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Naughty Behavior of Maize Minichromosomes in Meiosis

Imagine a plant genetics lab in the year 2020. After the genomics era, the function of nearly every plant gene is known. Inexpensive resequencing methods allow easy access to natural genetic variation and creation of multiple mutant alleles for each locus. Geneticists can pursue what has been a life-long dream for many of us: studying interactions between large numbers of genes. To do this, they use engineered minichromosomes that carry very large numbers of genes in a single genetic transformation vector.

When we think about the above scenario, it is hard not to get excited about the recent advances in the development of plant artificial chromosomes. Although development of plant minichromosomes was at first slower than in other organisms, the progress in the past few years has been amazing, and in early 2007, the group of Jim Birchler at the University of Missouri–Columbia reported creating minichromosomes in maize (Yu et al., 2007). One of the most important characteristics of a useful minichromosome is its stability through cell divisions and, in particular, its behavior during meiosis. Unfortunately, this is an area that we do not know much about. It is not understood what features of chromosomes determine their faithful transmission to the progeny, and it is not clear how we can affect the meiotic behavior of chromosomes. In this issue of *The Plant Cell*, **Han et al. (pages 3853–3863)** report on their efforts to close this knowledge gap. The authors generated a collection of 22 maize minichromosomes and observed them during meiosis. They report that many minichromosomes do not behave in exactly the same way as normal chromosomes, but this may not necessarily affect their ability to be transmitted to progeny.

CHROMOSOME B–DERIVED MINICHROMOSOMES

There are essentially two ways to create minichromosomes: bottom-up and top-down (Houben and Schubert, 2007). In the first approach, chromosomes, including centromeres and often telomeres, as well as selectable markers, and, most importantly, genes of interest are combined in vitro and then delivered into the cell. The second approach, pursued by Birchler and colleagues (Yu et al., 2007), is to create a minichromosome by truncating a regularly sized chromosome. However, instead of using one of the 10 normal maize chromosomes, they used the B chromosome. B chromosomes are supernumerary, nonessential chromosomes that are found in many species of plants, animals, and fungi (Jones et al., 2007). Maize B chromosomes are heavily heterochromatic and do not seem to carry any genes. The fact that B chromosomes are similar to normal chromosomes but devoid of genes makes them ideal as gene delivery vehicles because (1) transgenes can be inserted into them without disrupting endogenous genes, which is often the case on normal gene-carrying chromosomes, and (2) they can be added and removed without any consequences for the organism. B chromosomes have an interesting propensity to accumulate in large copy numbers. They often undergo nondisjunction in the second pollen mitosis, which is followed by a preferential fertilization of the B chromosome containing sperm (Jones et al., 2007).

How can the B chromosome be truncated to make a minichromosome? One way to do it is by using a genetic trick that relies on the breakage-fusion-bridge cycle phenomenon first described by Barbara McClintock in 1939 (McClintock, 1939; Han et al., 2006). The Birchler group used this approach starting with a B chromosome that had attached to it a duplicated segment of a normal maize chromosome 9. If during meiosis, the duplicated chromosome 9 segments recombine with each other, a dicentric chromosome is formed. Then, in the second division of meiosis, one centromere is pulled to one pole, while the second centromere is pulled to the opposite pole. As a result, the chromosome breaks in the region between the two centromeres. Then, two broken chromosome ends can fuse, which leads to a bridge in the following mitosis and another breakage. The cycle continues, and the chromosome gets smaller and smaller, until the broken end is healed by an addition of a telomere or until one of the two centromeres is inactivated (Han et al., 2007). Using this method, Han et al. amassed a collection of 22 small (mini) chromosomes. With this miniature but powerful resource in hand, the authors decided to address questions about behavior of minichromosomes during meiosis and features of B chromosomes that affect this behavior.

