IN THIS ISSUE

Homologous Recombination in Higher Plants: Clues from fasciata1-4, a New Chromatin Formation Mutant of Arabidopsis

Genetic recombination is one of the most fundamental and significant events in the history of life because it plays a central role in creating genetic diversity and safeguarding and maintaining genomic integrity. Homologous recombination (HR) was first described by Thomas Hunt Morgan (Morgan, 1916) and results from crossing-over of paired non-sister chromatids during prophase I of meiosis (see figure). HR has been extensively studied, and the functions of many of the key factors are well understood in a number of model organisms. All students of biology are familiar with the basic steps, which result in an exchange of information generating daughter chromosomes that comprise unique combinations of genetic loci. Any pair of homologous chromosomes may be expected to cross over multiple times during meiosis, and more advanced students will also be familiar with how this seemingly simple process becomes more and more complicated the more it is studied.

In fact, some essential aspects of HR remain murky. For example, although HR has been exploited for targeted gene replacement in a number of model organisms, such as yeast, mice, and Drosophila, higher plants remain largely recalcitrant to this approach, owing to low efficiencies of HR and poor reproducibility for reasons that are not well understood (reviewed in Hanin and Paszkowski, 2003; Puchta, 2003; Reiss, 2003). Targeted gene replacement is an exceptionally powerful tool that allows in-depth analysis of gene function through the creation of highly specific alleles. Transformation vectors are designed to facilitate HR and selection of recombinant progeny, resulting in genomic replacement of an endogenous gene with an in vitro-modified copy. Winzeler et al. (1999) used this approach to analyze the functions of nearly all of the 6200 open reading frames of the yeast Saccharomyces cerevisiae, and it is routinely used to investigate gene function in mice and Drosophila. Gene targeting has enormous potential for the precise engineering of transgenes for commercial agronomic and therapeutic applications, and its application in higher plants would be a most welcome addition to plant genomics.

Hanin et al. (2001) and Terada et al. (2002) reported relatively high efficiencies of gene targeting using HR in Arabidopsis and rice, respectively, but these reports did not lead to an immediate breakthrough in the technology, and reproducibility in both systems remained relatively low. Recently, a few more reports of successful gene targeting in plants have appeared, indicating that the technology is improving. Shaked et al. (2005) found that expression of the yeast RAD54 chromatin remodeling gene enhanced HR in Arabidopsis by one to two orders of magnitude, providing evidence that chromatin conformation is a factor restricting HR in plants. It is also known that HR can be enhanced by the creation of double-strand breaks at the target site (Puchta et al., 1996), and Wright et al. (2005) demonstrated the feasibility of designing zinc-finger nucleases to induce double-strand breaks and enhance HR at specific target sites in plants. Nonetheless, a deeper understanding of factors controlling and restricting HR in plants is needed.

Observations of high efficiencies of extrachromosomal HR in plants (which occurs, for example, in engineered recombination systems and between T-DNAs during Agrobacterium-mediated transformation), but routinely low efficiencies of chromosomal HR, have long suggested that chromatin might play a role in restricting HR in plant systems (reviewed in Reiss, 2003). In this issue of The Plant Cell, Kirik et al. (pages 2431–2442) provide further evidence that chromatin conformation is a major factor restricting HR in higher plants, through analysis of an Arabidopsis mutant with defects in chromatin assembly factor 1 (CAF-1), a heterotrimeric complex that is required for in vitro nucleosome assembly onto newly replicated chromatin in eukaryotic systems. The authors isolated a new allele of FASCIATA1 (FAS1), which encodes the p150 subunit of CAF-1. Plants containing the fas1–4 allele exhibit a severe developmental phenotype and reduced heterochromatin content compared with the wild type, along with a more open conformation of euchromatin, and, significantly, an ~100-fold enhanced rate of intrachromosomal HR, which is by far the strongest effect on intrachromosomal HR of all chromatin mutants analyzed in plants thus far. These results, together with nearly normal expression of several known HR genes in the fas1–4 mutant, suggest that chromatin conformation is a key factor limiting HR in plants.

