Chromosome Res (2015) 23:69–76
DOI 10.1007/s10577-014-9449-1

REVIEW

Engineering of plant chromosomes

Michael Florian Mette · Andreas Houben

Published online: 18 January 2015

Abstract Engineered minimal chromosomes with sufficient mitotic and meiotic stability have an enormous potential as vectors for stacking multiple genes required for complex traits in plant biotechnology. Proof of principle for essential steps in chromosome engineering such as truncation of chromosomes by T-DNA-mediated telomere seeding and de novo formation of centromeres by cenH3 fusion protein tethering has been recently obtained. In order to generate robust protocols for application in plant biotechnology, these steps need to be combined and supplemented with additional methods such as site-specific recombination for the directed transfer of multiple genes of interest on the minichromosomes. At the same time, the development of these methods allows new insight into basic aspects of plant chromosome functions such as how centromeres assure proper distribution of chromosomes to daughter cells or how telomeres serve to cap the chromosome ends to prevent shortening of ends over DNA replication cycles and chromosome end fusion.

Keywords Minichromosome · Telomere seeding · De novo centromere · Double-strand break · B chromosome

Responsible Editors: Natalay Kouprina and Vladimir Larionov

M. F. Mette (✉) · A. Houben
Research Group Chromosome Structure and Function, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Stadt Seeland OT Gatersleben, Germany
e-mail: mette@ipk-gatersleben.de

A. Houben
e-mail: houben@ipk-gatersleben.de

Abbreviations

BFB	Breakage-fusion-bridge
cenH3	Centromeric histone H3-variant
Cre	Phage P1 recombinase
CRISPR	Clustered regularly interspaced short palindromic repeats
DSB	Double-strand break
GFP	Green fluorescence protein
LacI	*Lac* inhibitor protein
lacO	*Lac* operator sequence
loxP	Locus of crossing-over on phage P1
PARC	Plant artificial ring chromosome

Introduction

In this review, we focus on recent approaches to manipulate individual plant chromosomes in an ideally targeted way in order to adapt them to needs of green biotechnology. In this context, we outline the advantages of engineering minichromosomes as vectors for transgenes in contrast to conventional transformation and discuss the basic strategies that can be followed to customise chromosomes in plants. Based on this, we summarise developments in practical implementation of engineered plant chromosomes and consider further steps required to get them established in broad application. What we did not include are aspects of manipulating ploidy levels or recombination in plants, which have been covered in an excellent recent review (Chan 2010).

Advantages of chromosome engineering in contrast to conventional transformation

Current plant biotechnology often involves the transfer of extraneous genes to establish desired traits. Conventional transformation methods such as biolistic or *Agrobacterium*-mediated DNA transfer result in integration of transgenes in the host genome at random (Yu et al. 2007a). For stacking transgenes encoding complex traits, however, coordinated transfer and inheritance of multiple genes is required (Naqvi et al. 2010). The use of engineered minimal (mini)chromosomes with sufficient mitotic and meiotic transmission, but not engaging in recombination with other chromosomes to ensure joint inheritance of multiple genes, would offer an enormous potential as vectors for plant biotechnology as exemplified in Acevedo-Garcia et al. (2013). Beyond application-related aspects, custom-designed minichromosomes would also form an excellent model system to study the mechanisms underlying chromosome function, such as how centromeres assure proper distribution of chromosomes to daughter cells or how telomeres serve to cap the chromosome ends, preventing shortening of ends over DNA replication cycles and chromosome end fusion.

"Bottom-up" versus "top-down" approach

Construction of minichromosomes can be approached in two ways, "bottom-up" by the assembly of all essential parts such as replication origins, centromeres and telomeres from cloned components, or "top-down" by modification of existing chromosomes in the organism of interest. While the bottom-up approach is well approved in yeast and animal cells, its applicability in plants, albeit reported (Carlson et al. 2007; Ananiev et al. 2009), is a matter of ongoing debate (reviewed in Houben et al. (2008) and Gaeta et al. (2012)). In contrast, the top-down approach has turned out to be robust in plants (reviewed in Birchler (2014)), making it the method of choice for the time being.