MEIOSIS AND CHROMOSOME PAIRING

The key to proper segregation of chromosomes in meiosis are processes that take place in early meiotic prophase I. The first of these processes is establishing meiosis-specific sister chromatid cohesion. After premitotic or premeiotic DNA replication, each chromosome consists of two identical chromatids that are connected by the cohesin complex. In meiosis, this complex consists of four main proteins, SMC1, SMC3, SCC3, and REC8 (Ronceret et al., 2007). The maize homolog of REC8 is AFD1 (Golubovskaya et al., 2006). AFD1 is installed along the chromosome axis during premeiotic interphase and leptotene. Later on, before anaphase I, the cohesins are removed, except for the chromosome regions surrounding the centromeres (Figure 1A), by the action of proteinase called separase and other separase-independent mechanisms (Liu and Makaroff, 2006). In the centromeric regions, sister chromatid cohesion is protected by the SHUGOSHIN1 (SGO1) protein and persists until anaphase II (Hamant et al., 2005). This intricate system allows homologous chromosomes to exchange parts of their chromatids but still retain both chromatids in the first meiotic division. The complete removal of the cohesin complex in the second meiotic

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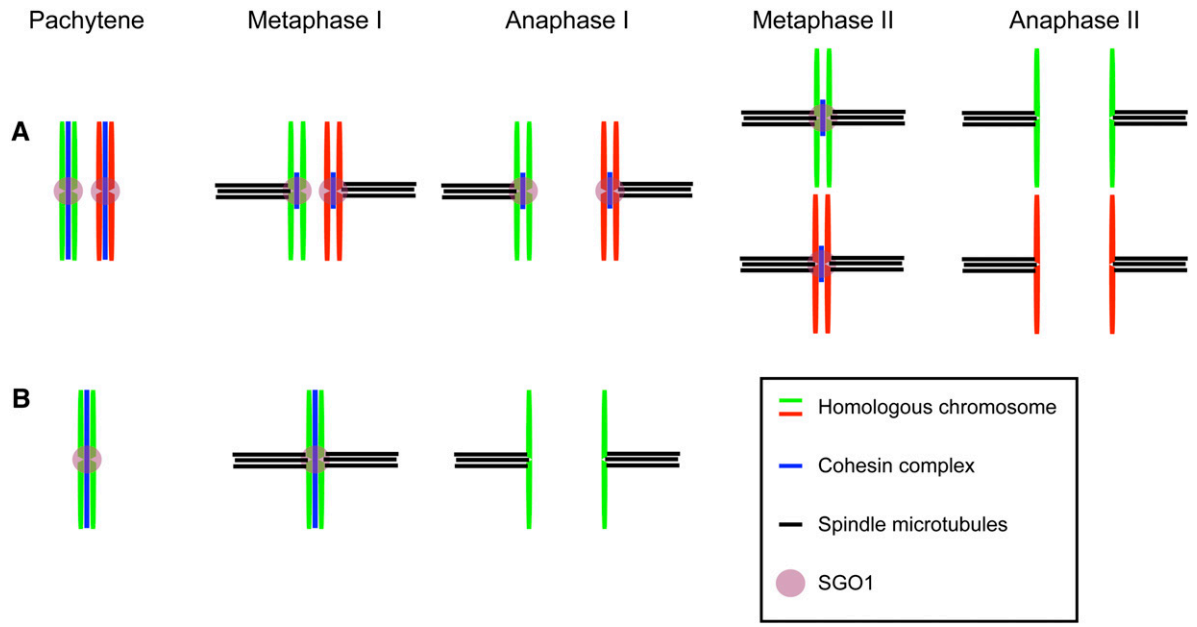

Figure 1. Sister Chromatid Cohesion.

A schematic representation of sister chromatid cohesions in normal meiosis **(A)** and of premature loss of cohesion **(B)** observed in some maize minichromosomes.

division allows sister chromatids to separate in anaphase II. In the absence of REC8, or in the case of a premature cohesin removal in the absence of SGO1, sister chromatids can segregate away precociously in meiosis I instead of meiosis II (Hamant et al., 2005; Golubovskaya et al., 2006).

Another meiotic process critical for proper chromosome segregation is homologous chromosome pairing. However, in contrast with most other meiotic processes, little is known about the mechanisms controlling chromosome pairing in plants. In most species, including plants, homologous pairing is dependent on the progression of meiotic recombination (Pawlowski and Cande, 2005). Several lines of evidence suggest that a process called single-end invasion is at the heart of chromosome homology recognition (Bozza and Pawlowski, 2008). In this process, single-stranded DNA overhangs, created by resection of meiotic double-strand breaks, probe double-stranded DNA searching for homology. However, how this microscale recognition relates to faithful pairing of entire chromosomes is less clear. Genomes of most plants, including maize, contain a large percentage of repetitive sequences that could obscure overall chromosome homology. In maize, there are ~500 single-end invasion events per cell (Franklin et al., 1999), but it is not known how many of them, if any, occur in repetitive DNA regions.

