International Journal of Modern Agriculture, Volume 9, No.3, 2020
ISSN: 2305-7246

Engineered Minichromosomes in Plants

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Abstract

In several plant species, modified minichromosomes were produced by chromosomal truncation telomere-mediated. This approach overcomes the complexities of the epigenetic nature of the centromere role in plants, which has stopped minichromosomes being generates in a plant cell by reintroducing centromere sequences. Gens are linked together with telomere repeats on the one side for cleaved chromosome attachment. When these constructs are implanted in plant cells, the genes are attached to the damaged chromosomes, but the repeats of telomere at the other end, at that stage, can catalyze the creation of a telomere. The chromosome truncation induced by telomers is sufficiently effective to generate very small chromosomes composed essentially of endogenous centromers and the transgenes attached. The transgenes introduced provide a framework for constructing a synthetic chromosome according to specification. The combination of evolved minichromosomes and double haploid replication will substantially speed up transfer of transgenes to new lines and the association of transgenes with genotypes in new background the discussion will focus on potential basic and applicable applications of synthetic chromosomes.

Key words: Artificial Chromosomes, B Chromosomes, Centromeres, Epigenetics, Gene Stacking, Genetic Engineering, Telomeres.

Introduction

The plasticity of the genome compared with other taxa and the ability to manipulate and interpret these chromosomes in the whole lifespan and in various stages of development would possibly lead to a number of applications in modified minichromosomes, better known as artificial clothes or synthetic chromosomes[1], [2]. Basically, the parameters that allow chromosomes to function and which are probably very useful for gene expression study and analysis of gene interactions at a large scale can be used. These can be used in helpful ways to incorporate multiple useful features in an isolated chromosome, which does not have a relation with the rest of the genome, which enables the transgene to be spread efficiently into several sections[3], [4]. There is an ability to incorporate entire bio-chemical pathways in plants to give them different properties, to use plants as factories for processing large quantities of novel proteins or metabolites and for moving mixed characteristics from one variety to another very rapidly among others. This paper addresses the methods used for generating modified minichromosomes in plants, why other approaches have struggled, minichromosomal behavior, conjunction with dual haploid replication. [5], [6]. This paper should help to promote other experiments. The assembly of centromere sections, an origin of replication, a selectable marker and the capping as a linear molecule by the telomeres on the terminal were first produced in budding yeast artificial assembly chromosomes. These

buildings would autonomously work as a chromosome separate from the rest of the genome if they were placed in the yeast cells [7]. In plant species, this experimental model served as a conceptual framework. The centromeres are however very distinct from budding yeast and plant. The budding leaves are centrifugal bases, which are about 125 bp base pairs, and consist of a single nucleosome. Therefore, when they are in the nucleus they influence the development of the kinetochore. In comparison, centromeres are usually geographic expanses and ranges of about 1 to several mega bases [8]–[10]. Furthermore, the basis of centromere activity in plants is strongly epigenetic because the sequences typically present in primary chromosome constraints are neither necessary nor sufficient for the organization of a kinetochore.

1. Epigenetics of Centromers in Plants:

Centromere sequences that can be used to organize the kinetochore to chromosome movements using a yeast model in plants could be based on the capacity to produce engineered minichromosomes. Nevertheless, the intentional transition of centromeric portions, recognized from the functional sections of centralomeric maize, which had hundreds of kilobases in size and had only resulted in a robust integration without autonomous functionality. Furthermore, the discovery that centromeres could be inactivated in systemic maize dicentrics as previously discovered in other taxa revealed that for centromere functions the existence of sequences alone could remain inactive and transmitted from it over the generations. Certain examples were recorded once the centromere inactivation was completed. The fact that centromeric sequences, first described by the plant kingdom for the barley, could be produced on chromosomal fragments without canonically centeromeric sequences, suggested, on the other hand, those specific centromeric sequences were not required. Certain cases of development of novo centromere in maize were now identified. De novo centromeres will carry and transport the otherwise structurally acentric fragment over centuries on chromosomal fragments without a regular centromer. Taken together, the results show that the location of a functioning centomer is determined by the preexisting site for each cell cycle, probably based on chromatin labeling, which apparently does not depend on the underlying DNA sequences. If activity is disrupted, centromere activity can be transferred permanently from one cell to another. This reality could not usually be established by the cell destruction, but this can be found by genetic trickery in structural dicentrics that can save a centromere connected to an active one that is inactivated. Neocentromeres can be introduced between cells and families, as shown by the replication of an otherwise acenteric fragment shortly after chromosome irradiation, and can then be inherited. In terms of the epigenetic complexity of the plant centomers, sequences are coordinated throughout the genome and tend to be located in central chromatin. An active retrotransposon (CRM2) Centromeric Retrotransposon of Maize 2, for instance, is one of the main components of centromere DNA in maize. Many retrotransposons are preferred in certain chromatin structures and new CRM2 inserts are mostly used at central positions. Centromere operation thus is epigenetically derminated and there are DNA elements stored. This remains an open question whether the DNA elements have characteristics which can be