Engineering of chromosomes with reduced size

Engineering of chromosomes in a top-down approach starting from the native chromosomes of a host plant requires maintaining essential functional components such as centromere and telomeres, while removing as much as possible of gene-containing parts in order to avoid gene dosage imbalances and thus make the minichromosomes-to-be phenotypically as neutral as possible (reviewed in Conant et al. (2014)). An important issue in this context is also size constrains that apply to chromosome function. While there is a well-defined upper limit for chromosome size as the longest chromosome arm must not exceed half of the length of the spindle axis in mitotic telophase (Schubert and Oud 1997), the potential lower size limit for minichromosomes is less clear and might be set by the requirements for proper bivalent stability or sister chromatid cohesion in meiosis, with possibly some dependence on host genome size (Schubert 2001). Thus, the desired small size is to be balanced with satisfactory mitotic and meiotic transmissibility for most optimal minichromosome-based vectors (reviewed in Birchler and Han (2013)).

Several approaches exist to reduce the size of native plant chromosomes. X-rays (Schubert 2001) or the application of gametocidal (Gc) chromosomes or Gc genes (reviewed in Endo (2007)) is an option to induce random chromosome tuncation events. However, chromosome fragmentation via the breakage-fusion-bridge (BFB) cycle involving dicentric chromosomes as intermediates is more efficient to downsize a particular chromosome (reviewed in Yu et al. (2007a)). The application of BFB cycles resulted in a series of midget chromosomes produced from the long arm of wheat chromosome 1B (Lukaszewski 1997). Based on a translocation between a supernumerary B chromosome and the short arm of the standard chromosome 9, several minichromosomes could be generated in maize via the BFB cycle (Han et al. 2006), which were transmissible through sexual reproduction, but showed compromised pairing or sister chromatid cohesion in meiosis (Han et al. 2007; Birchler and Han 2013; Zhang et al. 2014). Formation of minichromosomes, possibly involving some BFB cycle steps, has also been reported for an initially trisomic line of *Arabidopsis thaliana* (Murata 2014). These minichromosomes included linear as well as ring chromosomes, which all were transmitted in sexual reproduction, albeit with different efficiencies with regard to the genetic background and transmission via the male versus the female gametophyte. Interestingly, ring minichromosomes seem to be more stably transmitted than comparable linear ones.

Site-specific recombination using exogenous systems such as Cre recombinase combined with *loxP* locus of crossing-over sequences from phage P1 can be used to modify DNA sequences *in planta* (reviewed in Ow (2007)). Recombination requires the presence of two *loxP* sites. If they are positioned on one linear DNA molecule, recombination between them results in excision of the sequence flanked by them if they are in the same orientation and in inversion of the sequence flanked by them if they are in inverse orientation. Thus, Cre-mediated recombination has been successfully used to delete parts of plant chromosomes flanked by transgenic *loxP* sites in the same orientation (Stuurman et al. 1996). With the observation that ring minichromosomes might be more stable than linear ones that was mentioned above in mind (reviewed in Murata (2014)), an approach to the directed construction of a plant artificial ring chromosome (PARC) using the Cre/*loxP* system in *A. thaliana* has been taken. Site-specific recombination between two *loxP* sites on the same chromosome was used to release a large circular DNA fragment including a good part of the native centromere. The resulting PARC indeed showed substantial mitotic and meiotic transmission, although it did not engage in meiotic pairing (reviewed in Murata et al. (2013)).

A recently developed option to shorten chromosomes is the use of T-DNA-mediated telomere seeding (reviewed in Birchler et al. (2010)). As shown first by Farr et al. (1991), introduction of cloned telomeric repeats into cells may truncate randomly the distal portions of chromosomes by the formation of new telomeres at integration sites. Telomeres consist of tandem repeats of a conserved short sequence (5′-TTTAGGG-3′ in *A. thaliana*) at the ends of chromosomes that are synthesised by telomerase by adding repeat units to the ends of existing arrays. Thus, a DNA end carrying a tandem array of telomeric repeats can serve as seeding point for the formation of a telomere. Introduction of a T-DNA construct containing a block of telomeric repeats at one end via *Agrobacterium*-mediated DNA transfer can lead to the formation of T-DNA-associated de novo telomeres. If oriented properly, that is, with the T-DNA integrated in a way that the selection marker is at the side in the direction of the centromere, de novo telomere formation results in the formation of a stable truncated chromosome, while all parts distal to the site of telomere formation end up in an acentric fragment that is lost in subsequent cell divisions (Fig. 1a).

