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HIGHLIGHT

Centromere Epigenetics in Plants

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The centromere is an essential chromosome site at which the kinetochore forms and loads proteins needed for faithful segregation during the cell cycle and meiosis (Houben et al., 1999; Cleveland et al., 2003; Ma et al., 2007; Birchler and Han, 2009). Centromere specific sequences such as tandem repeats or transposable elements evolve quickly both within and between the species but have conserved kinetochore proteins (Henikoff and Furuyama, 2010). The universal feature of centromere function is a specific histone H3 variant CENH3 (Mendiburo et al., 2011).

In eukaryotes, there are two kinds of centromeres classified as simple and complex. The first defined centromere was a simple one, which is also called a point centromere, and was found in single-celled budding yeast (Furuyama and Biggins, 2007; Malik and Henikoff, 2009). It consists of a single nucleosome, binds one spindle fiber per chromosome and strictly depends on a specific DNA motif of 125 bp in length. The complex type, called regional centromere, contain tandem repeat satellite DNA and retrotransposon elements that are concentrated at all or most primary constrictions. Their sizes can range into megabases.

Maize has a long history as a model for chromosome study that facilitates the study of centromeres. It has 20 chromosomes called A chromosomes as well as a supernumerary or B chromosome. Both A and B chromosomes contain CentC (156 bp, tandem repeat in maize) and CRM (centromeric retrotransposon in maize) located in centromere regions (Jiang et al., 1996; Ananiev et al., 1998; Kato et al., 2004; Lamb et al., 2005).

Plant centromeric specific sequences cannot assemble a functional centromere when re-introduced into plant cells (Phan et al., 2007) unlike human satellite sequences that can be used to create an artificial chromosome in cell lines (Harrington et al., 1997). These results indicated that plant centromeres have a strong epigenetic component making it unclear if centromere sequences can organize a kinetochore in the absence of a pre-existing one (Birchler et al., 2011). However, in the last few years, centromere inactivation and reactivation have been described in maize and wheat (Stimpson and Sullivan, 2010; Birchler et al., 2011). Although plant centromeres are typically composed of tandem repeat sequences and retrotransposons (Ma et al., 2007), neocentromeres can form over unique sequences in barley and an oat−maize addition line (Nasuda et al., 2005; Topp et al., 2009).

CENTROMERE INACTIVATION

Typically one chromosome has one centromere. Dicentric chromosomes were first described in plants by McClintock who noted their unstable nature (McClintock, 1939, 1941). However, in human, several cases of inactive centromeres and further analyses indicated that the silenced centromere is accompanied by the loss of centromere chromatin (Kalitsis and Choo, 2012). In fission yeast, artificial dicentric chromosomes have been developed, which revealed that a centromere was inactivated epigenetically (Sato et al., 2012). In maize, stable dicentric chromosomes have been found and their centromeric DNA sequences and histone modification were studied in detail (Han et al., 2006, 2009; Gao et al., 2011). The inactive centromere shows no constriction and no evidence of CENH3 association.

The dicentric chromosome originally studied was minichromosome that originated from a chromosome type breakage-fusion-bridge cycle from TB-9Sb-Dp9 (Han et al., 2006). The five dicentric minichromosomes examined were stable because only one centromere was active (Han et al.,

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2006) (Fig. 1). It is interesting to note that centromere inactivation is not related to centromere size because one of them, dicentric minichromosome #5, contained one large and one small centromere, with the large one being inactive. The second type of dicentric chromosome was recovered from a centromere tug-of-war (Han et al., 2009). In these cases, the dicentric chromosome contained a 9S chromosome and formed a mirror image chromosome, but with one large and one small B centromere located at the ends. The small version of the B centromere was inactive based on the immunostaining for CENH3, CENP-C and phosphorylation of H3 at Ser-10 (Han et al., 2009).

The third type of dicentric chromosome was a maize A chromosome translocation involving chromosomes 1 and 5 (Gao et al., 2011). Both of the centromeres are large but one has lost centromeric function. The chromosome originated from irradiation decades ago and appeared to have captured a centromere between the breaks in chromosomes 1 and 5. This captured centromere must have become inactive when the translocation was formed. In some other cases we found a newly formed dicentric chromosome from the offspring of B9-Dp9, in which the new dicentric chromosome contains two normal B centromeres with one of them being inactive (Fig. 2). We also found one chromosome that contains three centromere regions with only one active and the other two inactive.