HOW DO MINICHROMOSOMES BEHAVE DURING MEIOSIS?

Our limited understanding of meiotic mechanisms prevents us from predicting the meiotic behavior of chromosomes based on their structural features. Consequently, the only way to know how minichromosomes will behave during meiosis is to test their behavior directly. This is what Han et al. have done, using fluorescence in situ hybridization probes specific to B chromosomes, which allowed them to follow the minichromosomes among all chromosomes in the cell. First, they looked at chromosome pairing in pachytene. They found that minichromosomes had lost the ability to pair with both their B and chromosome 9 progenitors. However, most B chromosomes (~64%) could still pair with each other when present in two copies per cell. Generally, larger minichromosomes were better at pairing. However, this was not always the case, one of the tiniest minichromosomes (#9) paired more frequently than some of the larger minichromosomes. Consequently, minichromosome size is not the only factor governing pairing.

Han et al. also looked at the disjunction behavior of minichromosomes in a situation with only one minichromosome present in a diploid cell. Two classes of minichromosomes could be distinguished on this basis. The first class showed regular disjunction behavior typical for univalent chromosomes (normal or B). The univalent moved to one pole during meiosis I, and sister chromatids separated at anaphase II. The second class showed an abnormal disjunction in which sister chromatids separated at meiosis I (Figure 1B). Interestingly, minichromosomes lost their autonomous ability for postmeiotic nondisjunction at the second pollen mitosis found in original B chromosomes. This mechanism of accumulation is known to require the tip of B chromosome long arm, which was absent

from the minichromosomes (Han et al., 2007). This property could be restored in trans by the addition of a full-size B chromosome.

Faced with the unusual behavior of minichromosomes, Han et al. initiated a study to understand its basis. In several lines showing premature sister chromatid separation, they followed the localization pattern of SGO1, which is thought to protect pericentromeric cohesion in meiosis I. In maize *afd1* mutants, when sister chromatids separate prematurely during anaphase I, SGO1 is not detected (Hamant et al., 2005). However, in the minichromosomes, the localization of SGO1 was normal, even though sister chromatids separated in meiosis I. They also did not find any differences between the minichromosomes and normal chromosomes for the phosphorylation pattern of the H3 histone at Ser-10, which is known to correlate with the status of sister chromatid cohesion (Kaszas and Cande, 2000).

MINICHROMOSOMES: THEY MAY BEHAVE STRANGELY, BUT THEY GET THE WORK DONE

The Han et al. study shows that minichromosomes often do not behave in the same way as normal chromosomes: they do not always pair and frequently undergo premature sister chromatid separation. However, in their abnormal way, they are still meiotically stable, and equational segregation of sister chromatids in meiosis I can alleviate the problem of reduced pairing. The fact that minichromosomes often do not pair may not be such a bad thing after all. If chromosomes do not pair, they also do not recombine, so two similar but not identical minichromosomes might be placed in one cell with no danger that their contents will eventually shuffle.

MEIOSIS' BASIC QUESTIONS

Although the immediate goal of Han et al. was to analyze the meiotic behavior of minichromosomes, several questions and answers raised by their study are brilliantly relevant for the quest to understand meiotic behavior of chromosomes in general. Because of their small size, minichromosomes could be more easily dissected to identify chromosome features that allow some of them to pair in meiosis while others do not. It will be also interesting to learn why the minichromosomes lost their ability to pair with their chromosome 9 and B chromosome progenitors. Examining whether the minichromosomes initiate recombination in the same way as normal chromosomes should be the first step of this analysis. Equally interesting will be further analysis of the authors' observation that the smallest minichromosomes are defective in meiotic sister chromatid cohesion, while the large chromosomes are not. The work of Han et al. provides a significant advancement for future efforts to design better minichromosomes and also contributes to the understanding of the behavior of normal chromosomes in meiosis.

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