Switching the centromeres on and off: epigenetic chromatin alterations provide plasticity in centromere activity stabilizing aberrant dicentric chromosomes

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Abstract

The kinetochore, which forms on a specific chromosomal locus called the centromere, mediates interactions between the chromosome and the spindle during mitosis and meiosis. Abnormal chromosome rearrangements and/or neocentromere formation can cause the presence of multiple centromeres on a single chromosome, which results in chromosome breakage or cell cycle arrest. Analyses of artificial dicentric chromosomes suggested that the activity of the centromere is regulated epigenetically; on some stably maintained dicentric chromosomes, one of the centromeres no longer functions as a platform for kinetochore formation, although the DNA sequence remains intact. Such epigenetic centromere inactivation occurs in cells of various eukaryotes harbouring 'regional centromeres', such as those of maize, fission yeast and humans, suggesting that the position of the active centromere is determined by epigenetic markers on a chromosome rather than the nucleotide sequence. Our recent findings in fission yeast revealed that epigenetic centromere inactivation consists of two steps: disassembly of the kinetochore initiates inactivation and subsequent heterochromatinization prevents revival of the inactivated centromere. Kinetochore disassembly followed by heterochromatinization is also observed in normal senescent human cells. Thus epigenetic centromere inactivation may not only stabilize abnormally generated dicentric chromosomes, but also be part of an intrinsic mechanism regulating cell proliferation.

Introduction

Inheritance of genetic information by daughter cells is ensured by the correct segregation of chromosome in eukaryotes. Among several essential elements composing the chromosome, the centromere is the key for correct chromosome segregation. The kinetochore, which is a multi-protein complex formed on the centromere DNA, mediates the physical interaction between the chromosome and the spindle, and thus plays a pivotal role in movement of the chromosome during mitosis and meiosis. Although there have been many studies in various model organisms to find elements determining centromere identity, it remains unclear how and why only a defined region of the chromosome can act as the centromere.

Analysis of centromeric DNA sequences in the budding yeast *Saccharomyces cerevisiae* suggested the presence of the DNA element necessary and sufficient for formation of a functional kinetochore [1]. As the element is relatively short (∼125 bp), the centromere in budding yeast is referred to as a 'point centromere'. However, short specific DNA sequences determining centromere identity have not been found in a

number of other organisms. Instead, the centromeres often contain large arrays of repetitive sequences expanding several tens to thousands of kilobases, and are therefore referred to as 'regional centromeres' [2,3]. Repetitive sequences composing centromeres are highly diverse among species. Despite the diversity of the centromere DNA sequences, proteins composing the kinetochore have been highly conserved during evolution. Among the many kinetochore proteins identified to date, CENP-A (centromere protein A) appears to be particularly important for centromere identity [4]. CENP-A is considered to be a centromere-specific histone H3 variant that replaces authentic histone H3 to form centromere-specific chromatin acting as a platform for kinetochore assembly.

Neocentromere formation is a phenomenon whereby a part of the chromosome other than the centromere gains the centromere function as a platform for kinetochore assembly [5]. The neocentromere was also found to be formed in the fission yeast *Schizosaccharomyces pombe* when the authentic centromere was artificially disrupted [6]. Although the DNA sequences of the neocentromeres do not share any similarities with those of the authentic centromeres, all of the essential kinetochore proteins gather on the neocentromere. Once established, the neocentromere is stably inherited by daughter cells throughout subsequent generations [7]. These findings strongly suggest that the site of kinetochore assembly is

determined by as yet unidentified epigenetic markers on the chromosome rather than the nucleotide sequence.

