A molecular view of plant centromeres

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Although plants were the organisms of choice in several classical centromere studies, molecular and biochemical studies of plant centromeres have lagged behind those in model animal species. However, in the past several years, several centromeric repetitive DNA elements have been isolated in plant species and their roles in centromere function have been demonstrated. Most significantly, a Ty3/*gypsy* class of centromere-specific retrotransposons, the CR family, was discovered in the grass species. The CR elements are highly enriched in chromatin domains associated with CENH3, the centromere-specific histone H3 variant. CR elements as well as their flanking centromeric satellite DNA are actively transcribed in maize. These data suggest that the deposition of centromeric histones might be a transcription-coupled event.

Centromeres are responsible for sister chromatid cohesion and are the sites for kinetochore assembly and spindle fiber attachment, thereby enabling faithful segregation of chromosomes during cell division. Although these functions are conserved among all eukaryotes, there is no conservation of centromeric DNA sequences: different organisms have strikingly different centromeric DNAs. This enigma has led to extensive studies in several model eukaryotes, including *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Drosophila melanogaster* and humans. Here we review the recent progress on centromere research in plants.

Centromeric histone H3 defines the boundaries of the centromere

Although centromeres can be defined cytologically or genetically, currently the most widely used definition of the centromere is biochemical: if a DNA sequence interacts with the kinetochore it is part of the functional centromere. At least 14 demonstrated or putative kinetochore proteins have been identified in plants, and these are generally localized in two domains, the inner and outer kinetochore [1–4]. The outer kinetochore proteins are transient and only necessary during chromosome segregation, whereas the inner kinetochore proteins recognize centromeric DNA and establish a specialized chromatin environment.

A fundamental feature of the inner kinetochore is a specialized histone H3 variant known as centromeric histone CENH3 [5,6]. The first CENH3 (CENP-A) was identified in humans [7,8], and homologous proteins have since been identified in all eukaryotes studied, including *Arabidopsis thaliana* [3] and maize [4]. CENH3 differs from the common form of histone H3 because it has a highly divergent N-terminal tail, which can vary substantially in length and composition even among closely related organisms [3,9]. CENH3 is present only in the functional centromeres of dicentric chromosomes in humans [10]. In addition, blocks of CENH3-associated nucleosomes and regular H3-associated nucleosomes are linearly interspersed in functional centromeres [11,12]. Thus, the boundary of the centromere can be defined by identifying DNA sequences that interact with CENH3.

Centromeric satellite arrays

The simple point centromeres of *S. cerevisiae* consist of only ~125 base pairs (bp) of unique sequence [13]. However, the centromeres in multicellular eukaryotic species are much larger and are embedded within megabases of highly repetitive DNA sequences. Satellite DNA is often the dominant DNA component in centromeres [14]. For example, the most abundant sequence in human centromeres is the ~171 bp α-satellite repeat, which is organized into long arrays of between ~250 kb and >4 Mb [15]. Human artificial chromosomes were successfully assembled using either synthetic or cloned α-satellite DNA, suggesting that long stretches of α-satellite DNA could act as a functional human centromere [16–18].

Satellite repeats associated with centromeres have been reported in several plant species (Table 1). These centromeric satellite repeats are highly abundant and can be readily visualized and cloned by restriction digestion of the genomic DNA [19–23]. Like the α-satellite in human centromeres, the centromeric satellites in plants are organized into arrays that can be several megabases long [24–26]. A common characteristic of centromeric satellite repeats is their rapid divergence [6]. Although weak homology can sometimes be detected between centromeric repeats of distantly related species (e.g. rice and maize [26]), most plant centromeric satellite repeats are specific to only closely related species [19–22,27,28]. In spite of their phenomenal rate of evolution, centromeric satellites appear to serve as the core of the centromere. In maize and *Arabidopsis*, chromatin immunoprecipitation

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Table 1. Centromere-associated repetitive DNA elements reported in plants