CAF-1 is evolutionarily conserved, and genes encoding the three subunits, p150, p60, and p48, are present in yeast and human cells as well as in plants. In human cells, CAF-1 interacts directly with proliferating cell nuclear antigen and functions to tether this complex to the growing replication fork during DNA replication. CAF-1 function has been linked to DNA replication, transcription, and error-free repair of DNA lesions. It is also well known that HR is influenced by the chromatin state, with euchromatic regions being more permissive for HR than heterochromatic regions. The authors propose that the defects in chromatin assembly factor 1 may affect CAF-1-mediated nucleosome assembly, thereby altering chromatin conformation and facilitating HR.

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Simple Scheme to Illustrate Crossing-Over of Chromosomes during Meiosis.

Reproduced from Morgan (1916), p. 132.
repair mechanisms, as defects in both yeast and human cells cause sensitivity to DNA damage. However, there is evidence of functional differences in CAF-1 between yeast and mammalian systems, and the full range of its function in plants is unknown. Arabidopsis fas1 and fas2 mutants, affecting genes encoding the p150 and p60 CAF-1 subunits, respectively, have been described by Leyser and Furner (1992) and Kaya et al. (2000, 2001). The fas1-1, fas1-2, and fas1-3 alleles show serrated leaves, fascination with broad stems, and abnormal phyllotaxy, which have been attributed to a destabilization of meristem identity gene expression leading to defects in the shoot and root apical meristems (Kaya et al., 2001). By contrast, the fas1-4 mutant described by Kirik et al. was severely retarded in growth of all organs and showed severe developmental abnormalities (the mutant plants nonetheless grew to maturity, flowered, and set seed, although with drastically reduced yield).

The reasons for the differences between fas1-4 and the other fas1 mutant alleles are not clear, as all of the alleles are believed to cause loss of function of CAF-1 (Kaya et al., 2001; Kirik et al., 2006). However, the fas1-4 mutation appears to be a stronger allele than the other fas1 mutant alleles, with effects that are not restricted to meristems. The development of techniques allowing subcellular assessment of the amount and localization of the CAF-1 complex might help to explain these differences as well as to further enhance our understanding of CAF-1 function. Another important test would be to measure intrachromosomal HR in the other fas1 and fas2 mutants. Recently, Shonrock et al. (2006) found that the CAF-1 defect in fas1-1 leads to a reduction of heterochromatin, although perhaps not as pronounced as that observed by Kirik et al. in the more severely affected fas1-4 mutants, again suggesting that CAF-1 affects chromatin conformation states.

Ono et al. (2006) observed released repression of transcriptional gene silencing in fas1-1 and fas2-1 mutants and concluded that CAF-1 ensures the fidelity of stable propagation of silent chromatin states during plant growth and development. Shonrock et al. (2006) concluded that silencing of most transposons and heterochromatic genes is independent of CAF-1, based on analysis of several pericentromeric loci and large-scale microarray gene expression analysis in CAF-1 mutants. However, Ono et al. (2006) examined expression of several typically silent genes (including CACTA and MUTI elements) and found evidence for stochastic derepression of gene silencing in the fas1 mutants. Genes at different chromosomal loci were derepressed independently and randomly in fas plants. These authors speculated that disruption of rapid nucleosome formation in the absence of CAF-1 might leave replicated DNA naked and easily accessible for a longer period of time, increasing the probability of accidental transpositional activation (Ono et al., 2006). All three groups (Kirik et al., 2006; Ono et al., 2006; Shonrock et al., 2006) found the gross level of centromeric DNA methylation to be unaffected in the fas1 mutants. These data are in accord with the conclusion of Kirik et al. that CAF-1 affects chromatin conformation independently of DNA methylation. It could therefore be of great interest to measure the rate of HR and the extent of open conformation of chromatin in these other fas mutants.

The work of Kirik et al. provides further insight into the role of chromatin states in controlling HR in plants, which should help to guide investigations aimed at improving gene targeting approaches, and enhances our knowledge of the fundamental process of genetic recombination.

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