Chromosome truncation by telomere seeding has been achieved by *Agrobacterium*-mediated transformation of maize (Yu et al. 2006; Vega et al. 2008), *A. thaliana* (Nelson et al. 2011; Teo et al. 2011) and barley (Kapusi et al. 2012) as well as biolistic transformation of rice (Xu et al. 2012) and maize (Gaeta et al. 2013). Chromosomes with extended deletions are usually not transmitted through sexual reproduction when generated in diploid material due to gametophyte lethality associated with the loss of essential genes. However, transfer of truncated chromosomes to the next generation is possible in lines containing backup chromosome copies. In autotetraploid material that either was used as target for transformation in the cases of *A. thaliana* (Nelson et al. 2011; Teo et al. 2011) and barley (Kapusi et al. 2012) or formed spontaneously in the process of transformation in the cases of barley (Kapusi et al. 2012) as well as maize (Yu et al. 2007b), truncated chromosomes were inherited across generations. A truncated chromosome that was recovered from a tetraploid maize plant could be transferred into diploid background by repeated backcrossing. Here, it showed stable transmission and failed to pair with its progenitor chromosome in meiosis, that is, the features required for a minichromosome-based vector (Yu et al. 2007b).

An interesting option in the context of avoiding deleterious effects of chromosome truncation is to use B chromosomes, which by definition are supernumerary and non-essential (reviewed in Houben and Schubert (2007) and Houben et al. (2014)), as targets of telomere seeding. Indeed, when a diploid maize line containing B chromosomes was biolistically transformed with telomere seeding constructs, multiple truncated B chromosomes were obtained that showed substantial transmission through sexual reproduction and expression of inserted reporter genes (Yu et al. 2007b).

A further advantage of using B chromosomes as starting point for chromosome engineering is that many of them possess “drive” mechanisms that promote their preferential inheritance and thus maintenance in the host plant population (reviwed in Houben et al. (2014)). Such a “drive” would also be very valuable for a chromosome-based vector. A prerequisite to exploit these drive mechanisms would be the ability to precisely target truncation of B chromosomes in order to preserve all chromosome regions required for the process of drive. However, as the insertion of exogenous DNA into plant chromosomes happens in general at random, transgene-associated telomere seeding and chromosome

