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Minichromosomes: The second generation genetic engineering tool

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Abstract

Genetic engineering is a scientific tool used in every field of science like plant, animal and human sciences. Plant genetic engineering technology has changed the face of plant sciences and the first generation of transgenic crops has become the most rapidly adopted technology in modern agriculture. But genetic engineering has some limitations and therefore still there is a clear need of new technologies to overcome issues like gene stacking, transgene position effects and insertion-site complexity. The recent strategy that researchers have developed to overcome those limitations is the development of plant artificial minichromosomes for delivery of large DNA sequences, including large genes, multigene complexes, or even complete metabolic pathways. A minichromosome is an extremely small version of a chromosome that have been produced by *de novo* construction using cloned components of chromosomes or through telomere-mediated truncation of endogenous chromosomes. After a successful experiment in maize with the help of minichromsomes by J. Birchler and colleagues (Yu et al., 2007a), a new paradigm have been set for all the agricultural researchers to use the minichromosome techniques for crop improvement. Engineered minichromosomes also offer an enormous opportunity to improve crop performance, as discussed by Houben and Schubert (2007). With rapidly expanding research in minichromosome as a second generation genetic engineering tool we can hope that it will bring a new generation of improved crop species to meet the global demands.

Keywords: Arabidopsis; B chromosomes; maize; mini B chromosome; minichromosomes

Introduction

Although there has been a tremendous revolution in the biological sciences in the past two decades, there is still a great deal that remains to be discovered. The completion of the sequencing of the human genome, as well as the genomes of most agriculturally and scientifically important plants and animals, has increased the possibilities of genetic research immeasurably. Genetic engineering is a powerful tool for improving crop quality and productivity, and reducing labor and resource utilization of farming (Ceccarelli et al., 1992). Traditonally genetic engineering is done by either *Agrobacterium*-mediated transformation (as reviewed by Opabode 2006) or direct transformation by particle bombard-

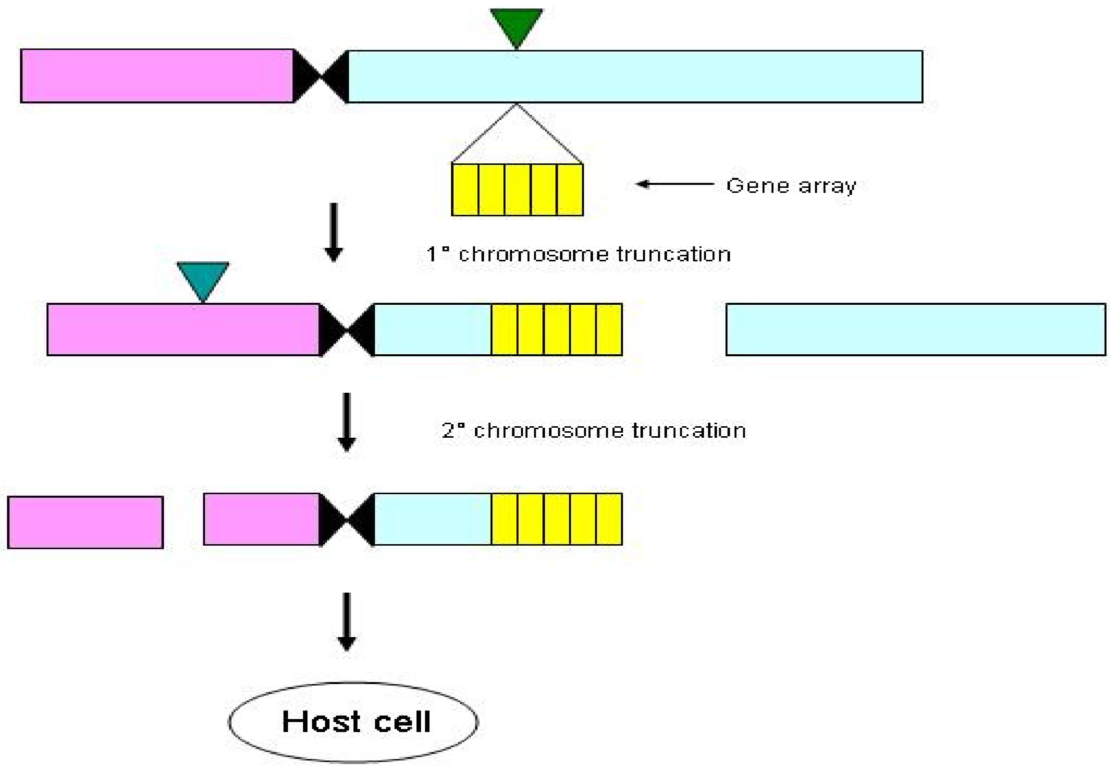

Fig 1. Minichromosomes can be produced by telomere mediated chromosome truncation.

