Normal chromosomes enter into meiosis following DNA replication. The homologous pairs find each other in the process of synapsis. Recombination exchanges parts of the homologs involving two of the four chromatids present. Then in anaphase I, each member of the pair segregates to opposite poles and into the two cells resulting from meiosis I. In contrast to mitosis, the sister chromatids of each homolog remain attached to each other. At meiosis II the sisters now separate to opposite poles. The consequence of this sequence of events is that segregation of the two cells resulting from meiosis I. In contrast to mitosis, the sister chromatids of each homolog remain attached to each other. At meiosis II the sisters now separate to opposite poles.

The typical behavior of chromosomes in meiosis is that homologous pairs synapse, recombine, and then separate at anaphase I. At anaphase II, sister chromatids separate. However, studies of small chromosomes in maize derived from a variety of sources typically have failure of sister chromatid cohesion at anaphase I. This failure occurs whether there is pairing of two copies of a minichromosome or not. These characteristics have implications for managing the transmission of the first generation artificial chromosomes in plants. Procedures to address these issues of minichromosomes are discussed.

Keywords: minichromosomes, maize, meiosis, sister chromatid cohesion, synapsis

RECENT STUDIES OF MINICHROMOSOMES IN MEIOSIS

A collection of minichromosomes of varying lengths was examined for their behavior in meiosis by Han et al. (2007). This collection consisting of 22 chromosomes was derived from a single progenitor chromosome that was undergoing the chromosome type of the breakage-fusion-bridge (BFB) cycle (Figure 1). Zheng et al. (1999) produced the progenitor chromosome which consists of a translocation between the supernumerary B chromosome and the short arm of chromosome 9 onto which had been recombined a foldback chromosome originally recovered by McClintock.

Before describing the behavior of this collection some background is provided. The B chromosome of maize is an extra...
chromosome that is basically inert (Carlson, 1986). It is not required nor is it detrimental in low copy number. It is preserved in maize populations because it has a drive mechanism. This mechanism consists of two parts. The centromere undergoes non-disjunction at the second pollen mitosis, which produces the two maize sperm. Then the sperm with the B chromosomes preferentially fertilizes the egg as opposed to the polar nuclei in the initial gametophyte generation but at the second pollen mitosis the B centromere will undergo non-disjunction. This will place two broken chromosomes in a sperm and thus following the subsequent fertilization event, these two broken chromosomes will set up the chromosome type of BFB cycle that will continue throughout development. Using this approach, Zheng et al. (1999) and Han et al. (2007) produced a collection of minichromosomes described in the text. B repeat is the B chromosome specific sequence present in and around the centromere of the B chromosome. Knob is the repeats of heterochromatin found on maize chromosomes including the short arm of chromosome 9.

FIGURE 1 | Initiating the chromosome type BFB cycle to generate minichromosomes. A translocation between the B chromosome and the short arm of chromosome 9 TB-9Sb, was recombined with a reverse duplication of 9S to generate TB-9Sb-Dp9. This chromosome can recombine with itself as depicted which will join the sister centromeres. At meiosis II the sister centromeres will separate and create a chromatin bridge that will rupture. This broken chromosome will fuse, form a bridge, and break through the gametophyte generation but at the second pollen mitosis the B centromere will undergo non-disjunction. This will place two broken chromosomes in a sperm and thus following the subsequent fertilization event, these two broken chromosomes will set up the chromosome type of BFB cycle that will continue throughout development. Using this approach, Zheng et al. (1999) and Han et al. (2007) produced a collection of minichromosomes described in the text. B repeat is the B chromosome specific sequence present in and around the centromere of the B chromosome. Knob is the repeats of heterochromatin found on maize chromosomes including the short arm of chromosome 9.

When plants that were undergoing this cycle where crossed, the next generation contained a variety of sizes of chromosomes derived from the TB-9Sb-Dp9 progenitor (Zheng et al., 1999; Kato et al., 2005; Han et al., 2007). Some consisted of basically the centromere of the B chromosome but others were of varying lengths. Five were stable dicentrics, which were found to contain one inactive centromere (Han et al., 2008), the first found in plants. This collection of chromosomes was examined for their behavior in meiosis. When studied as one copy in meiosis (Figures 2 and 3), eight of the 22 derivatives have sister chromatid cohesion at meiosis 1 as does the normal B chromosome when present as a singleton (Han et al., 2007). In contrast, 14 of the 22 had failure of sister cohesion. When these materials were self-pollinated and individuals with two copies were selected, the pairing of these chromosomes could be examined. The larger versions could find their homologous partner. Those of intermediate size showed pairing in a range of 25–100% of the cells containing two copies. For the tiny minichromosomes, they generally cannot find their pairing partner (Figures 4 and 5) although one of the smallest was an exception in that pairing regularly occurred. This small chromosome that showed pairing nevertheless exhibited a failure of sister chromatid cohesion. This fact illustrates that
the failure of sister cohesion is not dependent on homologous pairing.