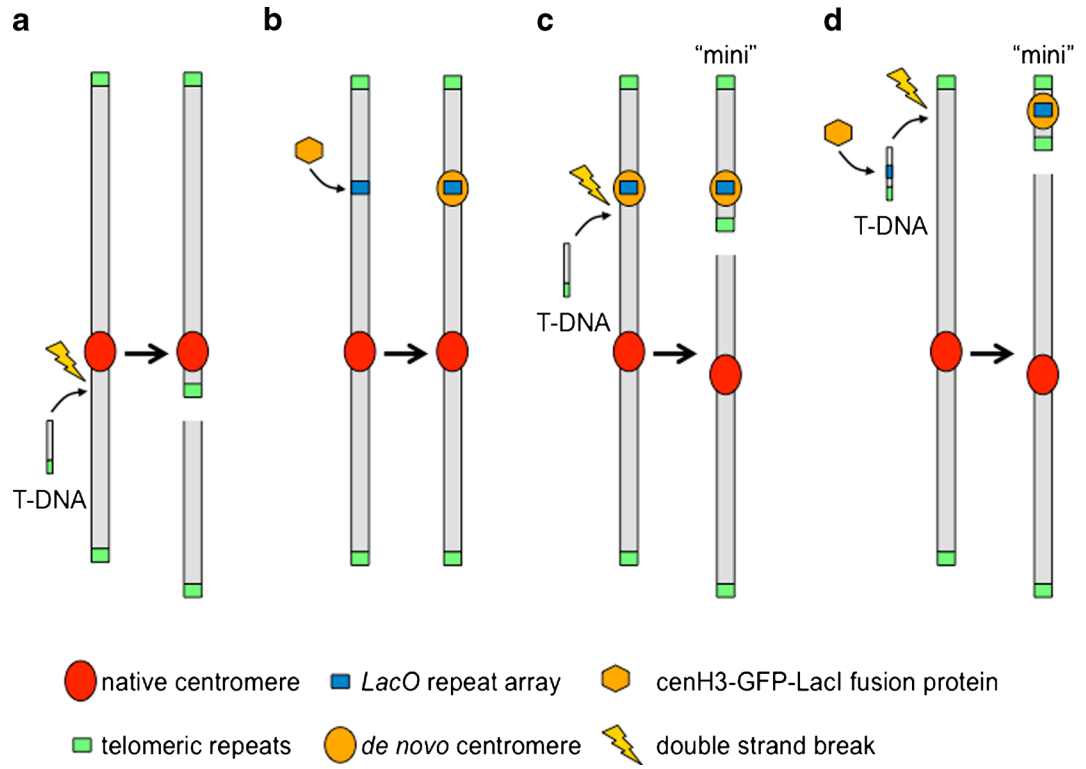

Fig. 1 Steps toward precision engineering of minichromosomes. **a** Chromosome truncation via T-DNA-mediated telomere seeding. The lower acentric fragment is lost in subsequent cell divisions. The site of truncation is random by default, but potentially can be targeted by simultaneously setting a double-strand break (DSB) by the use of a sequence-specific nuclease. **b** De novo centromere formation at tandem repeats via tethering of a cenH3 fusion protein. The presence of cenH3 at the ectopic site promotes the loading of further cenH3 and attraction of kinetochore proteins. **c** Combination of de novo centromere formation at tandem repeats already integrated in a host chromosome with targeted T-DNA-mediated telomere seeding proximal to the tandem repeats can release prototype minichromosomes (mini). However, their possible sizes are determined by the chromosomal positions of the tandem repeat insertion sites. **d** Including the tandem repeats for cenH3 tethering in the T-DNA construct for telomere seeding and simultaneously targeting T-DNA insertion to, for example, a subtelomeric region could generate minichromosomes of any desired size

truncation is random by default. Nevertheless, the insertion of T-DNA constructs introduced via *Agrobacterium*-mediated DNA transfer can be targeted to particular sites in the genome by simultaneous site-specific formation of DNA double-strand breaks (DSBs) (Chilton and Que 2003). Thus, the targeting of T-DNA-mediated telomere seeding to particular chromosomal sites via the site-specific introduction of DSB should be approached (Fig. 1a). There are now several tools for the sequence-specific induction of DSB in plants available, with CRISPR/Cas RNA-guided nucleases representing the most timely method (reviewed in Puchta and Fauser (2014)). Once such targeted telomere seeding would be available, targeted deletion of chromosome arms, while retaining other desired chromosome elements, would become feasible.

Induction of de novo centromere formation

Beside of using the existing centromeres of native chromosomes of the host plant, it would be very useful if centromeres could be generated de novo in plants. However, simply transferring centromeric sequences seemingly is not sufficient (Gaeta et al. 2012; Phan et al. 2007). Centromeres are marked by cenH3, a centromere-specific histone H3 variant (reviewed in Jiang et al. (2003)). In complex eukaryotes including plants, the presence of cenH3 at a particular DNA site is an epigenetic feature (reviewed in Burrack and Berman (2012)). That is, the cenH3 present prior to DNA replication serves as a signal for the loading of new cenH3 at the same site after DNA replication in order to maintain a constant cenH3 mark and thus centromere function

(Lermontova et al. 2006). Experimental tethering of cenH3, for example via expression of a cenH3-fusion protein comprising a sequence-specific DNA binding domain in an organism carrying the respective target sequence integrated in its chromosomes, can induce de novo centromere formation at a predefined site. This was first demonstrated in *Drosophila* (Mendiburo et al. 2011). Further, experimental tethering of accessory protein factors supports cenH3 loading to particular sequences in mammalian cells (Barnhart et al. 2011; Ohzeki et al. 2012). In a similar setup, cenH3-GFP-LacI fusion proteins could be targeted to *lacO* tandem repeat arrays in *A. thaliana* (Teo et al. 2013) (Fig. 1b). This cenH3 tethering was accompanied by the attraction of kinetochore-specific proteins to the sites of *lacO* tandem repeats, indicating the formation of ectopic centromere structures. The functionality of de novo centromeres was confirmed by the observation of anaphase bridges, a feature that arises in mitosis when chromosomes with two functional centromeres, in the current case the de novo and the native one, are at the same time pulled in the direction of both spindle poles.

Beyond the construction of chromosome-based vectors, this unique experimental system also has great potential in the analysis of the processes underlying plant centromere formation in general. Plant neocentromeres, that is, centromeres positioned at chromosomal sites that previously did not have centromere function, have been repeatedly reported, but the details of their formation still need to be studied (reviewed in Burrack and Berman (2012)). Chromatin immunoprecipitation employing antibodies against cenH3 combined with quantitative PCR for transgene and flanking chromosomal sequences or next-generation sequencing would allow following the development of induced de novo centromeres within and across generations. By combined determination of anaphase bridges as a functional test, centromere function could also be followed.