Plant species	Repeat and description	Refs
Arabidopsis arenosa	pAa214: 166–179-bp tandem repeat	[21]
Arabidopsis thaliana	pAL1: 180-bp tandem repeat	[66,67]
Beta species	pBV1: ~326-bp tandem repeat	[28]
	pT55: ~160-bp tandem repeat	
	ppHC8: 162-bp tandem repeat	
	pTS4.1: 312-bp tandem repeat	
	pBp10, pBv26: centromere-enriched retrotransposons	
Brachycome dichromosomatica	Bd49: 176-bp tandem repeat specific to the centromeres of B chromosomes	[68]
Brachypodium sylvaticum	CCS1: centromere-specific retrotransposon (CR family)	[44]
Brassica campestris	pBT11, pBcKB4: ~175-bp tandem repeat	[19,69]
Brassica oleracea	pBoKB1: ~171-bp tandem repeat	[19]
Hordeum vulgare (barley)	$(AGGGAG)_n$ satellite DNA	[47,70]
	Cereba: centromere-specific retrotransposon (CR family)	
Oryza sativa (rice)	CentO: 155-bp tandem repeat	[26,49,71]
	CRR: centromere-specific retrotransposon (CR family)	
Pennisetum glaucum	pPgKB19: 137-bp tandem repeat	[20]
Petunia hybrida	pBS-SB1-B5: 666-bp tandem repeat	[72]
Saccharum officinarum (sugar cane)	pSG1-2: 140-bp tandem repeat	[23]
Secale cereale (rye)	Bilby: centromere-specific retrotransposon	[73]
Sorghum bicolor (sorghum)	pSau3A10, pCEN38: ~137-bp tandem repeat	[27,45,46,74]
	pSau3A9, pHind22: centromere-specific retrotransposon (CR family)	
Triticum aestivum (wheat)	TaiII: 570-bp tandem repeat	[75,76]
	pBS301: 250-bp tandem repeat	
Vigna unguiculata	pVuKB1: 488-bp tandem repeat	[22]
Zea mays (maize)	CentC: 156-bp tandem repeat	[51,52,77,78]
	Cent4: ~740-bp tandem repeat specific to chromosome 4	
	CentA, CRM: centromere-specific retrotransposon (CR family)	
	B repeat: B centromere-specific tandem repeat	
Zingeria biebersteiniana	Zbcen1: 755-bp tandem repeat	[79]
	Zb47A: centromere-enriched retrotransposon	

(ChIP) experiments have demonstrated a clear interaction between CENH3 and centromeric satellites [4,29].

Another puzzling observation is that the quantity of centromeric satellites varies substantially from chromosome to chromosome. In most cases, centromeric satellites are present in vast excess. Each of the five *Arabidopsis* centromeres contains ~2–4 Mb of the 180-bp centromeric satellite repeat [30–32] and the centromeric region of the B chromosome in maize contains up to 9 Mb of the B-specific repeat [33]. A centromere can be broken in two by a process known as misdivision [34,35]. Using the B chromosome centromere of maize, which contains a unique repeat that serves as a specific marker, numerous misdivision derivatives were analyzed [33,36]. The B-specific repeat cluster in some rearranged B centromeres was reduced from 9 Mb to as small as 200–500 kb. Further misdivision derivatives of these small centromeres can be recovered, but the size of the repeat cluster remains similar or is increased (T. Phelps-Durr and J.A. Birchler, unpublished). ChIP studies also indicate that only a portion of the satellites in the cell can be precipitated by anti-CENH3 antibodies [4,29]. These results show that only a portion of the centromeric satellite is included in CENH3-associated centromeric chromatin.

The characteristic variability of centromeric satellites suggests that they might evolve by a process akin to meiotic drive [37], where chromosomes compete for access to the next generation by skewing mendelian segregation in their favor [38]. On the basis that the N-terminus and Loop 1 regions of CENH3 show evidence of adaptive evolution, Steve Henikoff and colleagues argued that CENH3 might be adapting to bind the rapidly evolving centromere [3,9]. Under this model, centromeric DNAs that are most efficient at binding to kinetochore proteins are likely to arrive at spindle poles first and be segregated to the functional megaspore (plants) or pronucleus (animals). The analogy is drawn to an arms race, where the satellites are evolving to bind more efficiently to the kinetochore and enhance their chances of being segregated to progeny whereas the host is evolving to modulate the interaction so that each of the chromosomes segregate with roughly the same efficiency. The result of the arms race is an ever-changing population of long uniform satellite arrays. Sequence data from *Arabidopsis*, derived from 457 satellite repeats, support this scenario [39]. Tandem repeats are well suited to this environment because of their susceptibility to unequal recombination [40], which can sweep new polymorphisms through an array in a relatively short time frame.