ment using gene gun (Klein et al., 1992; Kikkert et al., 2004; Altpeter et al., 2005). These methods have several limitations, since they allow insertion of single or few genes at random genomic positions and requires the simultaneous expression of multiple genes; but complex or combined traits cannot be transferred in a coordinated manner (Yu et al., 2007b). These methods are labor-intensive and time consuming processes and also require highly skilled personal and significant input for desirable results. Furthermore, a high number of phenotypicaly abnormal plants are recovered and often usefulness of host genome is seriously disturbed.

Minichromosome technology provides one solution to the stable expression and maintenance of multiple transgenes in one genome. In addition, plant artificial chromosomes or engineered minichromosomes represent a potentially powerful research tool for understanding chromosome structure and functions. Since it is technically difficult at present to introduce large repetitive DNA molecules into plant cells efficiently; minichromosomes, either those which occur naturally or those that are induced by irradiation, are another important alternative choice for determining minimum functional sizes of the centromeres and for constructing artificial chromosomes (Houben and Schubert 2007). Mammalian artificial minichromosomes also have several potential biotechnological and therapeutic applications arising from their ability to exist episomally, carry large DNA inserts, and allow expression of genes independently of the host genome (Irvine et al., 2005).

What is a minichromosome?

A minichromosome is an extremely small version of a chromosome, the thread-like linear or circular DNA and associated proteins that carry genes and functions in the transfer of genetic information. Mini chromosomes are plasmids that replicate autonomously from *oriC* (Hiraga 1976; Messer et al., 1978; von Meyen-

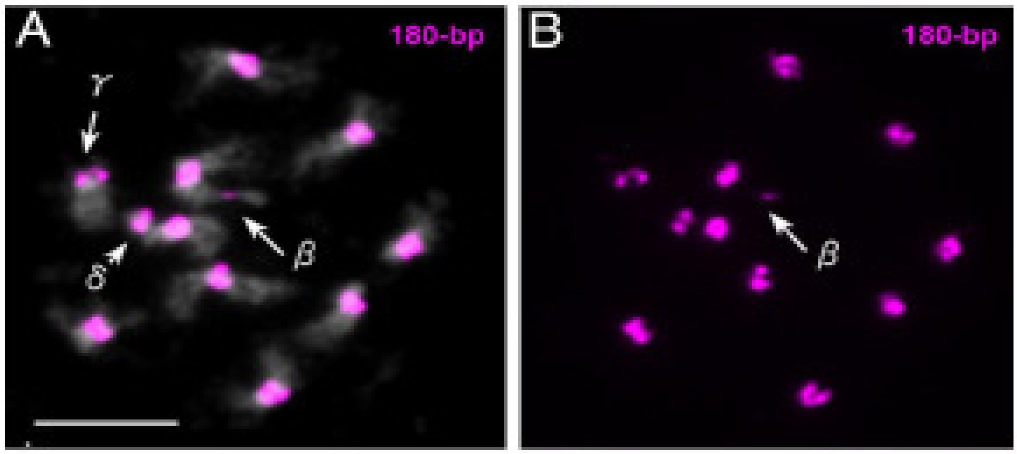

Fig 2. Cytological analysis of a G40 Arabidopsis cell containing minichromosomes α, β and δ. Source: Fig 3 (A & B) from Murata et al., (2008) PNAS USA. 105(21):7511-7516. Complete citation available in the reference section. Published with kind permission of the National Academy of Sciences, U. S. A. Copyright (2008) National Academy of Sciences, U. S. A.

burg et al., 1979). They depend on functional *DnaA* and *DnaC* products, *de novo* protein synthesis and RNA polymerase mediated transcription for initiation of bi-directional replication; thereby resembling their chromosomal counterparts (for review please see Messer and Weigel 1996). Minichromosomes are also known to be enriched with transposable or repetitive elements (Enkerli et al., 1997, 2000; Francis and Michelmore 1993; Kim et al., 1995; Nagy et al., 1995; Shiflett et al., 2002).