Comparing the efficiencies of centromere formation in wild type versus mutants, the roles of factors potentially regulating centromere formation and function in plants could be determined. This could also include the possible impact of epigenetic marks other than cenH3 on centromere formation. In plants, centromeric chromatin has been reported to be depleted in cytosine methylation and histone-H3-lysine-9-dimethylation (Zhang et al. 2008), while the *lacO* tandem repeats targeted for cenH3 tethering are associated with these marks typical for repressive chromatin (Watanabe et al. 2005; Jovtchev et al. 2008, 2011). Using mutant lines with reduced cytosine methylation and histone-H3-lysine-9-dimethylation at the *lacO* repeats (Watanabe et al. 2005; Jovtchev et al. 2011), it will be possible to directly test to what extent the presence of these marks interferes with centromere formation, and thus might contribute to shaping the extent of native centromeres. Whether de novo centromere formation can be achieved for available LacI-binding *lacO* sites in maize (Zhang et al. 2012) remains to be demonstrated.

Combination of approaches to custom-design chromosomes

Once fully optimised protocols for chromosome truncation at deliberate positions via DSB-targeted T-DNA-mediated telomere seeding and de novo centromere formation via cenH3 tethering will be available, the combination of the two methods will allow precision design of minichromosomes with predefined length and content. This would be possible using a sequential strategy, in which first a de novo centromere is induced at tandem repeats already integrated in a host plant chromosome via the expression of a cenH3-GFP-LacI fusion protein, and then a defined fragment including the de novo centromere is released via targeted telomere seeding (Fig. 1c). In this kind of approach, the T-DNA construct for telomere seeding could be kept simple, but the choice of possible centromere positions is limited by the available transgenic lines containing *lacO* tandem repeats (Matzke et al. 2005; Watanabe et al. 2005; Rosin et al. 2008). Alternatively, the telomeric repeats for telomere seeding and the *lacO* tandem repeats for cenH3 tethering could be integrated together into one T-DNA and be transformed into plants expressing cenH3-GFP-LacI fusion protein (Fig. 1d). In both cases, additional simultaneous expression of a CRISPR/Cas nuclease targeting the desired site of telomere seeding would be required.

With either method, scales of minichromosomes with defined size and sequence composition could be generated. After confirmation of their structure by molecular methods such as a telomere-specific PCR assay and gel blot analysis as well as microscopic methods such as immuno-staining and fluorescent in situ hybridisation (Teo et al. 2011, 2013), they could be used to determine the optimal chromosome-based vector design with

regard to mitotic and meiotic stability on the one hand and small size and thus phenotypic neutrality on the other.

Loading of genes of interest onto minichromosome-based vectors

Last but not least, once minichromosomes are available that fulfil all requirements for a suitable vector, efficient means for the transfer of expression cassettes for multiple genes of interest will be required. Again, site-specific recombination, for example via the Cre/*loxP* system, offers a possible solution. It has been demonstrated to allow the insertion of gene expression cassettes into transgenic *loxP* sites present in the plant genome, albeit with limited efficiency (Albert et al. 1995; Vergunst et al. 1998), which, however, can be improved by combining different *lox* sequence variants to favour integration over excision (Louwerse et al. 2007). The sequential insertion of multiple expression cassettes would also be feasible by using Cre/*loxP* in combination with alternative site-specific recombination systems (Ow 2007) such as, for example, phiC31 integrase (Rubtsova et al. 2008). In maize, the Cre/*loxP* system is active in modifying minichromosomes (Gaeta et al. 2013), and an elegant alternative way to use it to target sequences to minichromosomes has been explored by first integrating elements of interest at a separate chromosome end in the course of T-DNA-mediated telomere seeding and then transferring them by the exchange of chromosome ends via site-specific recombination (Yu et al. 2007b).

