Plant Centromere Biology

COPYRIGHTED MATERIAL

1 *Arabidopsis* Centromeres

Minoru Murata

Centromere DNA structure, 5
Cytosine methylation and heterochromatin, 6
Centromere proteins, 6
Functional domains, 9
Future prospects and conclusions, 11
Acknowledgments, 11
References, 11

Arabidopsis thaliana (L.) Heynh. is an annual flowering plant belonging to the family Brassicaceae. Since it has quite a small genome size and low amount of repetitive DNA sequences (see Meyerowitz, 1992, for the early history of the genome size estimation), it has become a model for molecular biological studies. Hence, its genome was the first among plant species to be sequenced (Arabidopsis Genome Initiative, 2000). This species has five pairs of chromosomes ($2n = 2x = 10$; Figure 1.1a), which is less than the chromosome number possessed by closely related species such as *A. lyrata* ($2n = 2x = 16$) and *A. arenosa* (*Cardaminopsis arenosa*; $2n = 2x = 16$). *A. suecica* ($2n = 4x = 26$) is an allotetraploid between *A. thaliana* and *A. arenosa* (Jakobsson et al., 2006).

Chromosome size, which is highly related to genome size, has made cytological analysis difficult in *Arabidopsis* species. Nevertheless, it is very surprising that the first accurate report regarding the chromosome number ($2n = 10$ for *A. thaliana*) was made in 1907 (Laibach, 1907). Although the properties that made this plant suitable for genetic studies have been recognized for more than half a century (cf. Redei, 1992), the cytogenetical approach had been quite limited until Sears's work (Steinitz-Sears, 1963; Sears and Lee-Chen, 1970). They assumed that the centromeres are located in or adjacent to the heterochromatic regions. Ambros and Schweizer (1976) applied Giemsa C-banding and confirmed that the centromeric regions of all chromosomes are heterochromatic. However, no DNA components of the centromeres had been revealed for a decade.

Plant Centromere Biology, First Edition. Edited by Jiming Jiang and James A. Birchler.
© 2013 John Wiley & Sons, Inc. Published 2013 by John Wiley & Sons, Inc.

Figure 1.1 (a) Schematic representation of the chromosome and centromere sizes of *Arabidopsis thaliana* (after Hosouchi et al., 2002). Orange box: genetically defined centromeric region; pink box: the central domain. (b) FISH image of a somatic prometaphase cell of *A. thaliana* ($2n = 10 + \text{mini}\alpha$), probed with the 180-bp repeats. Arrow indicates a minichromosome (miniα; Murata et al., 2008) carrying truncated 180-bp repeat array. Bar = 5 µm. (c) Consensus sequences of 178-bp repeats from 41 ecotypes (upper), Columbia (Col; middle) and Col-edge (lower). Blue, red, and green dotted boxes indicate conserved (C1, C2, and C3), variable (V1) regions (Hall et al., 2003), and conserved Box A and B (Heslop-Harrison et al., 1999), respectively. Cytosine residues of underlined nucleotides in light blue are possibly differentially methylated depending on the centromeric or pericentromeric location (Zhang et al., 2008). (d) Schematic representation of chromosome 2 and its derivatives, showing centromere sizes and HTR12 (CENH3) localization, based on our previous data (Murata et al., 2008; Yokota et al., 2011). (e) FISH image of a pachytene cell of *A. thaliana*, probed with four different BAC clones mapped on the short arm of chromosome 2. Upper: miniΔ; lower: chromosome 2. Bar = 1 µm. For color detail, please see color plate.

Centromere DNA structure

Regarding the centromeric DNA of *A. thaliana*, the first report was made by Martinez-Zapater and others (1986), which was followed by the work of Simoens and others (1988). Both research groups identified the same tandem repeat family, the unit size of which is approximately 180 bp (178~180 bp) and which constitutes approximately 0.8%–1.4% of the genome, among *Hin*dIII-digested DNA and the cosmid DNA library. The ladder pattern obtained via partial genome digestion by Southern blot analysis implied that the repetitive DNA sequences are arrayed in tandem. Although the former researchers speculated that the "180-bp family" lies within the heterochromatic blocks associated with centromeres or nucleolar organizing regions (Martinez-Zapater et al., 1986), neither research group could perform cytological analysis, due to the technical difficulty associated with the small size of chromosomes. Confirmation of the centromeric localization under microscopy had to wait for the establishment of the fluorescence in situ hybridization (FISH) technique. Using pAL1 as a probe, Maluszynska and Heslop-Harrison (1991) performed FISH and found that the FISH signals colocalize with the centromeric heterochromatin that could be visualized by DAPI-staining. A similar observation was made on mitotic metaphase cells using their own isolated two repetitive DNA sequences (pAtMr1 and pAtHr1) having high homology to pAL1 (Murata et al., 1994; see Figure 1.1b as an example). In addition, they extended their observation to the meiotic chromosomes (prophase I to metaphase I) and noted that the FISH signals preferentially appeared at a limited part of heterochromatic regions, that is, within the heterochromatic blocks that are extended well at zygotene to pachytene stages.

The pAL1-family repetitive DNA sequences were reported to be tandemly arrayed to form large clusters of more than 50 kb (Martinez-Zapater et al., 1986). Pulsed-field gel electrophoresis revealed that the centromere clusters exceeded 1 Mb (Murata et al., 1994). Similarly, the use of different restriction enzymes that are insensitive to cytosine methylation allowed Round et al. (1997) to report that the 180-bp repeats form large clusters up to 1 Mb and that large (>400 kb) restriction fragments containing 180-bp repeat arrays total over 3 Mb in length in ecotype Columbia. They also indicated that there are size polymorphisms in the 180-bp repeat arrays between two ecotypes, Columbia and Landsberg *erecta*, which made it possible to map the 180-bp repeat arrays in the *Arabidopsis* genetic map (Round et al., 1997).