The finding of neocentromere formation suggested that any region of a chromosome may have the potential to become a centromere, and thus multiple regions on a single chromosome could simultaneously act as centromeres by multiple neocentromere formation. However, as discussed below, the presence of multiple centromeres on a single chromosome is disruptive during mitosis, and thus the number of centromeres per chromosome is predicted to be somehow restricted. The occurrence of multiple centromeres on a single chromosome is caused not only by the formation of multiple neocentromeres, but also by improper DNA rearrangements leading to chromosome fusion. Clinical examples indicating the spontaneous occurrence of a dicentric chromosome (i.e. a chromosome harbouring two centromeres) due to translocation have been reported in humans [8,9]. Several groups, including ours, developed model systems generating an artificial dicentric chromosome, e.g. dicentric chromosomes were generated in budding yeast by integration of a conditionally inactivated centromere into the chromosome [10], whereas chromosome fusion forming a dicentric chromosome was induced by site-specific recombination in the fission yeast *S. pombe* and the fruitfly *Drosophila melanogaster* [11,12]. Transient expression of a dominant-negative mutant of telomere protein TRF2 in human cultured cells caused telomere de-protection resulting in dicentric chromosome formation [13]. These studies on dicentric chromosomes suggested that a proportion of dicentric chromosomes are somehow stabilized and properly segregated in mitosis. We found that, although most fission yeast cells failed to proliferate in the presence of an artificially generated dicentric chromosome, cell division resumed in some cells in which the dicentric chromosome was epigenetically stabilized [11]. In the present review, we summarize recent progress in understanding the epigenetic regulation of centromere activity. Epigenetic centromere inactivation, whereby the centromere loses its activity without any alterations in the DNA sequence, has been observed in a wide variety of organisms, suggesting that it is an evolutionarily conserved mechanism preventing loss of genetic information due to dicentric chromosome formation. It may also promote chromosomal alterations during evolution.

Consequences of dicentric chromosome formation

It has long been believed that dicentric chromosomes are broken apart when the centromeres are pulled towards the opposing spindle poles in mitosis and meiosis. As suggested in early studies in maize [14,15], if the broken end of the chromosome is not healed properly, the replicated sister chromatids are thought to fuse at the broken ends, and consequently another dicentric chromosome, which would be broken in the next round of chromosome

Figure 1 | Cell cycle arrest induced by a dicentric chromosome in fission yeast

Fission yeast cells are arrested in interphase when the dicentric chromosome is generated by artificial chromosome fusion. This cell cycle arrest requires DNA damage/replication checkpoint proteins (Rad3, Chk1 and Cds1), suggesting a novel checkpoint regulation mechanism for monitoring the number of centromeres per chromosome.
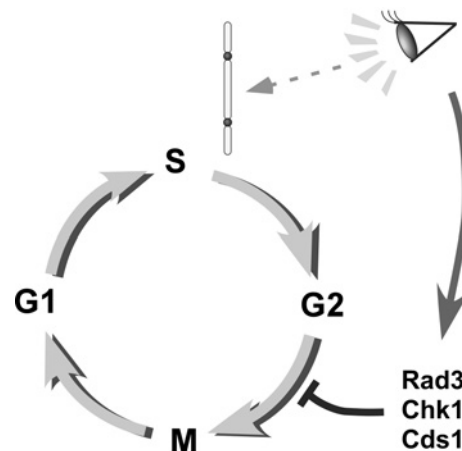

segregation, is generated. As breakage of the chromosome would cause loss and/or alteration of genetic information, the formation of dicentric chromosomes is potentially dangerous. Consistent with this suggestion, artificial dicentric chromosomes in budding yeast were found to be broken in mitosis [16], and to be consequently converted into two stable monocentric chromosomes if the broken ends were healed by *de novo* telomere addition [17,18]. Similarly, the dicentric chromosome in *D. melanogaster* showed abnormal 'chromosome bridge' stretching between the opposing spindle poles during mitotic anaphase and was subsequently broken [12]. These observations indicated that, in these organisms, the processes of mitotic chromosome segregation proceeds even in the presence of dicentric chromosomes, which are eventually torn apart by spindle pulling force.