Cross-lineage transfer of minichromosomes

For practical reasons, minichromosomes will be assembled in genetic backgrounds that are most accessible to the required molecular manipulations. Once established, they will need to be transferred into the cultivars most suitable for the desired applications. Provided that minichromosomes do not recombine with the native chromosomes of their host plants, this could be done by tedious and time-consuming repeated backcrossing over many generations. Single chromosomes may be transferred between different plant cells in a solely mechanic way by microscopic micromanipulation (reviewed in Houben and Schlegel (1991)). A more elegant way to solve this problem, however, would be to use haploid breeding which is routinely used in breeding (reviewed in Birchler (2014)). In maize haploid breeding, crosses between particular genotypes (haploid inducer lines) are used which result in specific elimination of the chromosomes of one of the crossing partners. In the formed haploid progeny plants, diploid status can be restored by genome doubling, for example via colchicine treatment. The loss of chromosomes from one parent, however, is not always complete. In maize, it has been demonstrated that a B chromosome present in a haploid inducer line could be retained in resulting haploids while the standard A chromosomes originating from the same parental genome were eliminated (Zhao et al. 2013). Thus, also selective transfer of minichromosomes, in particular if they are derived from B chromosomes, to cultivars of interest via haploid breeding seems feasible.

Conclusion

Proof of principle for essential steps in chromosome engineering such as chromosome truncation and de novo centromere formation has been obtained. Now, these steps need to be combined and supplemented with additional methods for the directed transfer of multiple genes of interest on the minichromosomes. At the same time, development of the methods for chromosome engineering allows new insights into many basic aspects of plant chromosome function.

Acknowledgments This work was supported by DFG (German Research Council) grant HO 1779/22-1.

References

Acevedo-Garcia J, Collins NC, Ahmadinejad N, Ma L, Houben A, Bednarek P, Benjdia M, Freialdenhoven A, Altmuller J, Nurnberg P, Reinhardt R, Schulze-Lefert P, Panstruga R (2013) Fine mapping and chromosome walking towards the Ror1 locus in barley (*Hordeum vulgare* L.). Theor Appl Genet 126:2969–2982

Albert H, Dale EC, Lee E, Ow DW (1995) Site-specific integration of DNA into wild-type and mutant lox sites placed in the plant genome. Plant J 7:649–659

Ananiev EV, Wu CC, Chamberlin MA, Svitashev S, Schwartz C, Gordon-Kamm W, Tingey S (2009) Artificial chromosome formation in maize (*Zea mays* L.). Chromosoma 118:157–177

Barnhart MC, Kuich PHJL, Stellfox ME, Ward JA, Bassett EA, Black BE, Foltz DR (2011) HJURP is a CENP-A chromatin assembly factor sufficient to form a functional de novo kinetochore. J Cell Biol 194:229–243

Birchler JA (2014) Engineered minichromosomes in plants. Curr Opin Plant Biol 19:76–80

Birchler JA, Han F (2013) Meiotic behavior of small chromosomes in maize. Front Plant Sci 4:505

Birchler JA, Krishnaswamy L, Gaeta RT, Masonbrink RE, Zhao CZ (2010) Engineered minichromosomes in plants. Crit Rev Plant Sci 29:135–147

Burrack LS, Berman J (2012) Neocentromeres and epigenetically inherited features of centromeres. Chromosom Res 20:607–619

Carlson SR, Rudgers GW, Zieler H, Mach JM, Luo S, Grunden E, Krol C, Copenhaver GP, Preuss D (2007) Meiotic transmission of an in vitro-assembled autonomous maize minichromosome. PLoS Genet 3:1965–1974

Chan SW (2010) Chromosome engineering: power tools for plant genetics. Trends Biotechnol 28:605–610

Chilton MD, Que Q (2003) Targeted integration of T-DNA into the tobacco genome at double-stranded breaks: new insights on the mechanism of T-DNA integration. Plant Physiol 133: 956–965

Conant GC, Birchler JA, Pires JC (2014) Dosage, duplication, and diploidization: clarifying the interplay of multiple models for duplicate gene evolution over time. Curr Opin Plant Biol 19: 91–98

Endo TR (2007) The gametocidal chromosome as a tool for chromosome manipulation in wheat. Chromosom Res 15: 67–75

Farr C, Fantes J, Goodfellow P, Cooke H (1991) Functional reintroduction of human telomeres into mammalian cells. Proc Natl Acad Sci U S A 88:7006–7010

Gaeta RT, Masonbrink RE, Krishnaswamy L, Zhao C, Birchler JA (2012) Synthetic chromosome platforms in plants. Annu Rev Plant Biol 63:307–330