Minichromosomes were usually produced by the radiation induced breakages. In the green alga, *Chlorella vulgaris* minichromosome was also produced by cell irradiation through electron beams (Yamada et al., 2003). In yeast, minichromosomes have been isolated by using metrizamide gradients (Shalitin and Vishlizky 1984). In other fungal members, minichromosomes has been defined as extra chromosomes composed primarily of DNA that is not present in all isolates of a species (Covert 1998). Through Restriction Fragment Length Polymorphism (RFLP) segregation analysis indicated that the minichromosomes in fungi underwent structural changes like deletions and duplications, not only in meiosis but also after meiosis Chuma et al., (2003). But in the protozoan *Trypanosoma brucei,* minichromosomes were investigated by *in situ* hybridization in combination with immuno fluorescence (Ersfeld and Gull, 1997). Mammalian and *Drosophila* minichromosomes analyses have been conducted by different research workers too (for details please see Han et al., 2007). Minichromosomes have been produced in *Drosophila* and mammalian cells through either *de novo* construction using the minimum constituent parts of chromosomes or telomere-mediated chromosomal truncation of existing chromosomes (Murphy and Karpan 1995; Harrington et al., 1997; Ebersole et al., 2000; Yang et al., 2000; Auriche et al., 2001), a schematic representation has been presented in Fig. 1. In humans, minichromosome techniques have been used to study the centromere and also for studying gene delivery process (Wong et al., 2002).

Meiotic behaviors of minichromosomes have been examined in detail in case of the fungus *Necteria haematococca* (Miao et al., 1991); the fungal pathogen of blackleg disease on *Brassica* sp., *Leptosphaeria maculans* (Leclair et al., 1996); and the prominent fungal rice pathogen, *Magnaporthe oryzae* (Chuma et al., 2003). In the Gram-negative bacteria, *Eschericia coli* the distribution of minichromosomes and its effect on replication was discovered with the help of green fluorescentprotein (GFP) (Løbner-Olesen 1999). The researchers

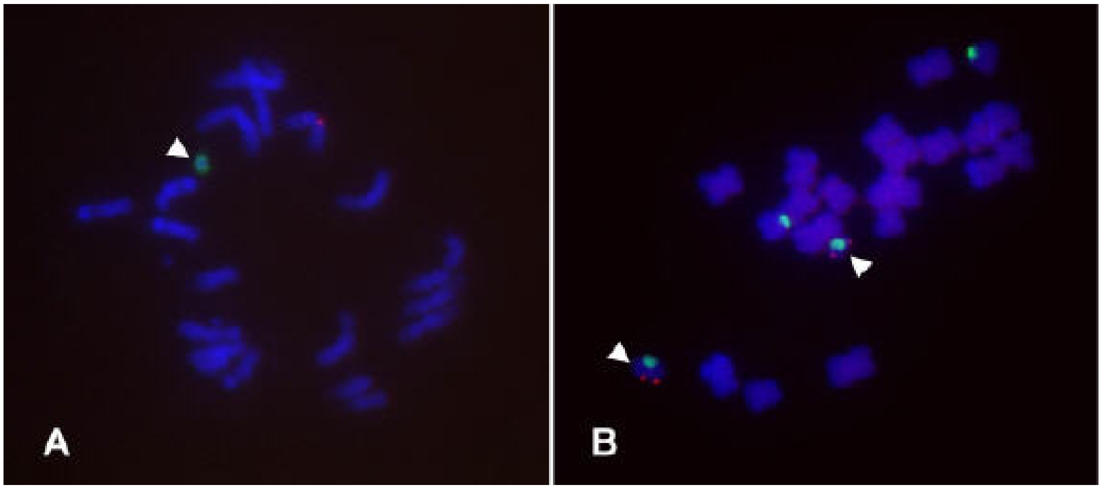

Fig 3. Minichromosomes produced from maize B chromosome truncation, arrows denote minichromosomes. Source: Fig 2 (A & B) from Yui et al., (2007a) PNAS USA. 104(21): 8924-8929. Complete citation available in the reference section. Published with kind permission of the National Academy of Sciences, U. S. A. Copyright (2007) National Academy of Sciences, U. S. A.

reported that the copy number distribution of minchromosomes is much wider than that of natural *E. coli* plasmids; and that the high copy number of minichromosomes leads to initiation of asynchrony in *E. coli.*

Minichromsome in plants

Minichromosomes technology is well known and successfully used in humans, fungi, yeast and other species as discussed above. In plant systems minichromosomes were discovered in the late nineties (Buchiwicz 1997). Earlier the function and use of minichromosomes were not clearly known or reported in primary literature. Later it was discovered that minichromosomes are very useful to understand the basics of chromosomal structure and for the purpose of use in genetic engineering of plants (Birchler et al., 2008; Houben et al., 2008). Recently, the minichromosome technology offers enormous opportunities to improve crop plants.