Copenhaver and others (1999) conducted a more extensive and accurate mapping of the centromeres and succeeded in connecting the centromeric contigs to the physical maps. In addition to the 180-bp repeat family, some other repeats such as 106A that have homology to the *Athila* retrotransposon were found to localize at the centromeric regions (Thompson et al., 1996; Brandes et al., 1997), but their participation in centromere function has not been demonstrated.

The genome project of *A. thaliana* was completed in December of 2000, and the 115.4-Mb region of the genome was recorded (Arabidopsis Genome Initiative, 2000). In the genome project, over 5 Mb of centromeric regions and over 3 Mb of repetitive arrays (the 180-bp repeats and 5S rDNA) were sequenced, and the results showed that the centromeric regions are rich in various kinds of repetitive DNA sequences similar to those of many higher eukaryotes. However, the core regions within the centromeres, consisting mainly of the homogeneous 180-bp repeats, remain unrecorded. This high homogenization of the repeats with the head-to-tail repeat unit organization has made it difficult to find landmarks within the sequences. It was reported that 95% of the nucleotides are conserved, and that there is 99% conservation in the two boxes 30- and 24-bp long (Heslop-Harrison et al., 1999; Heslop-Harrison et al., 2003). However, these two boxes were not highly conserved across 41 ecotypes (Hall et al., 2003), and instead three other conserved regions (C1, C2, and C3) with 95% conservation and one variable region (V1) were noted (Figure 1.1c).

Based on the molecular and cytogenetical analyses of the centromere of chromosome 1, Haupt and others (2001) first estimated the centromere sizes of all five chromosomes, ranging from 1.4 Mb (Chromosome 3) to 2.3 Mb (Chromosome 1). Since there were still large gaps uncovered with existing BAC clones in the middle of the centromeres, the overall organization of the centromeres was investigated by restriction analysis of large DNA fragments (Kumekawa et al., 2000, 2001; Hosouchi, 2002). As a result, genetically defined centromeric regions were determined to range from 4.0 to 9.0 Mb, while the sizes of the central domains composing the 180-bp repeats were found to be close to one another in the range 2.7 to 3.0 Mb (Figure 1.1a).

Cytosine methylation and heterochromatin

Cytological studies have shown that the centromeric regions of *Arabidopsis* chromosomes are heterochromatic (Sears and Lee-Chen, 1970) and stain deeply with DAPI (Maluszynska and Heslop-Harrison, 1991). Since the DNA of constitutive heterochromatin is known to be highly methylated on cytosines, the centromeric repetitive DNA sequences have also been thought to be methylated. The highly methylated status of the 180-bp repeats has been indicated since the first discovery of the repeats (Martinez-Zapater et al., 1986). The discovery was based on the use of the restriction enzymes *Hpa*II and *Msp*I, both of which recognize 5′-CCGG-3′, and the former is sensitive and latter insensitive to the second cytosine methylation. Although asymmetrical cytosine methylations are also common in the centromeric repeats and not all repeat units contain the 5′-CCGG-3′ sequence, this kind of symmetrical cytosine methylation has been used to screen the hypomethylation mutants in *A. thaliana* (Vongs et al., 1993).

Various approaches have been used to elucidate the relationship between the centromere, heterochromatin, and cytosine methylation as well as histone methylation (e.g, Luo et al., 2004). One of the most important findings regarding *Arabidopsis* centromere structure and functions concerns hypomethylation on the core regions of the centromeres, which are parts of the 180-bp repeat arrays and predominantly covered with the centromere-specific histone H3 (CENH3, HTR12, or CENP-A homologous in *A. thaliana*; Zhang et al., 2008). Using anti-5-methylcytosine antibody, it was shown that the 180-bp repeats associated with CENH3, which were referred to as the CEN chromatins, are distinctly hypomethylated, whereas the same repeat family in the pericentromeric heterochromatin is heavily methylated, and histone H3 dimetylated at lysine 9 (H3K9me2) is significantly reduced in the DNA-hypomethylated centromere regions. This differentiation in methylation status between the centromeric and pericentromeric regions might be related to differences in DNA sequence of the 180-bp repeats analyzed (Hall et al., 2003; Figure 1.1c). Since the CEN chromatins are flanked by heterochromatin enriched with H3K9me2, this situation is very similar to that in *S. pombe* (Partridge et al., 2000) and in *D. melanogaster* (Blower et al., 2002), although no DNA methylation is involved in *S. pombe*. DNA methylation and/or DNA-methylation-associated H3K9me2 or other histone modifications were suggested to act as a boundary to isolate the CEN chromatin (Zhang et al., 2008). In addition to the boundary role, heterochromatin at the pericentromeric regions could have additional roles in recruiting cohesin for sister chromatid cohesion (Gartenberg, 2009).

Centromere proteins

The centromere is a multifunctional complex, involving kinetochore formation, sister chromatid adhesion and separation, microtubule attachment, chromosome movement, heterochromatin

establishment, and mitotic checkpoint control. Among these functions, kinetochore formation is the most fundamental and essential. There are more than 60 constituent proteins of kinetochores in budding yeast (McAinsh et al., 2003), and more than twenty of these kinetochore proteins are conserved from yeasts to mammals (Amor et al., 2004; Table 1.1). This conservation is in striking contrast to the poor conservation of centromere DNA sequences (Henikoff et al., 2001).