Gaeta RT, Masonbrink RE, Zhao C, Sanyal A, Krishnaswamy L, Birchler JA (2013) In vivo modification of a maize engineered minichromosome. Chromosoma 122:221–232

Han F, Lamb JC, Birchler JA (2006) High frequency of centromere inactivation resulting in stable dicentric chromosomes of maize. Proc Natl Acad Sci U S A 103:3238–3243

Han FP, Gao Z, Yu WC, Birchler JA (2007) Minichromosome analysis of chromosome pairing, disjunction, and sister chromatid cohesion in maize. Plant Cell 19:3853–3863

Houben A, Schlegel R (1991) Chromosomentransfer bei Pflanzen. Wiss Fortschr 41:358–360

Houben A, Schubert I (2007) Engineered plant minichromosomes: a resurrection of B chromosomes? Plant Cell 19:2323–2327

Houben A, Dawe RK, Jiang JM, Schubert I (2008) Engineered plant minichromosomes: a bottom-up success? Plant Cell 20: 8–10

Houben A, Banaei-Moghaddam AM, Klemme S, Timmis JN (2014) Evolution and biology of supernumerary B chromosomes. Cell Mol Life Sci 71:467–478

Jiang J, Birchler JA, Parrott WA, Dawe RK (2003) A molecular view of plant centromeres. Trends Plant Sci 8:570–575

Jovtchev G, Watanabe K, Pecinka A, Rosin FM, Mette MF, Lam E, Schubert I (2008) Size and number of tandem repeat arrays can determine somatic homologous pairing of transgene loci mediated by epigenetic modifications in *Arabidopsis thaliana* nuclei. Chromosoma 117:267–276

Jovtchev G, Borisova BE, Kuhlmann M, Fuchs J, Watanabe K, Schubert I, Mette MF (2011) Pairing of lacO tandem repeats in *Arabidopsis thaliana* nuclei requires the presence of hypermethylated, large arrays at two chromosomal positions, but does not depend on H3-lysine-9-dimethylation. Chromosoma 120:609–619

Kapusi E, Ma L, Teo CH, Hensel G, Himmelbach A, Schubert I, Mette MF, Kumlehn J, Houben A (2012) Telomere-mediated truncation of barley chromosomes. Chromosoma 121:181–190

Lermontova I, Schubert V, Fuchs J, Klatte S, Macas J, Schubert I (2006) Loading of Arabidopsis centromeric histone CENH3 occurs mainly during G2 and requires the presence of the histone fold domain. Plant Cell 18:2443–2451

Louwerse JD, van Lier MCM, van der Steen DM, de Vlaam CMT, Hooykaas PJJ, Vergunst AC (2007) Stable recombinase-mediated cassette exchange in Arabidopsis using *Agrobacterium tumefaciens*. Plant Physiol 145:1282–1293

Lukaszewski AJ (1997) Construction of midget chromosomes in wheat. Genome 40:566–569

Matzke AJM, Huettel B, van der Winden J, Matzke M (2005) Use of two-color fluorescence-tagged transgenes to study interphase chromosomes in living plants. Plant Physiol 139: 1586–1596

Mendiburo MJ, Padeken J, Fulop S, Schepers A, Heun P (2011) Drosophila CENH3 is sufficient for centromere formation. Science 334:686–690

Murata M (2014) Minichromosomes and artificial chromosomes in Arabidopsis. Chromosome Res : Int J Mol Supramol Evol Aspects Chromosome Biol 22:167–178

Murata M, Shibata F, Hironaka A, Kashihara K, Fujimoto S, Yokota E, Nagaki K (2013) Generation of an artificial ring chromosome in Arabidopsis by Cre/LoxP-mediated recombination. Plant J 74:363–371

Naqvi S, Farre G, Sanahuja G, Capell T, Zhu C, Christou P (2010) When more is better: multigene engineering in plants. Trends Plant Sci 15:48–56

Nelson AD, Lamb JC, Kobrossly PS, Shippen DE (2011) Parameters affecting telomere-mediated chromosomal truncation in Arabidopsis. Plant Cell 23:2263–2272

Ohzeki J-I, Bergmann JH, Kouprina N, Noskov VN, Nakano M, Kimura H, Earnshaw WC, Larionov V, Masumoto H (2012) Breaking the HAC barrier: histone H3K9 acetyl/methyl balance regulates CENP-A assembly. EMBO J 31:2391–2402

