement by homologous recombination in plants.** *Plant Mol Biol* 2002, **48**:173-182.
 40. Hanin M, Paszkowski J: **Plant genome modification by homologous recombination.** *Curr Opin Plant Biol* 2003, **6**:157-162.

41. Lyznik LA, Gordon-Kamm WJ, Tao Y: **Site-specific recombination for genetic engineering in plants.** *Plant Cell Rep* 2003, **21**:925-932.
42. Iida S, Terada R: **Modification of endogenous natural genes by gene targeting in rice and other higher plants.** *Plant Mol Biol* 2005, **59**:205-219.
A comprehensive review on the progress made in gene targeting in plants.
43. Puchta H, Dujon B, Hohn B: **Homologous recombination in plant cells is enhanced by *in vivo* induction of double strand breaks into DNA by a site-specific endonuclease.** *Nucleic Acids Res* 1993, **21**:5034-5040.
44. Puchta H, Dujon B, Hohn B: **Two different but related mechanisms are used in plants for the repair of genomic double-strand breaks by homologous recombination.** *Proc Natl Acad Sci USA* 1996, **93**:5055-5060.
45. Lloyd A, Plaisier CL, Carroll D, Drews GN: **Targeted mutagenesis using zinc-finger nucleases in *Arabidopsis*.** *Proc Natl Acad Sci USA* 2005, **102**:2232-2237.
A recent paper describing the use of zinc-finger nuclease in targeted mutagenesis via homologous recombination in *Arabidopsis*.
46. Wright DA, Townsend JA, Winfrey RJ Jr, Irwin PA, Rajagopal J, Lonosky PM, Hall BD, Jondle MD, Voytas DF: **High-frequency homologous recombination in plants mediated by zinc-finger nucleases.** *Plant J* 2005, **44**:693-705.
A recent paper describing the use of zinc-finger nuclease in gene targeting via homologous recombination in tobacco.
47. Durai S, Mani M, Kandavelou K, Wu J, Porteus MH, Chandrasegaran S: **Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells.** *Nucleic Acids Res* 2005, **33**:5978-5990.
A review focusing on the principal of zinc-finger motif design, current progress, obstacles and possible solutions.
48. Smith J, Bibikova M, Whitby FG, Reddy AR, Chandrasegaran S, Carroll D: **Requirements for double-strand cleavage by chimeric restriction enzymes with zinc finger DNA-recognition domains.** *Nucleic Acids Res* 2000, **28**:3361-3369.
49. Mani M, Smith J, Kandavelou K, Berg JM, Chandrasegaran S: **Binding of two zinc finger nuclease monomers to two specific sites is required for effective double-strand DNA cleavage.** *Biochem Biophys Res Commun* 2005, **334**:1191-1197.
50. Reiss B, Schubert I, Kopchen K, Wendeler E, Schell J, Puchta H: **RecA stimulates sister chromatid exchange and the fidelity of double-strand break repair, but not gene targeting, in plants transformed by *Agrobacterium*.** *Proc Natl Acad Sci USA* 2000, **97**:3358-3363.
51. Shalev G, Sitrit Y, Avivi-Ragolski N, Lichtenstein C, Levy AA: **Stimulation of homologous recombination in plants by expression of the bacterial resolvase *ruvC*.** *Proc Natl Acad Sci USA* 1999, **96**:7398-7402.
52. Shaked H, Melamed-Bessudo C, Levy AA: **High-frequency gene targeting in *Arabidopsis* plants expressing the yeast *RAD54* gene.** *Proc Natl Acad Sci USA* 2005, **102**:12265-12269.
53. Krappmann S, Sasse C, Braus GH: **Gene targeting in *Aspergillus fumigatus* by homologous recombination is facilitated in a nonhomologous end-joining-deficient genetic background.** *Eukaryot Cell* 2006, **5**:212-215.
A recent paper describing the application of zinc-finger nuclease in gene targeting via targeted mutagenesis in *Arabidopsis*.
54. Takahashi T, Masuda T, Koyama Y: **Enhanced gene targeting frequency in *ku70* and *ku80* disruption mutants of *Aspergillus sojae* and *Aspergillus oryzae*.** *Mol Genet Genomics* 2006, **275**:460-470.
55. Gallego ME, Bleuyard JY, Daoudal-Cotterell S, Jallut N, White Cl: **Ku80 plays a role in non-homologous recombination but is not required for T-DNA integration in *Arabidopsis*.** *Plant J* 2003, **35**:557-565.
56. Tamura K, Adachi Y, Chiba K, Oguchi K, Takahashi H: **Identification of *Ku70* and *Ku80* homologues in *Arabidopsis thaliana*: evidence for a role in the repair of DNA double-strand breaks.** *Plant J* 2002, **29**:771-781.