Although studies on kinetochore proteins have been performed mainly in yeasts and mammals, some of the plant counterparts have been identified since the pioneering work on maize CENP-C (Dawe et al., 1999). In *A. thaliana*, Talbert and colleagues (2002) first identified the HTR12 protein as a centromere-specific histone H3 variant (CENH3), which corresponds to CENP-A in mammals. This report certainly accelerated subsequent centromere studies, since CENP-A or its orthologues are present in all eukaryotes that have been investigated to date, and are only detected on functional centromeres (Warburton et al., 1997). Interestingly, HTR12 is detected on all centromeres in *A. suecica* (allotetraploid, $2n = 4x = 26$) and *A. thaliana* ($2n = 2x = 10$) but not in *A. arenosa* ($2n = 2x = 16$) that is another parent of *A. suecica*. This suggests a unique evolutionary force important for the centromere proteins. The close interaction of HTR12 with the 180-bp repeats was shown by the chromatin immunoprecipitation (ChIP) assay, but an interaction with *Athila*, a *Ty3*/gypsy-type retroelement, was not detected (Nagaki et al., 2003).

A gene (AT2G06660) encoding CENP-B-like protein was thought to exist in the *Arabidopsis* genome, but this is now doubtful since its homology to CENP-B of mammals and Abp1, Cbh1, and Cbh2 of fission yeast is unclear, and no distinct transcription and/or translation from the CENP-B-like gene has been confirmed (Murata, 2002). The *Arabidopsis* counterpart of CENP-C (AtCENP-C) was identified based on the homology to DNA sequences of maize CENP-C (Ogura et al., 2004; Talbert et al., 2004). Human CENP-C is one of the few centromere proteins having DNA-binding ability, and its close association to CENP-A has been suggested (Perpelescu and Fukagawa, 2011). Although the C-terminal amino acid sequence of AtCENP-C was conserved among plant species, no similarity to animal or fungal CENP-Cs was found, except for the CENP-C motif (Talbert et al., 2004).

Mis12 was first identified as one of the kinetochore proteins in *S. pombe* (Goshima et al., 1999), and its human orthologue was shown to be a component of the Mis12/MIND complex comprising Mis12, Dsn1, Nnf1, and Nsl1 (Perpelescu and Fukagawa, 2011). Despite the poor overall similarity to fission yeast and human Mis12, Goshima and colleagues (2003) predicted the Mis12 homologue in *A. thaliana* using Block Maker (Henikoff et al., 1998) and MAST (Bailey and Gribskov, 1998) analysis. The centromere localization of the putative AtMIS12 was confirmed by immunostaining with the antibody raised against a peptide synthesized from the putative amino acid sequence (Sato et al., 2005).

For other kinetochore proteins, orthologues have not been identified in *Arabidopsis* until recently, mainly due to the rapid findings of novel kinetochore proteins in humans and yeasts and their poor homologies to plant orthologues. Very recently, however, six counterparts were identified based on InterPro domain analysis (D. Li, personal communication) and added to the TAIR database (http://arabidopsis.org). To date, 11 centromere proteins have been listed in *A. thaliana* (Table 1.1), although the centromere localization and function of the newly-added proteins have not yet been revealed. In the inner centromere structure, three of four components except CENP-B have been identified among human, fly, fission and budding yeasts, and *Arabidopsis*. Since CENP-B or its homologues have been shown to be inessential in mice and fission yeasts (Kapoor et al., 1998; Perez-Castro et al., 1998; Baum and Clarke, 2000), it is not surprising that no CENP-B counterparts have been detected in *Arabidopsis* or other eukaryotes. This fact suggests that the inner centromere structure is conserved well from yeasts to animals and plants. Similarly, the structure of the outer

Table 1.1 Centromere proteins of *A. thaliana* and four other species

	Species					
Localization (Network)*	*H. sapiens*	*D. melanogaster*	*S. pombe*	*S. cerevisiae*	*A. thaliana*	**
IC	INCENP	INCENP	Pic1	Sli15	AtINCENP	1
IC	AuroraB	IPLI	Ark1	Ipl1	AtAUR3	2
IC	CENP-A	CID	Cnp1	Cse4	HTR12	3
IC	CENP-B	—	Abp1, Cbh1,2	—	—	
IK (CCAN)	CENP-C	CENP-C	Cnp3	Mif2	AtCENP-C	4
IK (CCAN)	CENP-H		Fta3	Mcm16		
IK (CCAN)	CENP-I		Mis6	Ctf3		
IK (CCAN)	CENP-K		Sim4			
IK (CCAN)	CENP-L		Fta1			
IK (CCAN)	CENP-M		Mis17	Iml3		
IK (CCAN)	CENP-N		Mis15			
IK (CCAN)	CENP-O		Mal2	Mcm21	AtCENP-O	5
IK (CCAN)	CENP-P		Fta2			
IK (CCAN)	CENP-Q		Fta7			
IK (CCAN)	CENP-R					
IK (CCAN)	CENP-U					
IK (CCAN)	CENP-S			YOL86-A		
IK (CCAN)	CENP-X					
IK (CCAN)	CENP-T		SpBC800			
IK (CCAN)	CENP-W					
OK (KMNN)	hMis12	CG18156	Mis12	Mtw1	AtMIS12	6
OK (KMNN)	DSN1		Dsn1/Mis13	Dsn1		
OK (KMNN)	NNF1	CGI13434	Nnf1	Nnf1		
OK (KMNN)	NSL1	CGI1558	Nsl1/Mis14	Nsl1		
OK (KMNN)	Hec1/NDC80	Ndc80	Ndc80	Tid3	AtNDC80	7
OK (KMNN)	NUF2	Nuf2	Nuf2	Nuf2	AtNUF2	8
OK (KMNN)	SPC24	(GI12063)	Spc24	Spc24		
OK (KMNN)	SPC25	CG7242	Spc25	Spc25	AtSPC25	9
OK (KMNN)	KNL1	CG11451	Spc7	Spc105		
(facultative)	CENP-E	CENP-meta	Tea2	Kip2	AtCENP-E	10
(facultative)	CENP-F	Spn		Atg11		
(facultative)	CENP-V					
(CACE)	HJURP	(CAL1?)	YK12	Scm3		
(CACE)	Mis18α		Mis18			
(CACE)	Mis18β		Mis18			
(CACE)	M18BP1		Mis18			
(CACE)	RbAp48	RbAp48	Mis16	Msi1	AtMSI1	11
(CACE)	RbAp46		Mis16	Msi1		