Ow DW (2007) GM maize from site-specific recombination technology, what next? Curr Opin Biotechnol 18:115–120

Phan BH, Jin W, Topp CN, Zhong CX, Jiang J, Dawe RK, Parrott WA (2007) Transformation of rice with long DNA-segments consisting of random genomic DNA or centromere-specific DNA. Transgenic Res 16:341–351

Puchta H, Fauser F (2014) Synthetic nucleases for genome engineering in plants: prospects for a bright future. Plant J 78: 727–741

Rosin FM, Watanabe N, Cacas JL, Kato N, Arroyo JM, Fang Y, May B, Vaughn M, Simorowski J, Ramu U, McCombie RW, Spector DL, Martienssen RA, Lam E (2008) Genome-wide transposon tagging reveals location-dependent effects on transcription and chromatin organization in Arabidopsis. Plant J 55:514–525

Rubtsova M, Kempe K, Gils A, Ismagul A, Weyen J, Gils M (2008) Expression of active Streptomyces phage phiC31 integrase in transgenic wheat plants. Plant Cell Rep 27: 1821–1831

Schubert I (2001) Alteration of chromosome numbers by generation of minichromosomes—is there a lower limit of chromosome size for stable segregation? Cytogenet Cell Genet 93: 175–181

Schubert I, Oud JL (1997) There is an upper limit of chromosome size for normal development of an organism. Cell 88:515–520

Stuurman J, de Vroomen MJ, Nijkamp HJ, van Haaren MJ (1996) Single-site manipulation of tomato chromosomes in vitro and in vivo using Cre-lox site-specific recombination. Plant Mol Biol 32:901–913

Teo CH, Ma L, Kapusi E, Hensel G, Kumlehn J, Schubert I, Houben A, Mette MF (2011) Induction of telomere-mediated chromosomal truncation and stability of truncated chromosomes in *Arabidopsis thaliana*. Plant J 68:28–39

Teo CH, Lermontova I, Houben A, Mette MF, Schubert I (2013) De novo generation of plant centromeres at tandem repeats. Chromosoma 122:233–241

Vega JM, Yu WC, Han FP, Kato A, Peters EM, Zhang ZJ, Birchler JA (2008) Agrobacterium-mediated transformation of maize (Zea mays) with Cre-lox site specific recombination cassettes in BIBAC vectors. Plant Mol Biol 66:587–598

Vergunst AC, Jansen LET, Hooykaas PJJ (1998) Site-specific integration of Agrobacterium T-DNA in *Arabidopsis thaliana* mediated by Cre recombinase. Nucleic Acids Res 26:2729–2734

Watanabe K, Pecinka A, Meister A, Schubert I, Lam E (2005) DNA hypomethylation reduces homologous pairing of inserted tandem repeat arrays in somatic nuclei of *Arabidopsis thaliana*. Plant J 44:531–540

Xu C, Cheng Z, Yu W (2012) Construction of rice mini-chromosomes by telomere-mediated chromosomal truncation. Plant J 70:1070–1079

Yu WC, Lamb JC, Han FP, Birchler JA (2006) Telomere-mediated chromosomal truncation in maize. Proc Natl Acad Sci U S A 103:17331–17336

Yu WC, Han FP, Birchler JA (2007a) Engineered minichromosomes in plants. Curr Opin Biotechnol 18:425–431

Yu WC, Han FP, Gao Z, Vega JM, Birchler JA (2007b) Construction and behavior of engineered minichromosomes in maize. Proc Natl Acad Sci U S A 104:8924–8929

Zhang W, Lee HR, Koo DH, Jiang J (2008) Epigenetic modification of centromeric chromatin: hypomethylation of DNA sequences in the CENH3-associated chromatin in *Arabidopsis thaliana* and maize. Plant Cell 20:25–34

Zhang H, Phan BH, Wang K, Artelt BJ, Jiang J, Parrott WA, Dawe RK (2012) Stable integration of an engineered megabase repeat array into the maize genome. Plant J 70:357–365

Zhang J, Zhang B, Su H, Birchler JA, Han F (2014) Molecular mechanisms of homologous chromosome pairing and segregation in plants. J Genet Genomics = Yi Chuan Xue Bao 41: 117–123

Zhao X, Xu XW, Xie HX, Chen SJ, Jin WW (2013) Fertilization and uniparental chromosome elimination during crosses with maize haploid inducers. Plant Physiol 163:721–731