In contrast with the above-mentioned results in maize, budding yeast and fruitflies, we recently found that the presence of a dicentric chromosome caused cell cycle arrest in interphase in fission yeast [11] (Figure 1). When the artificial dicentric chromosome was generated by induced site-directed recombination that fuses the ends of chromosomes 1 and 2, *S. pombe* cells showed elongated cell morphology characteristic of cells in cell cycle arrest. A cytoplasmic array of microtubules was observed in these elongated cells, indicating that these cells were arrested before the onset of mitosis. This interphase arrest induced by the presence of the dicentric chromosome was dependent on genes involved in the DNA structure checkpoint, but not the mitotic checkpoint; cells lacking a gene related to DNA damage and/or replication checkpoint (*rad3*[+], *chk1*[+] or *cds1*[+]) appeared to keep dividing even after the dicentric chromosome was generated by induced chromosome fusion, whereas cells lacking the *mad2*[+] gene, which is essential for

Figure 2 | **Possible mechanisms stabilizing dicentric chromosomes**

(**A**) Schematic representations of a stabilized dicentric chromosome. Dicentric chromosomes generated by chromosome fusion are stabilized in three ways: (a) epigenetic centromere inactivation, (b) elimination of the centromere by DNA rearrangement, and (c) chromosome breakage into two monocentric chromosomes. (**B**) Proportions of the types of stabilized dicentric chromosomes in fission yeast. Epigenetic centromere inactivation occurred in more than three-quarters of the stabilized dicentric chromosomes [11].
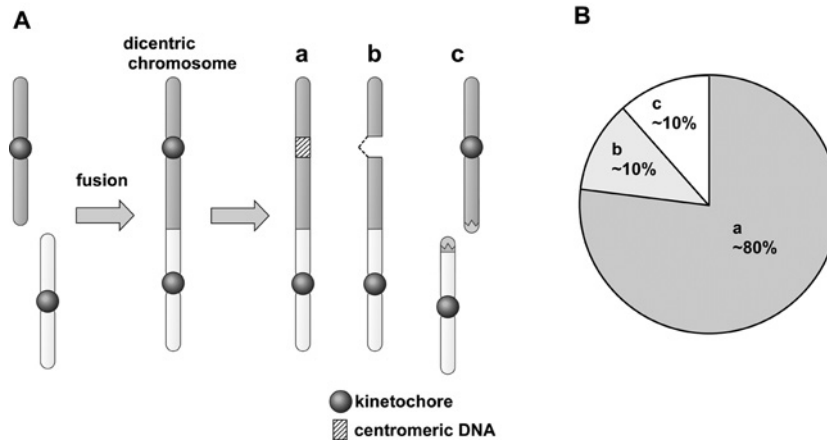
the mitotic spindle checkpoint, stopped dividing and showed an elongated cell morphology similar to that of the wild-type cells. Our observations suggested that, in fission yeast, the number of centromeres per chromosome may somehow be monitored by a novel DNA structure checkpoint that blocks cell cycle progression to avoid mitotic chromosome breakage in cases where multiple centromeres are detected on a single chromosome. It is also possible that the ends of the broken dicentric chromosome, which activates the DNA damage checkpoint, could hardly be healed in *S. pombe* for as yet unknown reasons. Although the consequences of dicentric chromosome formation vary among species, its presence greatly endangers normal cell proliferation in all cases. Thus a 'repair' mechanism may exist for restoring cell proliferation in the event that a dicentric chromosome is spontaneously formed.

Dicentric chromosomes are stabilized by epigenetic centromere inactivation

Although the majority of dicentric chromosomes are broken into monocentric chromosomes or cause permanent cell cycle arrest, a small portion (less than 1%) of *S. pombe* cells proliferated and maintained the dicentric fused chromosome stably for multiple generations [11]. Detailed analyses indicated that the stably maintained dicentric chromosomes became functionally monocentric because of either epigenetic centromere inactivation as described below or DNA rearrangements deleting one of the two centromeres (Figure 2). Whereas dicentric chromosomes were stabilized in budding yeast exclusively by DNA rearrangements leading to centromere deletion [17,19], epigenetic centromere inactivation was also observed in plants (maize and wheat) [20–23] and humans [13,24–27].

In 70–80% of the stabilized dicentric chromosomes in fission yeast, one of the two centromeres appeared to be inactivated epigenetically; although the DNA sequence of the inactivated centromere was intact, it showed no accumulation of CENP-A, which is essential for formation of the functional kinetochore [11]. Consistent with the absence of CENP-A, the epigenetically inactivated centromere no longer bound to the spindle microtubules during mitosis and segregated as part of a chromosome arm.