*IC = inner centromere; IK = inner kinetochore; OK = outer kinetochore; CACE = CENP-A chromatin establishment; CCAN = constitutively centromere-associated network; KMNN = KNL1/Mis12 complex/Ndc80 complex network.

**Loci and references: 1. AT5G55820; Kirioukhova et al., 2011. 2. AT2G45490; Kurihara et al., 2006. 3. AT1G01370; Talbert et al., 2002. 4. AT1G15560; Ogura et al., 2004; Talbert et al., 2004. 5. AT5G10710; Direct submission to TAIR database, Swarbreck et al., 2011. 6. AT5G35520; Sato et al., 2005. 7. AT3G54630. 8. AT1G61000. 9. AT3G48210. 10. AT2G21380. 11. AT5G58230; Direct submission to TAIR database, Swarbreck et al., 2011.

kinetochore seems conserved among the eukaryotes, since most of the constituent protein counterparts have been identified, even in *Arabidopsis* (four of nine counterparts). On the other hand, it is difficult to determine the components of the inner kinetochore in *Arabidopsis*, except AtCENP-C and -O. Although a group of those components, called the constitutive centromere-associated network, are conserved in vertebrates, these orthologues have seldom been identified in *D. melanogaster* or *C. elegans* (Perpelescu and Fukagawa, 2011). For example, the CENP-H/I complex was shown to be necessary for centromere-targeting of newly-synthesized CENP-A (Okada et al., 2006), but in *A. thaliana,* the CENP-I/Mis6 homologues remain unidentified (Sato et al., 2005). These data suggest the possibility that plants, as well as some invertebrates, have different kinetochore structures from those of vertebrates, and this idea is supported by the finding that the classical tri-layer structure of vertebrate kinetochores has not been detected in plants (Wilson, 1968; Dawe et al., 2005).

CENP-A or CENH3 is a key protein that interacts with centromeric DNA sequences (Henikoff et al., 2001). Its necessity for kinetochore assembly was first shown in mouse null mutants for Cenpa (Howman et al., 2000), and was also confirmed in *A. thaliana* using its tetraploid plants (Ravi et al., 2010; please see Chapter 13 for details). Therefore, it is very important to know the process of CENP-A chromatin establishment for kinetochore formation, which is divided into centromere priming, CENP-A uploading, and maintenance (Perpelescu and Fukagawa, 2011). In the process, three to five components have been identified in humans and fission yeasts (Table 1.1). Among them, HJURP is the most important component, working as a CENP-A-specific chaperone. In *Drosophila*, however, it has just been reported that CAL1 (Chromosome ALignment defect 1), whose amino acid sequence has diverged from that of HJURP and its yeast counterparts, has similar functions to HJURP and Scm3 (Mellone et al., 2011). This sort of divergence might make it difficult to determine the HJURP/Scm3 counterpart in *Arabidopsis*.

Functional domains

As described above, the *Arabidopsis* centromeric regions are preferentially occupied by the "180-bp repeat" family. Since the array size of the 180-bp cores has been estimated to be about 2.7–3 Mb for all five chromosomes (Kumekawa et al., 2000, 2001; Hosouchi et al., 2002), this size seems important for centromere functionality and accurate chromatid segregation during cell division. However, ChIP assays suggested that only subsets of the 180-bp repeat arrays are involved in centromere function (Nagaki et al., 2003). More direct evidence was obtained from chromatin-fiber immunolabeling and the FISH technique, which demonstrated that HTR12 proteins localize only on a limited number of copies of the 180-bp repeats (Shibata and Murata, 2004).

Minichromosomes with truncated centromeres are quite useful for elucidating the relationship between the size of repeat arrays and functionality, as shown in fruit fly (Sun et al., 2003) and humans (Spence et al., 2002). In *A. thaliana*, several minichromosomes have been isolated (Table 1.2). Since most of these are relatively stable and transmissible to the next generation, they are maintained as partial trisomic lines. All of these minichromosomes were found to carry a shorter array of the centromeric satellite, and they are valuable for analyzing centromere function (Murata et al., 2006; Murata et al., 2008; Yokota et al., 2011). The minichromosome mini4S was found in progeny of telotrisomic Tr1A plants of Landsberg *erecta* and was shown to have originated from the short arm of chromosome 4 and possesses a truncated centromere (Murata et al., 2006). This "mini4S," the size of which was estimated to be approximately 7.5 Mb, contains only about 1 Mb,