Centromere-specific retrotransposons in plants

In general, the abundance of retrotransposons is much lower in centromeres than would be expected based on their frequency in other parts of the genome [41,42]. Recent characterization of the centromere of human X chromosome showed that the functional domain contains highly homogenized α-satellite arrays and lacks transposon insertions, whereas the flanking domains contain diverged α-satellite arrays and significantly more transposons [43]. These results imply that transposon invasion interferes with centromere function or that the satellites in the functional core domain are homogenized by frequent unequal recombination, a process that would also remove inserted transposons.

In contrast to the low abundance of many retrotransposon families in plant centromeres [42], there are a few plant retrotransposon families that are significantly enriched in the centromeric regions or highly specific to the centromeres (Table 1). A particularly intriguing class is the centromeric retrotransposon (CR) family discovered in grass species. Sequences derived from the CR family were first isolated from *Brachypodium sylvaticum* [44] and sorghum [45]. These sequences were later found to be derived from a Ty3/*gypsy* class of retrotransposon [46–48]. Unlike other retrotransposon families that diverge rapidly during evolution, the CR family has been found in the centromeres of all grass species studied [46], including the centromeres of B chromosomes [49] and a significantly rearranged rye centromere [50]. Highly conserved motifs were found in the long terminal repeats (LTRs) of the CR elements from rice, maize and barley [51]. CR elements are frequently found inserted into centromeric satellites [26,51]. CR elements can also insert into each other, and often cluster together in long arrays [26].

In maize, CRM (the maize subfamily of CR) elements are common and extensively intermingled with CentC, a 156-bp centromeric satellite repeat [52] (Figure 1). Such intermingled CRM and CentC sequences stretch from 300 kb to >2 Mb in the core domains of maize centromeres (W.W. Jin and J. Jiang, unpublished) (Figure 1). Importantly, CRM elements are immunoprecipitated by CENH3 antibodies as efficiently as CentC [4], indicating that the CRM elements are functional components of maize centromeres. Thus, CRM elements appear to have adapted to the special environment of the centromeric chromatin. The adaptation might be as simple as carefully targeting the centromere [48] and transposing at a rate that is faster than the rate of removal by unequal recombination. Alternatively, the transposon might contribute in a positive manner to centromere function and provide the host with a selective advantage.