Epigenetic centromere inactivation has been observed in various organisms other than fission yeast. In maize, cytological analysis of stabilized dicentric chromosomes derived from the non-essential B-chromosome indicated that the kinetochore protein CENH3 (centromere-specific variant of histone H3, the maize orthologue of CENP-A) accumulated on only one of two centromeres, although both of the centromeres contained the identical centromere-specific repeat sequences [20]. When the two B-chromosome-derived centromeres were different in size, the smaller centromere was always inactivated; however, when separated from the active centromere by intrachromosomal recombination, the inactivated smaller centromere regained the activity to act as a platform for kinetochore formation, suggesting that the smaller centromere was epigenetically marked not to function as a kinetochore-forming platform as long as another active centromere was present on the same chromosome [21]. In some patient-derived spontaneous human fused chromosomes, although two distinct arrays of α-satellite DNA repeats (a hallmark of the human centromere) were clearly detected by FISH (fluorescence *in situ* hybridization), the kinetochore proteins, such as CENP-A and CENP-C, were localized on only one of these two arrays [25,26]. Similarly, some freshly generated human artificial dicentric chromosomes were converted into functionally monocentric

chromosomes by dissociation of the kinetochore components from one of the two centromeres, in both of which α-satellite arrays appeared intact [13]. Thus epigenetic inhibition of centromere activity is commonly observed in stabilization of dicentric chromosomes in fission yeast, plants and humans. Such epigenetic regulation of centromere activity may ensure that each chromosome contains a single functional centromere. It remains unknown why centromere inactivation occurs only at low frequency in dicentric chromosomes, the majority of which are broken or cause cell-cycle arrest. As centromere inactivation is predicted to be harmful, if it would occur in normal monocentric chromosome, its frequency might be kept low so as not to occur accidentally.

Although further studies are required to determine the molecular mechanism underlying epigenetic regulation of centromere function, our study suggested that disassembly of the kinetochore complex may initiate centromere inactivation in fission yeast [11] (Figure 3); mutations destabilizing the kinetochore complex markedly increased (10–50-fold) the probability of epigenetic centromere inactivation stabilizing the dicentric chromosome. A previous study in cultured human cells suggested that most of the dicentric chromosomes generated *de novo* were stably maintained for more than 100 cell divisions with the kinetochore proteins assembling on both centromeres before stabilization by centromere inactivation [13]. This observation suggests that, at least in humans, centromere inactivation may not be an instant phenomenon, but may rather consist of hierarchical processes taking multiple cell divisions for completion.

Heterochromatin formation and histone deacetylation prevents the reactivation of inactivated centromeres

The fission yeast centromere consists of two domains: the CENP-A-binding central core domain and the flanking heterochromatinized domain [28]. The core domain of the inactivated centromere in fission yeast was shown to contain histone H3 with methylation of Lys[9] and Swi6 (fission yeast homologue of heterochromatin protein HP-1), neither of which is found on the active centromere, indicating that the inactivated centromeres were heterochromatinized in fission yeast [11] (Figure 3).

Similarly, the inactivated centromere in maize appeared to be heterochromatinized. The centromere of the maize B-chromosome (B-centromere) spans ~700 kb of DNA, and consists of five arrays of ZmB repeats separated by intermingled CentC satellite repeats and CRM (centromeric retrotransposon of maize) sequences [29]. Although cytosine residues in CentC and CRM sequences are hypomethylated in the functional B-centromere, they were found to be highly methylated on the inactivated B-centromere of the dicentric chromosome [22]. As hypermethylation of the cytosine residue is commonly found in heterochromatin in higher