Table 1.2 Minichromosomes in *A. thaliana*

Name	Origin	Size (Mb) Chromosome	Size (Mb) Centromere	Shape	Reference
mini 4S	Chr. 4S	7.5	1	Linear	1
mini α	Chr. 2S/1T	8.8	0.7	Linear	2, 3
mini δ	Chr. 2S	5.6	0.5 × 2	Circular	2, 4
mini δ1	Chr. 2S	3.8–5.0	0.27 × 2	Circular	4
mini δ1-1	Chr. 2S	1.7	0.25	Circular	4
ARC1	Chr. 2L	2.85	0.25	Circular	5

1. Murata et al., 2006. 2. Murata et al., 2008. 3. Yokota et al., 2010. 4. Yokota et al., 2011. 5. Murata et al., unpublished data.

or about one-third of the amount of centromeric 180-bp repeats in the normal chromosome 4. However, it is relatively stable at mitosis, particularly in the Columbia background, and the transmission rate to the next generation was comparable to that of chromosome 4 in a primary trisomic Tr4. In addition, HTR12 was found to colocalize with the 180-bp repeats on mini4S. These data indicate that the centromere function of mini4S is normal, despite more than a 2-Mb deletion of the 180-bp repeats.

Two other minichromosomes (miniα and miniδ) have been produced by T-DNA insertion within the centromere of chromosome 2, in addition to two other aberrant chromosomes (β and γ; Murata et al., 2008; Yokota et al., 2010). These centromeres allow estimation of the minimal region that encompasses the functional domain of the centromere of chromosome 2 (Figure 1.1d). Translocation with another T-DNA inserted on chromosome 1 split the 3-Mb centromere (180-bp repeat array) into two fragments comprising 0.7 and 2.3 Mb. The former was retained in miniα while the latter was retained in chromosome γ. Each of the two centromeres of dicentric ring miniδ (Figure 1.1e) was found to contain 0.5 Mb of the 180-bp repeats.

Chromosome β had two 180-bp repeat arrays: one derived from chromosome 1 and the other from chromosome 2. The latter was estimated to be only 0.2 Mb, with no HTR12 being detected, despite originating from the central part of the original chromosome-2 centromere. These results indicate that a 180-bp repeat array larger than 500 kb is required for centromere function.

A shorter functional domain was indicated by the detailed analysis of smaller ring minichromosomes (miniδ1 and miniδ1-1) derived from miniδ (Yokota et al., 2011). The estimated sizes of miniδ1 and miniδ1-1 were approximately 3.8–5.0 and 1.7 Mb, respectively, which were shorter than miniδ (5.6 Mb; Table 1.2). Although miniδ1-1 is a little unstable at mitosis and the number was variable, HTR12 (CENH3) was found on the centromere, indicating that the centromere domain, which was estimated to be about 250 kb, is sufficient for loading the kinetochore protein. Although other factors such sequence differences and epigenetic modifications could not be excluded, 200–250 kb of the 180-bp repeat arrays would be a critical size for centromere functions.

It should be noted that all miniδ and its derivatives contain the pericentromeric region in addition to the 180-bp repeats greater than 250 kb from the edge of the short arm of chromosome 2 (2S-edge). This suggests that the pericentromeric region is also needed for cohesion and subsequent accurate separation of sister chromatids, since similar ring chromosomes have recently been created from a 2L-edge containing roughly 250 kb-long 180-bp repeats (Murata et al., unpublished data). This situation is similar to that in human X minichromosomes, having an active subdomain

anchored about 150 kb from the Xp-edge (Spence et al., 2002). Interestingly, a major site of topoisomerase II cleavage was found within the domain, but no such site(s) has been detected within the 180-bp repeat arrays in *A. thaliana*.

Future prospects and conclusions

The centromere domains of *A. thaliana* comprising mostly 180-bp repeats remain as black boxes. However, an increasing amount of information on the centromeres is accumulating as described above. To advance studies on plant centromeres, effective techniques for manipulating chromosomes and truncating centromeres should be established. Telomere-mediated chromosome truncation (TCT) adapted to plants by Birchler and his colleagues is quite attractive (Yu et al., 2006), but centromere truncations by TCT rarely occurred in *A. thaliana* (Nelson et al., 2011; Teo et al., 2011). Therefore, the development of alternative techniques for effective induction of centromere truncation is needed. Sequence-specific recombination such as the Cre/LoxP system might be one of the possible choices, since our preliminary attempts using this system seem effective in inducing centromere deletion. These techniques must also be useful for constructing plant artificial chromosomes.

In contrast to the top-down approaches mentioned above, the bottom-up approach for constructing artificial chromosomes remains undeveloped, although two reports on maize artificial chromosomes have already been published (Carlson et al., 2007; Ananiev et al., 2009). In *A. thaliana*, direct gene transfer to protoplasts was reported more than 20 years ago (Damm et al., 1989). However, no success in artificial chromosome formation by introducing large DNA molecules comprising the centromere-specific 180-bp repeats has been made to date. In such cases, there is a possibility that the 180-bp repeats introduced contain no unidentified subdomain(s) that is essential for de novo kinetochore formation, like CENP-B-box of α-satellites in human artificial chromosome formation (Ikeno et al., 1998; Masumoto et al., 1998; Ohzeki et al., 2002). This sort of limitation would be solved using the top-down approach by producing minichromosomes with truncated centromeres and identifying the functional subdomain(s).

Like other higher eukaryotic centromeres, the function of *Arabidopsis* centromeres is specified by proteins that bind to centromere DNA as well as epigenetic modifications. More detailed studies on the chromatin status of the centromeres such as H3K4 me2 (Bergmann et al., 2011) are therefore urgently needed to meet the great demands for plant artificial chromosomes as a new vector.

