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The road to crossovers: plants have their say

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Crossovers involve the reciprocal exchange of large fragments of genetic material between homologous chromosomes during meiosis. In this way, crossovers are the basis of genetics. Remarkably, the number and distribution of crossovers on chromosomes are closely controlled. Data from various model organisms (notably *Saccharomyces cerevisiae*) show that the distribution of crossovers results from a series of tightly regulated events involving the formation and repair of double-strand breaks and interference. Recent advances in genetic and cytological tools, particularly for studying *Arabidopsis thaliana*, have enabled crossover control in plants to be studied in more detail. In this article, we discuss the contribution of plant studies to meiosis research, particularly to our understanding of crossover control and interference, and we evaluate models of interference.

Introduction

The crossover (see Glossary) and its cytological signature, the chiasma, are major features of genetics. Our knowledge of the molecular mechanisms of crossover formation has increased considerably in the past decade, owing to studies of fungi [1,2]. Nevertheless, little is known about controlling the number of crossovers and their distribution along chromosomes, except for the remarkable observation that crossovers and/or chiasma are not randomly distributed [3]. However, it has been observed that when this control is affected, the missegregation of chromosomes markedly increases, resulting in aneuploid gametes in organisms as diverse as yeast, nematodes, mammals and plants [4]. The current view is that the distribution of crossovers results from three constraints. First, each pair of chromosomes, regardless of size, has at least one chiasma (known as the obligatory chiasma), which is essential for the segregation of homologous chromosomes at the first meiotic division. Second, crossovers are not independent of each other: the occurrence of one crossover inhibits the occurrence of another such event in a distance-dependent manner, resulting in crossovers being spaced more evenly along the chromosomes than would be expected if they occurred independently. This phenomenon is known as 'positive' interference and is referred to as interference throughout this article [3]. Third, separately from the influence of interference, the densities of crossovers along chromosomes vary greatly, so there is a nonhomogeneous distribution of crossovers.

Plants have always been at the forefront of the study of heredity (Box 1). Now, plant models can be used to study meiosis and recombination. This is because they not only enable use of the rare combination of both genetic and cytological approaches but also, owing mainly to technological progress with *Arabidopsis thaliana*, a full range of molecular approaches can be used. The data obtained in plants contribute to our general understanding of meiosis,

Glossary

Chiasma: the cytological signature of a crossover. Chiasmata are observed as connections between the homologous chromosomes in bivalents during meiosis, from diakinesis to metaphase I.

Crossovers: one of the products of meiotic DSB repair. The repair process, through breaking and rejoining DNA molecules, results in the reciprocal exchange of large fragments of genetic material (i.e. the exchange of homologous regions). Therefore, the genetic outcome of crossovers is the reassociation of genetic markers located on both sides of the crossover point. Also called crossing-overs.

Gene conversion: the nonreciprocal exchange of small fragments of genetic material (i.e. homologous regions), resulting in a non-mendelian segregation of genetic markers. Gene conversions are formed by the repair of a short region around a DSB site by homologous recombination, using the chromatid of the homologous partner chromosome as a template. They can be 'single' or associated with a crossover.

Leptotene: the first of the four substages of prophase in the first meiotic division (i.e. prophase I). During this substage, individual chromosomes condense into long strands. Initiation of recombination (i.e. DSBs) occurs at this stage.

Noncrossovers: the subset of meiotic DSB-repair events that is not associated with crossovers. Noncrossovers can be genetically detectable, in which case they are called single gene-conversion events. Whether noncrossovers are genetically detectable depends on the presence or absence of a polymorphism in the conversion tract (i.e. the fragment of DNA that has been exchanged in the process).

Pachytene: the third of the four substages of prophase in the first meiotic division (i.e. prophase I). This substage is characterized by completion of the polymerization of the synaptonemal complex along homologous chromosomes. Recombination is completed at this stage.

Recombination nodules: electron-dense structures located on chromosomes. These are observed in electron micrographs of meiotic chromosomes during prophase I. During zygotene, the early nodules (ENs) have a variable shape: for example, round and small in tomato (*Solanum lycopersicum*), and ellipsoidal and spherical in *Allium* species. These nodules disappear early in pachytene. The nodules that remain at pachytene are called late nodules (LNs), and they are larger and are usually ellipsoidal.

Synaptonemal complexes: structures that are specific to meiosis. By electron microscopy, a synaptonemal complex appears as a ladder-like structure that forms at zygotene, and formation of this complex is completed at pachytene. The two uprights of the ladder consist of each axial element (the continuous protein structures formed along each chromosome at leptotene) of the two homologous chromosomes (at this stage called lateral elements), and the rungs consist of a dimer of the protein Zip1. The function of synaptonemal complexes has not been determined.

Zygotene: the second of the four substages of prophase in the first meiotic division (i.e. prophase I). This substage is characterized by formation of the synaptonemal complex, which progressively links closely the pairs of homologous chromosomes. Recombination progresses during this stage.

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Box 1. Genetics in plants: from *Pisum sativum* to *Arabidopsis thaliana*

Since Mendel established the law of segregation of independent characteristics using the pea (*Pisum sativum*), plants have been at the heart of the history of genetics. Mendel's work was ignored for three decades, until his laws were rediscovered independently through studies of a large range of plants, including *Trifolium pratense* (red clover), *Silene latifolia* subsp. alba (white campion), *Papaver somniferum* (opium poppy), *Zea mays* (maize) and *P. sativum* [69,70]. The first partial linkage was found in *Lathyrus odoratus* (sweet pea) [71]. Although the data were not initially interpreted as a meiotic reassociation of characteristics, this was the first report of a genetic crossover. The first demonstration that crossovers are associated with physical recombination of chromosomes was in *Z. mays* [72], and the first evidence for chiasmata interference, predicted from crossover interference, was obtained in *Vicia faba* (fava bean) [70,73].