Studies from other species have identified some of the molecules involved with the cohesion properties of chromosomes in meiosis (Kitajima et al., 2004; Vaiz et al., 2005; Watanabe, 2005a,b; Kurihara et al., 2006). The Separase enzyme dissolves cohesion at anaphase I allowing the sisters to dissociate. The cohesion complex in mitosis and meiosis is distinct with Rad21, the mitotic component, being replaced by Rec8 in meiosis. The Rec8 homolog in maize has been identified as the absence of the first division (afD1) mutation (Hamant et al., 2005). In meiosis, the Shugoshin protein protects the centromere so that cohesion is maintained in meiosis I and then dissociates in anaphase II, when Shugoshin is degraded.

Interestingly, immunostaining for Shugoshin of normal B chromosomes and the minichromosomes demonstrated the presence of this protein in both cases during meiosis I even though the small chromosomes had sister separation. Apparently, chromosome size plays a role, as well as perhaps a need for adjacent pericentromeric regions, for proper establishment of cohesion.

Small chromosomes derived from telomere-mediated chromosomal truncation also exhibit a failure of sister cohesion (Yu et al., 2007; Masonbrink and Birchler, 2012; Gaeta et al., 2013). Minichromosomes derived from both the B chromosome (Yu et al., 2007) or an A chromosome (Gaeta et al., 2013) show the same behavior. A small fragment of chromosome arm 3L with a de novo centromere also shows failure of sister cohesion in meiosis I (Yu et al., 2013).

**IMPLICATIONS FOR ENGINEERED MINICHROMOSOMES**

The properties of minichromosomes have implications for the use of engineered minichromosomes. The generalized failure of pairing would indicate that a pair of minichromosomes would not segregate from each other at anaphase I but instead would proceed to the poles independently. Secondly, the failure of sister cohesion would also prevent the predictable transmission of a pair of minichromosomes, whether there is pairing or not. Of course, for vegetatively propagated species with a bypass of meiosis, there is no issue because minichromosomes with an endogenous centromere typically have good mitotic stability.

Nevertheless, while these considerations must be taken into account, these obstacles should be able to be overcome. One possible way is to use a truncated B chromosome that still contains substantial portions of the long arm present. These longer B chromosomes do not undergo non-disjunction at the second pollen mitosis because they are missing the distal tip. However, they are large enough to exhibit homolog pairing and to have faithful sister chromatid cohesion at meiosis I (Han et al., 2007). Because they are B chromosomes, they are basically inert and so the chromatin present is unlikely to have any impact on plants. Yet, their termini are engineered in a manner that will allow the addition of new DNA to them in order to grow them as one might prescribe.

A second approach that could overcome this issue would be to place a gametophyte selection on the minichromosome in one copy at every generation. The transmission of a single copy of a minichromosome is usually at a workable frequency. If a gene were placed on the minichromosome that would allow it to survive in the pollen but other grains did not, then a single such engineered minichromosome used as a male parent in each generation would place a full representation of the minichromosome into the next generation. A potential example would be to place the restorer of fertility, Rf3, of the cytoplasmic male sterility S (cms-S) system
As the minichromosome manipulations continue to advance, it might become possible in the future to engineer a system to overcome the cohesion and pairing issues. Clearly, in the distant future, if one can contemplate growing back a chromosome to sufficient length to provide cohesion and pairing properties, then such synthetic chromosomes would be expected to transmit well. We do not know, however, at this time how a synthetic chromosome might behave in terms of compaction and other properties in the absence of evolutionary selection. The promise of the engineered chromosome field is that we will learn these parameters in the future.

ACKNOWLEDGMENTS

This work was supported by National Science Foundation grants (DBI 0922703, DBI 0701297, and IOS1339198) to James A. Birchler and the National Basic Research Program of China (973 Program 2011CB944601) and the National Natural Science Foundation of China (3122103912) to Fangpu Han.

AUTHOR CONTRIBUTIONS

James A. Birchler and Fangpu Han wrote the paper.

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onto the minichromosome in a background of cmi-S. The cmi-S cytoplasm is a mendelian mutation that causes pollen sterility. The Rf3 restorer acts in individual pollen grains to provide viability. Thus, if a minichromosome carrying Rf3 were present in a background of cmi-S and is crossed as a male parent to females carrying the male sterile cmi-S at each generation, the transmission should be complete at each step.
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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 October 2013; paper pending published: 23 October 2013; accepted: 25 November 2013; published online: 17 December 2013.

Citation: Birchler JA and Han F (2013) Meiotic behavior of small chromosomes in maize. Front. Plant Sci. 4:505. doi: 10.3389/fpls.2013.00505

This article was submitted to Plant Genetics and Genomics, a section of the journal Frontiers in Plant Science.

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