Figure 3 | Two-step model of epigenetic centromere inactivation in fission yeast

Genetic analysis in fission yeast suggests that the epigenetic centromere inactivation consists of two steps. Epigenetic inactivation is thought to be initiated by dissociation of kinetochore components from the centromere. Subsequently, heterochromatin may expand from flanking regions to the core region of the inactivated centromere, accompanying histone deacetylation, which prevents reassembly of the kinetochore.
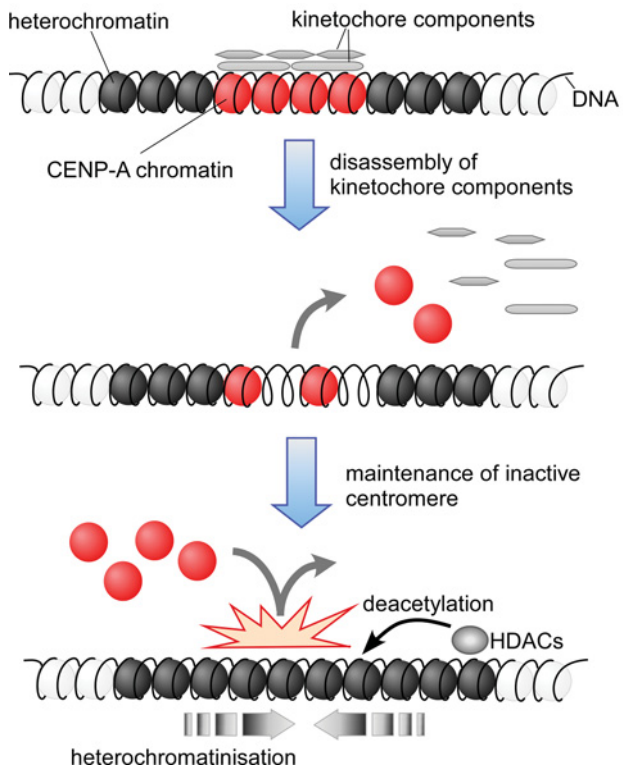
eukaryotes, the inactivated centromere in maize may also be fully covered with heterochromatin.

Our genetic analysis in fission yeast indicated that heterochromatin formation was not essential for initiating epigenetic centromere inactivation; as seen in the wild-type cells, a centromere on the stabilized dicentric chromosomes was also epigenetically inactivated in mutants defective in heterochromatin formation [11]. Heterochromatin appeared to play an essential role in preventing reactivation of inactivated centromeres. The once-inactivated centromere on the stabilized dicentric chromosome was frequently reactivated in heterochromatin-defective mutant *S. pombe* cells when the active centromere was removed, whereas it was hardly reactivated in wild-type cells. The frequency of reactivation of the once-inactivated centromere was markedly elevated by disruption of HDAC (histone deacetylase) genes, suggesting that histone deacetylation accompanying heterochromatinization prevents revival of the inactivated centromere.

De novo CENP-A incorporation and assembly of the kinetochore on human artificial chromosomes were promoted by acetylation of Lys[9] of histone H3, and suppressed

by its methylation, which occurs in heterochromatin [30]. Deposition of newly synthesized CENP-A on to the human centromere requires hMIS18 and RbAp46/48, which regulate acetylation of histones in the centromere [31]. Thus heterochromatin formation and subsequent histone deacetylation may prevent CENP-A incorporation into the centromere in humans. Whereas the precise roles of histone deacetylation and heterochromatin formation in epigenetic centromere inactivation in higher eukaryotes remain elusive, they may prevent reassembly of the kinetochore on the once-inactivated centromere, as suggested in fission yeast.

Centromere inactivation down-regulates cell division in senescent human cells

Centromere inactivation appears to be important not only for avoiding loss of genetic information due to dicentric chromosome formation, but also for attenuating cell proliferation in senescent cells. Kinetochore proteins including CENP-A were found to be dissociated from the centromeres in senescent human fibroblasts, which remain metabolically active, but stop undergoing cell division [32]. CENP-A-lacking centromeres in the senescent human cells were covered by heterochromatin proteins, such as HP-1β, HP-1γ and CENP-B, similarly to the inactivated centromeres on the stabilized dicentric chromosome in fission yeast. Interestingly, forced depletion of CENP-A using shRNA caused premature cellular senescence in a manner dependent on the tumour suppressor p53, suggesting that a positive feedback circuit may exist between the kinetochore disassembly and induction of cellular senescence. The p53-dependent cellular senescence induced by kinetochore disassembly may be a self-defence mechanism preventing proliferation of cells with chromosomal abnormalities.