Plants have been fundamental in numerous other genetic discoveries. Mobile DNA elements were first reported in *Z. mays* [74]. RNA-mediated silencing was first discovered in transgenic *Petunia hybrida*, and plants still have a central role in deciphering the underlying mechanisms of gene silencing [75]. One of the current favorite models of plant geneticists is *Arabidopsis thaliana* because of its suitability for combined molecular genetic and cytological analyses.

particularly owing to the comparisons that can be made with data obtained in other model organisms. Here, we review recent findings on crossovers in plants, particularly those concerning key genes in *Arabidopsis*, and we relate these findings to the numerous genetic and cytological data from other species, including yeast, on the distribution of crossovers and the role of interference.

Different manifestations of recombination: from yeast to plants

Recombination was first defined as the generation of a new combination of genes [5]. Now, this definition can be extended to a reassortment of markers. In meiosis, this can arise either by crossover or by 'single' gene conversion. Recently, the term noncrossover has been introduced to designate DNA double-strand break (DSB)-repair events that are not associated with crossovers. Noncrossovers include single gene-conversion events and events that are not genetically detectable. Crossover and noncrossover events are products of the repair of programmed DSBs [6]. It is also probable that some repair events occur on the sister chromatid [sister chromatid exchange (i.e. SCE)]; however, these events are difficult to analyze, so their frequency is not easy to estimate [7]. In budding yeast (*Saccharomyces cerevisiae*), meiotic DSBs are caused by a set of 11 proteins, including Spo11, which directly generates the DSBs [6] (Figure 1). Only four of these proteins (Spo11, Mre11, Rad50 and Ski8) are conserved in *Arabidopsis*. However, there are three Spo11 homologs in *Arabidopsis*. The *Arabidopsis* proteins AtSPO11-1 and AtSPO11-2 are required to initiate meiotic recombination [8,9]. Mre11 and Rad50 are involved in both the formation and the repair of DSBs in yeast, but their orthologs are not required for DSB formation in *Arabidopsis* [10,11]. It is also clear that the homolog of Ski8 is not required for meiosis in plants [12]. Although the mechanism of initiation of DSB formation is conserved across all species, there are clear differences in the control of this process.

Numerous data on crossover distribution in plants are available, largely because crossovers are the basis of genetic maps. By contrast, information about noncrossover distribution is poor, because these events are difficult to visualize by classical genetic analyses and have no (or little) impact on genetic maps. Crossovers can also be studied by cytology, visualized as chiasmata or as late recombination nodules (LNs). Chiasmata reflect the same molecular event as crossovers [3] and are powerful and easy markers for counting the number of crossovers per cell and per chromosome. However, counting in this way could result in a slight underestimation, because, even in plants with large genomes, chiasmata that are close together cannot be resolved unambiguously [13]. Individual chiasmata can be visualized only in a few organisms, such as grasshoppers [3]. LNs lie on the central region of synaptonemal complexes during pachytene and closely reflect the sites of crossovers, providing a much better resolution than that provided by chiasmata [14,15]. However, the technique for efficient visualization of LNs is applicable to only a few species. At an earlier substage in prophase I (zygotene), there is a much larger population of nodules, called early recombination nodules (ENs). Their distribution, their protein content and the timing of their appearance indicate that ENs might mark the sites of recombination intermediates [14]. The recombinases Rad51 and Dmc1 are involved in meiotic DSB repair and colocalize with ENs [16]. These two proteins are not observed in all ENs at a given time, but this is probably because ENs have progressed through the process of DSB repair to different extents. The genes *ATRAD51* and *ATDMC1* have been functionally characterized in *Arabidopsis*. As expected, the *atrad51* mutant shows strong meiotic fragmentation of its chromosomes [17]. Surprisingly, in *Arabidopsis atdmc1* mutants, DSBs seem to be repaired using the sister chromatid, although they are not repaired in similar yeast mutants [18,19]. Another interesting difference is that recombination defects result in apoptosis in mammals, whereas, in *Arabidopsis*, meiosis progresses regardless of the defect, allowing access to more information about the mutant phenotype.

Crossover frequency varies along chromosomes

In all eukaryotes that have been studied, including plants, the distribution of crossovers or LNs along chromosomes is not homogeneous [15,20], on either a megabase or a kilobase scale (Figure 2). That is, the local probability of a crossover varies among different chromosome intervals. This nonhomogeneity is the basis of the definition of 'hot' and 'cold' regions (which have significantly high and low crossover frequencies, respectively). A general rule is that centromeres are cold regions [21].

Plants have diverse chromosome sizes and structures, and, in each type of plant, these hot and cold regions are distributed in a particular way. In some plants – including wheat (*Triticum aestivum*), maize (*Zea mays*) and barley (*Hordeum vulgare*) – the crossover frequency tends to increase with the relative physical distance from the centromere. By contrast, in *Allium fistulosum* (Welsh onion), crossovers seem to cluster close to centromeres [3]. For other plants – for example, *Arabidopsis*, rice (*Oryza sativa*)