Acknowledgments

Our recent work with *Arabidopsis* is supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry and JSPS KAKENHI 22310129.

References

Ambros, P., and Schweizer, D. (1976). The Geimsa C-banded karyotype of *Arabidopsis thaliana* (L.) Heynh. Arabidopsis Inf Serv **13**, 167–171.

Amor, D.J., Kalitsis, P., Sumer, H., and Choo, K.H. (2004). Building the centromere: from foundation proteins to 3D organization. Trends Cell Biol **14**, 359–368.

Ananiev, E.V., Wu, C., Chamberlin, M.A., Svitashev, S., Schwartz, C., Gordon-Kamm, W., and Tingey, S. (2009). Artificial chromosome formation in maize (*Zea mays* L.). Chromosoma **118**, 157–177.

Arabidopsis Genome Initiative. (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature **408**, 796–815.

Bailey, T.L., and Gribskov, M. (1998). Methods and statistics for combining motif match scores. J Comput Biol **5**, 211–221.

Baum, M., and Clarke, L. (2000). Fission yeast homologs of human CENP-B have redundant functions affecting cell growth and chromosome segregation. Mol Cell Biol **20**, 2852–2864.

Bergmann, J.H., Rodriguez, M.G., Martins, N.M., Kimura, H., Kelly, D.A., Masumoto, H., Larionov, V., Jansen, L.E., and Earnshaw, W.C. (2011). Epigenetic engineering shows H3K4me2 is required for HJURP targeting and CENP-A assembly on a synthetic human kinetochore. Embo J **30**, 328–340.

Blower, M.D., Sullivan, B.A., and Karpen, G.H. (2002). Conserved organization of centromeric chromatin in flies and humans. Dev. Cell **2**, 319–330.

Brandes, A., Thompson, H., Dean, C., and Heslop-Harrison, J.S. (1997). Multiple repetitive DNA sequences in the paracentromeric regions of *Arabidopsis thaliana* L. Chromosome Res **5**, 238–246.

Carlson, S.R., Rudgers, G.W., Zieler, H., Mach, J.M., Luo, S., Grunden, E., Krol, C., Copenhaver, G.P., and Preuss, D. (2007). Meiotic transmission of an in vitro–assembled autonomous maize minichromosome. PLoS Genet **3**, 1965–1974.

Copenhaver, G.P., Nickel, K., Kuromori, T., Benito, M.I., Kaul, S., Lin, X., Bevan, M., Murphy, G., Harris, B., Parnell, L.D., McCombie, W.R., Martienssen, R.A., Marra, M., and Preuss, D. (1999). Genetic definition and sequence analysis of *Arabidopsis* centromeres. Science **286**, 2468–2474.

Damm, B., Schmidt, R., and Willmitzer, L. (1989). Efficient transformation of *Arabidopsis thaliana* using direct gene transfer to protoplasts. Mol Gen Genet **217**, 6–12.

Dawe, R.K., Reed, L.M., Yu, H.G., Muszynski, M.G., and Hiatt, E.N. (1999). A maize homolog of mammalian CENPC is a constitutive component of the inner kinetochore. Plant Cell **11**, 1227–1238.

Dawe, R.K., Richardson, E.A., and Zhang, X. (2005). The simple ultrastructure of the maize kinetochore fits a two-domain model. Cytogenet Genome Res **109**, 128–133.

Gartenberg, M. (2009). Heterochromatin and the cohesion of sister chromatids. Chromosome Res **17**, 229–238.

Goshima, G., Kiyomitsu, T., Yoda, K., and Yanagida, M. (2003). Human centromere chromatin protein hMis12, essential for equal segregation, is independent of CENP-A loading pathway. J Cell Biol **160**, 25–39.

Goshima, G., Saitoh, S., and Yanagida, M. (1999). Proper metaphase spindle length is determined by centromere proteins Mis12 and Mis6 required for faithful chromosome segregation. Genes Dev **13**, 1664–1677.

Hall, S.E., Kettler, G., and Preuss, D. (2003). Centromere satellites from *Arabidopsis* populations: maintenance of conserved and variable domains. Genome Res **13**, 195–205.

Haupt, W., Fischer, T.C., Winderl, S., Fransz, P., and Torres-Ruiz, R.A. (2001). The centromere1 (CEN1) region of *Arabidopsis thaliana*: architecture and functional impact of chromatin. Plant J **27**, 285–296.

Henikoff, S., Ahmad, K., and Malik, H.S. (2001). The centromere paradox: stable inheritance with rapidly evolving DNA. Science **293**, 1098–1102.

Henikoff, S., Pietrokovski, S., and Henikoff, J.G. (1998). Superior performance in protein homology detection with the Blocks Database servers. Nucleic Acids Res **26**, 309–312.

Heslop-Harrison, J.S., Brandes, A., and Schwarzacher, T. (2003). Tandemly repeated DNA sequences and centromeric chromosomal regions of *Arabidopsis* species. Chromosome Res **11**, 241–253.

Heslop-Harrison, J.S., Murata, M., Ogura, Y., Schwarzacher, T., and Motoyoshi, F. (1999). Polymorphisms and genomic organization of repetitive DNA from centromeric regions of *Arabidopsis* chromosomes. Plant Cell **11**, 31–42.

Hosouchi, T., Kumekawa, N., Tsuruoka, H., and Kotani, H. (2002). Physical map-based sizes of the centromeric regions of *Arabidopsis thaliana* chromosomes 1, 2, and 3. DNA Res. **9**, 117–121.