identified with a specific histone H3 form, which is always present in active centers of any kind. Thus centromer sequences are not used to activate centromere activity if the possible artificial chromosome structure inserted in plant cells is placed. This approach has been tested many times but it has been reported that the results are negative. Two positive reports have been made, but as stated in depth elsewhere and these cases are unlikely to be autonomous. Another technique was necessary to make engineered minichromosomes.

2. Chromosomal Truncation Mediated by Telomere:

The alternative approach was introduced because of the epigenetic problems in the synthesis of modified minichromosomes. In this case, sequences from telomeres were asymmetrically arranged in the plant cells on transgenes. It was established that telomeres caused the telomere capping reaction genetically and separated the chromosome at the introductory site. In addition the chromosome is split off at the insertion site in a fraction of the chosen processing events where repetitive telomere has been placed inside the right boundary of Agrobacterium Ti plasmids. The chromosome will possibly be cleared during fusion and the plasmid DNA will be connected to the left boundary of the chromosome. Nevertheless, the chromosome split is hindered by the creation of the telomere when the telomere is replicated close to the right boundary. The transforming plasmid events have been observed in subsequent generations and seem stable, meaning that the reactive truncation occurs during the time the T-DNA is inserted into the chromosome. The transforming plasmid is not truncated. The structure of these truncation processes showed that at the newly formed end of the chromosome the genes placed on the transforming plasmid are present. This position can therefore help to further integrate transgenes by placing sequences there for potential exploitation. In the initial instances, cassettes have been positioned on the terminus for the recombination site. These have been shown to be recombined. The genes placed at the site on the chromosomes showed good expression.

3. B Chromosomes Uses:

The original truncations resulted in the usual maize chromosomes. Nonetheless, there is a supernumerary chromosome that has been used as a good platform for engineered minichromosomes in this species B chromosomes are additional chromosomes that are not required and usually relatively inert in a varietal number of plants and animals. The chromosome of maize B does not need to be developed nor does it harm the low number of copies. B chromosomes have developed aggregation processes, which retain them despite their dispensable presence in populations. This consists of the one mitosis in the life cycle that generates the two sperm, namely the second microspore division, for the B chromosome of maize. In the process of double fertilization the sperm with the B chromosome fertilizes the egg in comparison to the polar nuclei. The maize B is essentially a telocentric chromosome, with the tip of the long arm in a nucleus identical to the centromere to the non-disjunction. In other words, a transmission system occurs where the components of this process are at opposite ends to the chromosome, which, given its almost inert existence, retains the identity of the chromosome in normal conditions. In the absence of a long arm tip

of the chromosome the truncation of the B chromosome eliminates the non-disjunction process. This is a valuable situation in the sense of formed minichromosomes. Biolistic transformation of truncating structures by target cells of many B chromosomes has shown a regular truncation. However, in contrast with the A chromosomes, more active B chromosomes have been detected than truncated A chromosomes, even though the overall chromosome length is shorter for B chromosomes. Researchers assume it is because of the truncation of A chromosomes that a segmental monosomy condition is induced, which is typically negative. Therefore, while a number of chromosome truncation events are detected, certain fractions are likely not able to complete the selection process. On the other hand, chromosome truncation of B has no effect on the cells and these events can be quickly recovered and selected.

4. Methods for Recovering Truncation:

Specific methods for promoting the recovery of truncated chromosomes were effective in organisms lacking B chromosomes. Tetraploid material was used in the production of truncated transgenes in barley and Arabidopsis. The tetraploid comprises four copies of each chromosome under this situation. Therefore, three intact copies remain when one of these copies is truncated. The aneuploidy produced is therefore not as badly as diploid and a separate picture is given to allow the recovery of truncated chromosomes by the new, intact copy of genes that are not required for gametophytic functions. In a maize example, a spontaneous tetraploid has been developed that enables a truncated chromosome to recover with a break close to the centromere. The truncated chromosome was added to a diploid history and was viewed as an external chromosome with a sequence of crosses in a diploid parent. The aneuploidy of an extra chromosome was not serious due to the truncation. These truncated chromosomes can be used by telomere transformation to isolate the other arm. In the use of tetraploids, truncated chromosomes were discovered, and both arms were separated following contamination of diploid matter. If truncated chromosome was found. The experiment consisted of the synthesis of the target genes and free telomers. The collection was made of a minichromosome that did not contain any known chromosome genes, but that was endogenous with a diploid centromere in combination with the transgenes. It is not possible to reconstruct the sequence of events that lead this minichromosome but it is probable that both arms of the chromosome is cleaved off and that the desired genes are attached to the remainder of the minichromosome. The researchers assume that there must have been a monosomal situation but that the remaining chromosomes must have doubled their copy number to the diploid state of choice. Tetraploids are not needed for extremely truncated chromosomes to be obtained from B chromosomes.