Concluding remarks

As discussed in the present review, the presence of multiple centromeres on a single chromosome disturbs the normal progression of mitosis and/or the cell cycle in eukaryotes, and therefore surplus centromeres must be inactivated epigenetically or by chromosome rearrangements. Our study on fission yeast artificial dicentric chromosomes demonstrated that epigenetic centromere inactivation is initiated by removal of kinetochore components from the centromere [11] (Figure 3). Subsequent heterochromatinization and histone deacetylation on the inactivated centromere may suppress reassembly of the kinetochore complex, fixing the inactive state of the once-inactivated centromere. Further detailed studies are required to understand how the presence of a dicentric chromosome is recognized, and what triggers kinetochore disassembly leading to centromere inactivation. Comparative karyotype analyses among related species indicate that chromosome fusion, which is thought to promote karyotype alteration resulting in establishment of new species, occurred frequently during evolution [33,34]. Epigenetic centromere inactivation, which stabilizes fused dicentric chromosomes, may therefore play an important role in eukaryote genome evolution.

Funding

References

1 Koshland, D., Rutledge, L., Fitzgerald-Hayes, M. and Hartwell, L.H. (1987) A genetic analysis of dicentric minichromosomes in *Saccharomyces cerevisiae*. Cell **48**, 801–812

2 Pluta, A.F., Mackay, A.M., Ainsztein, A.M., Goldberg, I.G. and Earnshaw, W.C. (1995) The centromere: hub of chromosomal activities. Science **270**, 1591–1594

3 Meraldi, P., McAinsh, A.D., Rheinbay, E. and Sorger, P.K. (2006) Phylogenetic and structural analysis of centromeric DNA and kinetochore proteins. Genome Biol. **7**, R23

4 De Rop, V., Padeganeh, A. and Maddox, P.S. (2012) CENP-A: the key player behind centromere identity, propagation, and kinetochore assembly. Chromosoma **121**, 527–538

5 Marshall, O.J., Chueh, A.C., Wong, L.H. and Choo, K.H. (2008) Neocentromeres: new insights into centromere structure, disease development, and karyotype evolution. Am. J. Hum. Genet. **82**, 261–282

6 Ishii, K., Ogiyama, Y., Chikashige, Y., Soejima, S., Masuda, F., Kakuma, T., Hiraoka, Y. and Takahashi, K. (2008) Heterochromatin integrity affects chromosome reorganization after centromere dysfunction. Science **321**, 1088–1091

7 Saffery, R., Irvine, D.V., Griffiths, B., Kalitsis, P., Wordeman, L. and Choo, K.H. (2000) Human centromeres and neocentromeres show identical distribution patterns of >20 functionally important kinetochore-associated proteins. Hum. Mol. Genet. **9**, 175–185

8 Daniel, A. and Lam-Po-Tang, P.R. (1976) Structure and inheritance of some heterozygous Robertsonian translocation in man. J. Med. Genet. **13**, 381–388

9 de la Chapelle, A. and Stenstrand, K. (1974) Dicentric human X chromosomes. Hereditas **76**, 259–268

10 Hill, A. and Bloom, K. (1989) Acquisition and processing of a conditional dicentric chromosome in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9**, 1368–1370

11 Sato, H., Masuda, F., Takayama, Y., Takahashi, K. and Saitoh, S. (2012) Epigenetic inactivation and subsequent heterochromatinization of a centromere stabilize dicentric chromosomes. Curr. Biol. **22**, 658–667

12 Ahmad, K. and Golic, K.G. (1998) The transmission of fragmented chromosomes in *Drosophila melanogaster*. Genetics **148**, 775–792

13 Stimpson, K.M., Song, I.Y., Jauch, A., Holtgreve-Grez, H., Hayden, K.E., Bridger, J.M. and Sullivan, B.A. (2010) Telomere disruption results in non-random formation of *de novo* dicentric chromosomes involving acrocentric human chromosomes. PLoS Genet. **6**, e1001061

14 McClintock, B. (1939) The behavior in successive nuclear divisions of a chromosome broken at meiosis. Proc. Natl. Acad. Sci. U.S.A. **25**, 405–416

15 McClintock, B. (1941) The stability of broken ends of chromosomes in *Zea mays*. Genetics **26**, 234–282

16 Brock, J.A. and Bloom, K. (1994) A chromosome breakage assay to monitor mitotic forces in budding yeast. J. Cell Sci. **107**, 891–902