Howman, E.V., Fowler, K.J., Newson, A.J., Redward, S., MacDonald, A.C., Kalitsis, P., and Choo, K.H. (2000). Early disruption of centromeric chromatin organization in centromere protein A (Cenpa) null mice. Proc Natl Acad Sci USA **97**, 1148–1153.

Ikeno, M., Grimes, B., Okazaki, T., Nakano, M., Saitoh, K., Hoshino, H., McGill, N.I., Cooke, H., and Masumoto, H. (1998). Construction of YAC-based mammalian artificial chromosomes. Nat Biotechnol **16**, 431–439.

Jakobsson, M., Hagenblad, J., Tavare, S., Sall, T., Hallden, C., Lind-Hallden, C., and Nordborg, M. (2006). A unique recent origin of the allotetraploid species *Arabidopsis suecica*: evidence from nuclear DNA markers. Mol Biol Evol **23**, 1217–1231.

Kapoor, M., Montes de Oca Luna, R., Liu, G., Lozano, G., Cummings, C., Mancini, M., Ouspenski, I., Brinkley, B.R., and May, G.S. (1998). The cenpB gene is not essential in mice. Chromosoma **107**, 570–576.

Kirioukhova, O., Johnston, A.J., Kleen, D., Kagi, C., Baskar, R., Moore, J.M., Baumlein, H., Gross-Hardt, R., and Grossniklaus, U. (2011). Female gametophytic cell specification and seed development require the function of the putative *Arabidopsis* INCENP ortholog WYRD. Development **138**, 3409–3420.

Kumekawa, N., Hosouchi, T., Tsuruoka, H., and Kotani, H. (2000). The size and sequence organization of the centromeric region of *Arabidopsis thaliana* chromosome 5. DNA Res **7**, 315–321.

Kumekawa, N., Hosouchi, T., Tsuruoka, H., and Kotani, H. (2001). The size and sequence organization of the centromeric region of *Arabidopsis thaliana* chromosome 4. DNA Res **8**, 285–290.

Kurihara, D., Matsunaga, S., Kawabe, A., Fujimoto, S., Noda, M., Uchiyama, S., and Fukui, K. (2006). Aurora kinase is required for chromosome segregation in tobacco BY-2 cells. Plant J **48**, 572–580.

Laibach, F. (1907). Zur Frage nach der Indivisualitat der Chromosomen im Pflanzenreich. Beih Bot Cbl **22**, 191–210.

Luo, S., Hall, A.E., Hall, S.E., and Preuss, D. (2004). Whole-genome fractionation rapidly purifies DNA from centromeric regions. Nat Methods **1**, 67–71.

Maluszynska, J., and Heslop-Harrison, J.S. (1991). Localization of tandemly repeated DNA sequences in *Arabidopsis thaliana*. Plant J **1**, 159–166.

Martinez-Zapater, J.M., Estelle, M.A., and Somervilee, C.R. (1986). A highly repeated DNA sequence in *Arabidopsis thaliana*. Mol Gen Genet **204**, 417–423.

Masumoto, H., Ikeno, M., Nakano, M., Okazaki, T., Grimes, B., Cooke, H., and Suzuki, N. (1998). Assay of centromere function using a human artificial chromosome. Chromosoma **107**, 406–416.

McAinsh, A.D., Tytell, J.D., and Sorger, P.K. (2003). Structure, function, and regulation of budding yeast kinetochores. Annu Rev Cell Dev Biol **19**, 519–539.

Mellone, B.G., Grive, K.J., Shteyn, V., Bowers, S.R., Oderberg, I., and Karpen, G.H. (2011). Assembly of *Drosophila* centromeric chromatin proteins during mitosis. PLoS Genet **7**, e1002068.

Meyerowitz, E.M. (1992). Introduction to the Arabidopsis genome. In Methods in Arabidopsis Research (eds C. Koncz, N.-H. Chua, and J. Schell). World Scientific, Singapore, New Jersey, London, Hong Kong, pp. 100–118.

Murata, M. (2002). Telomeres and centromeres in plants. Curr Genomics **3**, 527–538.

Murata, M., Ogura, Y., and Motoyoshi, F. (1994). Centromeric repetitive sequences in *Arabidopsis thaliana*. Jpn J Genet **69**, 361–370.

Murata, M., Shibata, F., and Yokota, E. (2006). The origin, meiotic behavior, and transmission of a novel minichromosome in *Arabidopsis thaliana*. Chromosoma **115**, 311–319.

Murata, M., Yokota, E., Shibata, F., and Kashihara, K. (2008). Functional analysis of the *Arabidopsis* centromere by T-DNA insertion-induced centromere breakage. Proc Natl Acad Sci USA **105**, 7511–7516.

Nagaki, K., Talbert, P.B., Zhong, C.X., Dawe, R.K., Henikoff, S., and Jiang, J. (2003). Chromatin immunoprecipitation reveals that the 180-bp satellite repeat is the key functional DNA element of *Arabidopsis thaliana* centromeres. Genetics **163**, 1221–1225.

Nelson, A.D., Lamb, J.C., Kobrossly, P.S., and Shippen, D.E. (2011). Parameters affecting telomere-mediated chromosomal truncation in *Arabidopsis*. Plant Cell **23**, 2263–2272.

Ogura, Y., Shibata, F., Sato, H., and Murata, M. (2004). Characterization of a CENP-C homolog in *Arabidopsis thaliana*. Genes Genet Syst **79**, 139–144.