Minichromosomes in *Arabidopsis*

The DNA structures of centromeres have been studied extensively in case of *Arabidopsis* (Copenhaver et al., 1999; Heslop- Harrison et al., 1999; The Arabidopsis Genome Initiative 2000; Kumekawa et al., 2000, 2001; Hosouchi et al., 2002; Hall et al., 2003, 2005). Since very small genomes (like that in *Arabidopsis*) has relatively small chromosomes; the DNA is estimated to be 17.5–29.1 Mb only (The Arabidopsis Genome Imitative 2000); but it is still large to be easily manipulated *in vitro*. In teleocentric line of *A. thaliana,* a minichromsome was identified through Fluorescence *In Situ* Hybridization (FISH) approach and it reveled that it was from the short arm of chromosome number 4 (Murata et al., 2006). The size of this “mini4S” chromosome was estimated to be ~7.5 Mb on the basis of previously reported data and the amount of the centromeric major satellite (180-bp family) present, which was determined to be about 1 Mb, or about one third of that in the normal chromosome 4. The researchers also reported the size, centromeric function and the meiotic behavior of minichromosome. Recently, two more minichromosome (α, β and δ) have also been discovered by the same research group (Fig 2; Murata et al., 2008). These two minichromosomes were found in a transgenic *Arabidopsis* plant produced by *in planta* vacuum infiltration technique.

Minichromosome in maize

The maize B chromosome exists in only some varieties of maize (Carlson, 1978). The properties and function of B chromosomes in maize were discovered by Carlson and Roseman (1992) and rediscovered by Ronceret et al., in 2007 in the light of minichromosomes. Recently, maize minichromosomes were engineered by modifying A and B chromosome using telomere-mediated chromosome truncation (Fig. 3; Yu et al., 2007a). These minichromosomes were transferred to a diploid background by repeated backcrossing and were stably maintained. By using the same set of constructs, they targeted the maize B chromosome with biolistic-mediated gene transformation. Truncated B chromosomes were recovered with much greater efficiency. The sizes of the mini B chromosomes ranged from very small (Fig. 3) to almost the full size of the normal B chromosome. Although they produced A and B minichromosomes by this method but they were more interested in B chromosome based minichromosomes, because B chromosomes has many interesting properties (Kato et al., 2005), such as: (i) the truncation of B chromosomes will not cause developmental or transmission problems as A chromosomes do; (ii) the B chromosome derivatives can be distinguished by their shape and the presence of a B chromosome specific repeat in and around the centromeric region; and (iii) the size of mini B chromosomes is not crucial because there will be no residual endogenous genes that might interfere with plant development and transgene expression. Recently, Carlson et al., (2007) developed maize minichromosomes (MMCs) and demonstrated that autonomous MMCs can be mitotically and meiotically maintained.

Future prospects of minichromosomes

Engineered minichromosomes can be used in all areas of future genetic engineering. Minichromosomes can be used in site-specific recombination or retrofitting the minichromosomes with additional foreign genes (Ow 2007). Minichromosomes can also be used for gene stacking in plants, which is currently considered as challenging for plant biotechnology (Halpin 2005). Minichromosomes can also facilitate an understanding of fundamental questions about chromosomal structure and function, such as for centromeres, neocentromeres, B chromosome non-disjunction as well as chromosomal behavior in general., In addition, it might be possible to develop a mini B chromosome-based genomic cloning system for capturing large chromosome fragments. The B chromosomes in maize can accumulate up to many copies. Because mini-B chromosomes can non-disjoin in the presence of normal B chromosomes, it may be possible to accumulate higher numbers of mini B chromosomes than normal B chromosomes.
Recently a private company called CHROMATIN® (for details please refer www.chromatininc.com).got three patents from "United States Patent and Trademark Office" for their minichromosome technology (http://patft.uspto.gov/; U.S. Patent Nos. 7,227,057 and 7,226,782 and 7,193,128). The Chromatin® technology uses a single heritable piece of the plant's own DNA to generate a minichromosome. The issued patents describe minichromosome DNA sequences and the use of those sequences to incorporate genes to the plants. Chromatin® Inc. develops and markets novel proprietary technology that enables entire chromosomes to be designed and incorporated into plant cells. These minichromosomes can be used in any plant or crop to simultaneously introduce multiple genes while maintaining precise control of gene expression. Chromatin's minichromosome technology can be used to deliver genes that benefit the agricultural, nutritional, energy, pharmaceutical, and chemical sectors.

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