17 Jager, D. and Philippsen, P. (1989) Stabilization of dicentric chromosomes in *Saccharomyces cerevisiae* by telomere addition to broken ends or by centromere deletion. EMBO J. **8**, 247–254

18 Pobiega, S. and Marcand, S. (2010) Dicentric breakage at telomere fusions. Genes Dev. **24**, 720–733

19 Pennaneach, V. and Kolodner, R.D. (2009) Stabilization of dicentric translocations through secondary rearrangements mediated by multiple mechanisms in *S. cerevisiae*. PLoS ONE **4**, e6389

20 Han, F., Lamb, J.C. and Birchler, J.A. (2006) High frequency of centromere inactivation resulting in stable dicentric chromosomes of maize. Proc. Natl. Acad. Sci. U.S.A. **103**, 3238–3243

21 Han, F., Gao, Z. and Birchler, J.A. (2009) Reactivation of an inactive centromere reveals epigenetic and structural components for centromere specification in maize. Plant Cell **21**, 1929–1939

22 Koo, D.H., Han, F., Birchler, J.A. and Jiang, J. (2011) Distinct DNA methylation patterns associated with active and inactive centromeres of the maize B chromosome. Genome Res. **21**, 908–914

23 Zhang, W., Friebe, B., Gill, B.S. and Jiang, J. (2010) Centromere inactivation and epigenetic modifications of a plant chromosome with three functional centromeres. Chromosoma **119**, 553–563

24 Maraschio, P., Zuffardi, O., Caiulo, A., Dainotti, E., Piantanida, M., Rivera, H. and Tupler, R. (1990) Deletion of specific sequences or modification of centromeric chromatin are responsible for Y chromosome centromere inactivation. Hum. Genet. **85**, 491–494

25 Sullivan, B.A. and Schwartz, S. (1995) Identification of centromeric antigens in dicentric Robertsonian translocations: CENP-C and CENP-E are necessary components of functional centromeres. Hum. Mol. Genet. **4**, 2189–2197

26 Warburton, P.E., Cooke, C.A., Bourassa, S., Vafa, O., Sullivan, B.A., Stetten, G., Gimelli, G., Warburton, D., Tyler-Smith, C., Sullivan, K.F. et al. (1997) Immunolocalization of CENP-A suggests a distinct nucleosome structure at the inner kinetochore plate of active centromeres. Curr. Biol. **7**, 901–904

27 Higgins, A.W., Gustashaw, K.M. and Willard, H.F. (2005) Engineered human dicentric chromosomes show centromere plasticity. Chromosome Res. **13**, 745–762

28 Partridge, J.F., Borgstrom, B. and Allshire, R.C. (2000) Distinct protein interaction domains and protein spreading in a complex centromere. Genes Dev. **14**, 783–791

29 Jin, W., Lamb, J.C., Vega, J.M., Dawe, R.K., Birchler, J.A. and Jiang, J. (2005) Molecular and functional dissection of the maize B chromosome centromere. Plant Cell **17**, 1412–1423

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

31 Fujita, Y., Hayashi, T., Kiyomitsu, T., Toyoda, Y., Kokubu, A., Obuse, C. and Yanagida, M. (2007) Priming of centromere for CENP-A recruitment by human hMis18α, hMis18β, and M18BP1. Dev. Cell **12**, 17–30

32 Maehara, K., Takahashi, K. and Saitoh, S. (2010) CENP-A reduction induces a p53-dependent cellular senescence response to protect cells from executing defective mitoses. Mol. Cell. Biol. **30**, 2090–2104

33 Allshire, R.C., Gosden, J.R., Cross, S.H., Cranston, G., Rout, D., Sugawara, N., Szostak, J.W., Fantes, P.A. and Hastie, N.D. (1988) Telomeric repeat from *T. thermophila* cross hybridizes with human telomeres. Nature **332**, 656–659

34 Ferguson-Smith, M.A. and Trifonov, V. (2007) Mammalian karyotype evolution. Nat. Rev. Genet. **8**, 950–962