CENTROMERE REACTIVATION

The most common types of DNA sequences in centromere regions are tandem repeats and retrotransposon elements. However, neocentromeres can form anew at sites without these repeats. This raises the question of whether the repeats can foster a centromere or whether they accumulate at the sites of centromeric chromatin. In maize, an inactive centromere can be reactivated through intrachromosomal recombination (Han et al., 2009). A new dicentric chromosome was released from recombination in a fold back chromosome resulting from the centromere tug-of-war described above. This forms a mirror image chromosome with only the small inactive centromeres, but the core DNA sequences such as CentC, CRM and B repeats are present. When the small inactive centromere separated from this dicentric condition, its centromere function was regained in some cells and was stably transmitted to the next generation. In this resurrection process, the centromeric core DNA sequences do not change based on FISH (fluorescence *in situ* hybridization) detection.

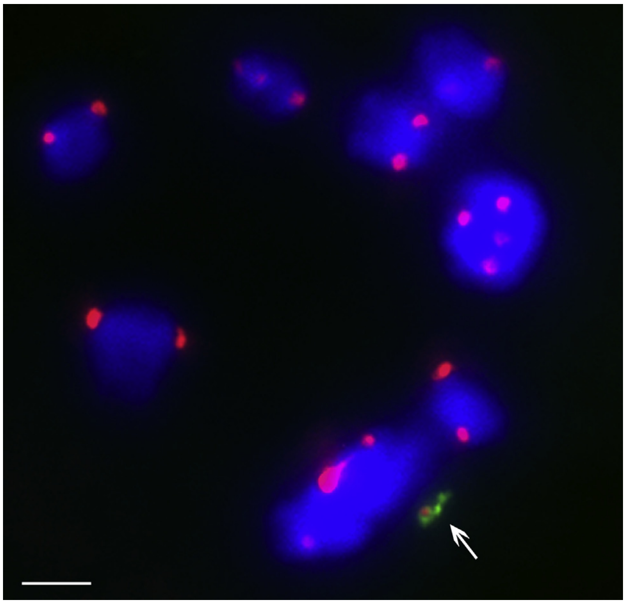

Fig. 1. Immunostaining of CENH3 in dicentric minichromosome at diakinesis of meiosis.
Red represents the signal of CENH3, which is a marker for centromere activity; DAPI-stained chromosomes are blue; green is B specific centromere repeat signal. The arrows denote the dicentric chromosome with one site of CENH3 binding. Bar = 10 μm.

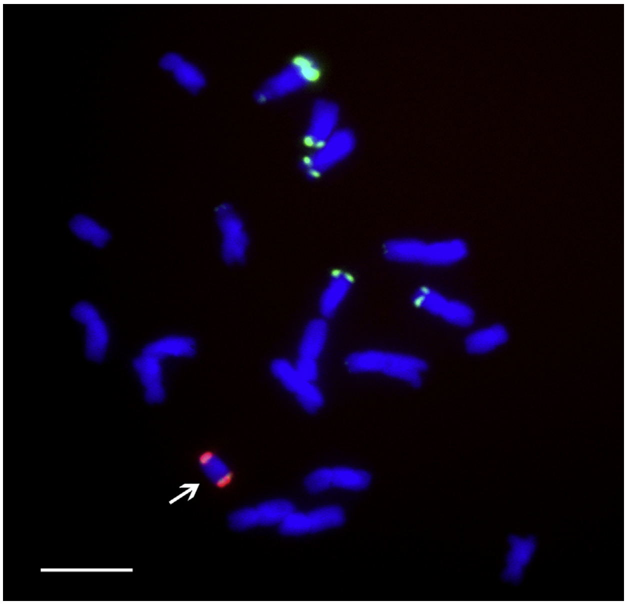

Fig. 2. FISH pattern of a newly formed dicentric chromosome.
Arrow indicates a newly formed dicentric chromosome, which contained two similar sized centromere regions. Red is B specific centromere repeat signals; green is knob and DAPI-stained chromosomes are blue. Bars = 10 μm.

NONDISJUNCTION OF THE B CENTROMERE

B chromosomes (Bs), also called supernumerary chromosomes, are dispensable components in the genomes of plants, fungi and animals. B chromosomes do not confer any advantages on the organism and have a non-Mendelian transmission. They are considered selfish elements that maintain a high transmission in populations (Jones and Houben, 2003; Banaei-Moghaddam et al., 2012). In plants, the B chromosomes undergo nondisjunction constituting a post-meiotic drive. It occurs at the first pollen mitosis in rye and second pollen mitosis in maize.

The availability of an inactive B centromere on chromosome arm 9S provided an opportunity to examine whether centromere function is necessary for nondisjunction. The normal nondisjunction property of the B centromere involves