Ohzeki, J., Nakano, M., Okada, T., and Masumoto, H. (2002). CENP-B box is required for de novo centromere chromatin assembly on human alphoid DNA. J Cell Biol **159**, 765–775.

Okada, M., Cheeseman, I.M., Hori, T., Okawa, K., McLeod, I.X., Yates, J.R. III, Desai, A., and Fukagawa, T. (2006). The CENP-H-I complex is required for the efficient incorporation of newly synthesized CENP-A into centromeres. Nat Cell Biol **8**, 446–457.

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.

Perez-Castro, A.V., Shamanski, F.L., Meneses, J.J., Lovato, T.L., Vogel, K.G., Moyzis, R.K., and Pedersen, R. (1998). Centromeric protein B null mice are viable with no apparent abnormalities. Dev Biol **201**, 135–143.

Perpelescu, M., and Fukagawa, T. (2011). The ABCs of CENPs. Chromosoma **120**, 425–446.

Ravi, M., Kwong, P.N., Menorca, R.M., Valencia, J.T., Ramahi, J.S., Stewart, J.L., Tran, R.K., Sundaresan, V., Comai, L., and Chan, S.W. (2010). The rapidly evolving centromere-specific histone has stringent functional requirements in *Arabidopsis thaliana*. Genetics **186**, 461–471.

Redei, G.P. (1992). A heuristic glance at the past of Arabidopsis genetics. In Methods in Arabidopsis Research (eds C. Koncz, N.-H. Chua, and J. Schell). World Scientific, Singapore, New Jersey, London, Hong Kong, pp. 1–15.

Round, E.K., Flowers, S.K., and Richards, E.J. (1997). *Arabidopsis thaliana* centromere regions: genetic map positions and repetitive DNA structure. Genome Res **7**, 1045–1053.

Sato, H., Shibata, F., and Murata, M. (2005). Characterization of a Mis12 homologue in *Arabidopsis thaliana*. Chromosome Res **13**, 827–834.

Sears, L.M.S., and Lee-Chen, S. (1970). Cytogenetic studies in *Arabidopsis*. Can J Genet Cytol **12**, 217–223.

Shibata, F., and Murata, M. (2004). Differential localization of the centromere-specific proteins in the major centromeric satellite of *Arabidopsis thaliana*. J Cell Sci **117**, 2963–2970.

Simoens, C.R., Gielen, J., Van Montagu, M., and Inze, D. (1988). Characterization of highly repetitive sequences of *Arabidopsis thaliana*. Nucleic Acids Res **16**, 6753–6766.

Spence, J.M., Critcher, R., Ebersole, T.A., Valdivia, M.M., Earnshaw, W.C., Fukagawa, T., and Farr, C.J. (2002). Co-localization of centromere activity, proteins and topoisomerase II within a subdomain of the major human X alpha-satellite array. Embo J **21**, 5269–5280.

Steinitz-Sears, L.M. (1963). Chromosome studies in *Arabidopsis thaliana*. Genetics **48**, 483–490.

Sun, X., Le, H.D., Wahlstrom, J.M., and Karpen, G.H. (2003). Sequence analysis of a functional *Drosophila* centromere. Genome Res **13**, 182–194.

Talbert, P.B., Bryson, T.D., and Henikoff, S. (2004). Adaptive evolution of centromere proteins in plants and animals. J Biol **3**, 18.

Talbert, P.B., Masuelli, R., Tyagi, A.P., Comai, L., and Henikoff, S. (2002). Centromeric localization and adaptive evolution of an *Arabidopsis* histone H3 variant. Plant Cell **14**, 1053–1066.

Teo, C.H., Ma, L., Kapusi, E., Hensel, G., Kumlehn, J., Schubert, I., Houben, A., and Mette, M.F. (2011). Induction of telomere-mediated chromosomal truncation and stability of truncated chromosomes in *Arabidopsis thaliana*. Plant J **68**, 28–39.

Thompson, H., Schmidt, R., Brandes, A., Heslop-Harrison, J.S., and Dean, C. (1996). A novel repetitive sequence associated with the centromeric regions of *Arabidopsis thaliana* chromosomes. Mol Gen Genet **253**, 247–252.

Vongs, A., Kakutani, T., Martienssen, R.A., and Richards, E.J. (1993). *Arabidopsis thaliana* DNA methylation mutants. Science **260**, 1926–1928.

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., Poirier, G.G., and Earnshaw, W.C. (1997). Immunolocalization of CENP-A suggests a distinct nucleosome structure at the inner kinetochore plate of active centromeres. Curr Biol **7**, 901–904.

Wilson, H.J. (1968). The fine structure of the kinetochoare in meiotic cells of *Tradescantia*. Planta **78**, 379–385.

Yokota, E., Nagaki, K., and Murata, M. (2010). Minichromosome stability induced by partial genome duplication in *Arabidopsis thaliana*. Chromosoma **119**, 361–369.

Yokota, E., Shibata, F., Nagaki, K., and Murata, M. (2011). Stability of monocentric and dicentric ring minichromosomes in *Arabidopsis*. Chromosome Res **19**, 999–1012.

Yu, W., Lamb, J.C., Han, F., and Birchler, J.A. (2006). Telomere-mediated chromosomal truncation in maize. Proc Natl Acad Sci USA **103**, 17331–17336.

Zhang, W., Lee, H.R., Koo, D.H., and Jiang, J. (2008). Epigenetic modification of centromeric chromatin: hypomethylation of DNA sequences in the CENH3-associated chromatin in *Arabidopsis thaliana* and maize. Plant Cell **20**, 25–34.