5. Dosage Manipulation:

The minichromosomes can be modified in a formulation for controlling the copy number of the engineered minichromosomes of organisms in which the chromosome B can be used as the starting matter, such as maize and rye, or those in which the minichromosomes B have been used like oat and wheat. The truncated B chromosomes of maize have been paired with

an intact B chromosome that provides the functionality of no disjunction. The usual B then provides the transitive function to itself and the non-autonomous truncated minichromosomes for non-disjunction. Afterwards, the number of engineered minichromosomes has been increased up to a maximum of 19 copies by selecting themselves for an increased copying number of minichromosomes over generations. This principle evidence shows that the production of a minichromosome can be improved by modulating its dosage if gene silencing can be prevented.

6. Minichromosomes and Site-Specific Recombination:

As mentioned above, a site specific recombination sequence in the DNA attached to the bottom of cleaved chromosome was found in the first example of a telomere induced truncation in maize. The recombination site for the Cre / lox system was included in this scenario. The activation of the red fluorescent protein was observed in the progeny when this truncated chromosome was converted into another line that expresses Cre recombinase with an additional lox site. PCR amplification across the recombination site junctions followed by sequencing confirmed the exchange occurred. This example showed that the recombination of a particular site through chromosomes could occur and that the transition in a minichromosome could occur near the end of a site. In addition, a site specific recombination was used as evidence to demonstrate that a minichromosome in vivo can remove the marker genes. A minichromosome containing a powerful promoter for transgenic selection of bialophos was made. The gene of the selection was flanked by lox sites with the intention to generate a period of the gene to extract it from the minichromosome, which left a single lox site with a transcription series, behind the promoter. PCR analysis showed that the subset of the gene was eliminated but other parts remained. The minichromosome was subjected to a modified Cre recombinase in a mixture in which they were fused together. This example showed the possibility to change in vivo minichromosomes.

7. Small Chromosomes Behavior:

The endogenous centromere carries an optimized minichromosome as mentioned above. Consequently, all steps that have been applied for transmission through mitosis from the life cycle are usual. Seedlings equipped to hold a minichromosome must present them during the meiosis, which will be studied to demonstrate the faithful maintenance of the growth. For meiosis the action of modified minichromosomes has been observed and all small chromosomes are used in corn. Small chromosomes never locate their partners in a meiosis prophase when two copies are present, as first recorded by Barbara McClintock. Various chromosome sizes of truncated B are studied. It seems that roughly half the size of the usual B-Chromosome is needed to find a pair successfully in a meiocyte. Minichromosome copies of the usual A chromosome homologues are normally distributed at random to the cells.

8. Combination with Methods of Genome Editing:

Recently, methods for genome editing have been extended to plants. The techniques are based on the formation of chromosome breaks when a deletes, template changes, or removes genetic material is used for repairing. This procedure may be used to alter minichromosomes in the same manner as it is used to modify normal chromosomes. Nevertheless, these technologies are constrained in their size in order to alter a genome. The ultimate objective of minichromosome research is to generate transgenic organisms in the hundreds of kilo bases and potentially Mega bases ranges.

Conclusion

Site-specific recombination systems have been used for targeted inserts and genome processing technologies may also exist. Consequently, it seems certain that continuous genes will be added to minichromosomes. Of course, as mentioned above a long-term goal is to build forms in which the minichromosomes can obtain much greater cargo. Using BiBAC technology to provide foreign DNA over 100 kb, combined with techniques for the efficient composition of many fragments, the minichromosomes can be incorporated to help the fragments grow by bounds and leaps. In the current state of the art of minichromosomes, and processes in plants, the development of technologically evolved minichromosomes can be envisioned, which include a wide variety of genes that confer specific useful properties confer various pesticide resistances, yield improvements and nutritional improvements. But some issues that must be taken into account as technology is moving forward can be predicted. For instance, it is not clear what the action of a chromosome is if it consists only of genes without the several transposons that normally fill plant chromosomes. These transposons produce chromatin changes that are likely to influence condensation of the chromosome, which is an exploration pathway for how a minichromosome is growing if it is deficient.

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