De novo generation of plant centromeres at tandem repeats

Chee How Teo · Inna Lermontova · Andreas Houben ·
Michael Florian Mette · Ingo Schubert

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Abstract Artificial minichromosomes are highly desirable tools for basic research, breeding, and biotechnology purposes. We present an option to generate plant artificial minichromosomes via de novo engineering of plant centromeres in Arabidopsis thaliana by targeting kinetochore proteins to tandem repeat arrays at non-centromeric positions. We employed the bacterial lactose repressor/lactose operator system to guide derivatives of the centromeric histone H3 variant cenH3 to LacO operator sequences. Tethering of cenH3 to non-centromeric loci led to de novo assembly of kinetochore proteins and to dicentric carrier chromosomes which potentially form anaphase bridges. This approach will be further developed and may contribute to generating minichromosomes from preselected genomic regions, potentially even in a diploid background.

Keywords cenH3 targeting · De novo centromere formation · Kinetochore proteins · Lac repressor · Lac operator system · Technical advance

Introduction

Different approaches have been described toward developing chromosome-based vector systems for complex gene transfer, either by artificial composition of cloned chromosomal constituents into functional chromosomes (“bottom-up” approach) or by engineering endogenous chromosomes (“top-down” approach). Engineered minichromosomes have been used for development of chromosome-based vector systems (Grimes and Monaco 2005) and to study the function of specific chromosomal domains (e.g., centromeres) (Nakano et al. 2008).

The “bottom-up” approach relies on cell-mediated chromosome assembly after transfection of a cell with recombinant constructs comprising centromeric sequences, a marker gene, telomeric repeats, and other genomic DNA (Clarke and Carbon 1980; Murray and Szostak 1983; Harrington et al. 1997; Ikono et al. 1998). For plants, the “bottom-up” approach has not yet yielded robust solutions (reviewed in Houben et al. 2008; Birchler et al. 2010). To circumvent the necessity of de novo centromere formation, modification of existing chromosomes can be achieved by chromosome truncation. In this “top-down” approach, as shown first for mammals, transformation of cells with cloned telomeric repeats may truncate the distal portion of a chromosome by the formation of a new telomere at the integration site (Fan et al. 1991). In plants, telomere seeding for the formation of truncated chromosomes was successfully applied for maize (Yu et al. 2006), Arabidopsis thaliana (Teo et al. 2011; Nelson et al. 2011), barley (Kapusi et al. 2012), and rice (Xu et al. 2012).

An alternative approach uses functional synthetic kinetochore components generated by tethering the centromeric histone H3 variant cenH3 (Mendiburo et al. 2011), a cenH3 assembly factor (Barnhart et al. 2011), kinetochore components...