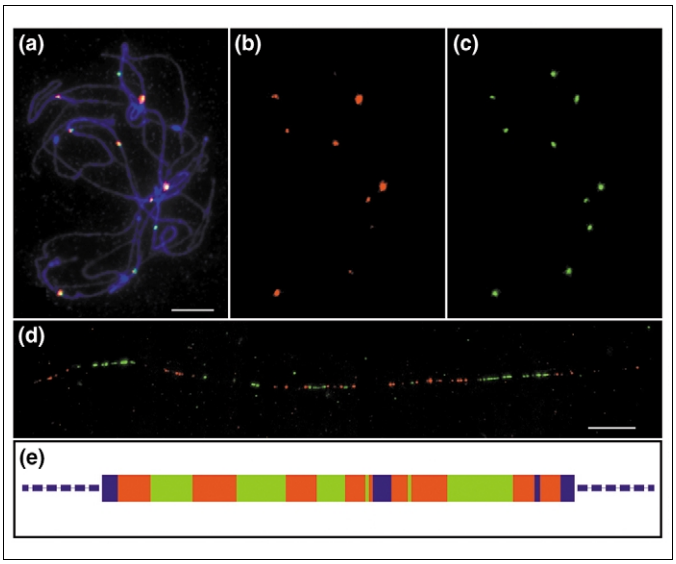

Figure 1. Structure and organization of centromeric DNA in maize. **(a)** Fluorescence *in situ* hybridization (FISH) of centromeric DNA on maize pachytene chromosomes. The maize centromeric satellite CentC [52] is visualized in red and the maize centromere-specific retrotransposon CRM is visualized in green. Scale bar = 10 μm. **(b)** Digitally separated FISH signals derived from CentC. **(c)** Digitally separated FISH signals derived from CRM. Both CentC and CRM are highly specific to the centromeric regions. The amount of CentC varies significantly among different maize centromeres. The size and intensities of the FISH signals from CRM are relatively similar among different centromeres. **(d)** Organization of the centromeric DNA of maize chromosome 6 revealed by FISH analysis on extended DNA fibers (fiber-FISH). DNA fibers were prepared from an oat–maize chromosome addition line 6 [80] and probed with CentC (green) and CRM (red) probes. The CRM sequences are clustered and intermingled with the CentC satellite. Each micrometer of the fiber-FISH signals represents ~3.2 kb DNA [81]. Scale bar = 10 μm. **(e)** A diagram of the DNA structure of maize centromere 6. Green and red bars represent CentC and CRM sequences, respectively. Blue bars represent unknown sequences. The FISH images in (a), (b), (c) and (d) are courtesy of Weiwei Jin.

Centromere–kinetochore interface – how is centromeric DNA recognized?

In the past few years, our understanding of kinetochore proteins and the underlying centromeric DNAs has expanded at a remarkable pace, but we have been unable to answer the fundamental question of how the centromere and inner kinetochore recognize each other. Most authors agree that interactions must be epigenetic (sequence independent), but because the centromeres are reliably maintained at the same loci, this strongly suggests some sort of DNA–protein recognition process. In humans, sequence specificity is conferred in part by the kinetochore protein CENP-B, which is thought to have evolved from a transposable element. CENP-B has significant homology to Mariner transposases [53] and binds to a 17-bp motif in the α-satellite DNA (presumed remnant of a Mariner TIR) that is required for efficient artificial chromosome formation in humans [54]. What might have begun as the invasion of the centromere by a transposable element, seems to have evolved into a mutualistic relationship where both the centromere and (a descendent of) the transposable element benefit. CENP-B and its interaction with the centromere provides a precedent for the idea that transposons might be selected for a role in chromosome segregation [53].

Maize and rice CR elements have substantial homology in the LTR [4,51], suggesting that there has been selective pressure at the nucleotide level. Because the transposon does not appear to be mimicking the satellite DNAs, either in size or in primary sequence, the selective pressure might be on the capacity to initiate RNA (i.e. the promoter) or the RNA itself [55]. CR elements in both maize and rice are indeed expressed, as judged by the presence of CR-homologous ESTs in GenBank. CentC RNA, although not detectable on northern blots or EST databases (only a single cDNA clone reported), can be readily detected within the nucleus and on chromatin immunoprecipitated with CENH3 antibodies (C.X. Zhong *et al*., unpublished). These results parallel a recent study in humans showing that selection for transcription near an array of α-satellite repeats can induce the formation of a functional centromere [56]. The CR elements might be under selection for their capacity to initiate transcription through flanking satellite DNA. Any centromere-embedded transcription units, either from transposons or cryptic promoters within satellite repeats, could fulfill the same function.

In animals, CENH3 is incorporated into chromatin in a replication-independent fashion [11,57]. Henikoff and colleagues have suggested that histone replacement is often associated with active transcription – a time at which nucleosomes are destabilized (or fully displaced)

and subsequently reassembled [58]. The chromatin disruption caused by transcription provides an ideal time for histone replacement. Assuming a slight binding preference of CENH3 for satellite DNA, transcription of the centromere might be sufficient to establish a CENH3-centered chromatin environment. However, at least in maize, much of the centromeric RNA remains associated with centromeric chromatin. The high local concentration of RNA might help to recruit CENH3, similar to the role of human Xist RNA in facilitating the replacement of histone H2 with macroH2 on inactive X chromosomes [59]. Several chromatin proteins are known to bind RNA [60,61], including histones themselves [62]. One can imagine that centromeric RNA has an affinity for chromatin remodeling proteins, other kinetochore proteins or CENH3 itself. As a result, centromere-associated RNA could facilitate chromosome segregation by helping to recruit necessary proteins to the centromere (Figure 2a,b).

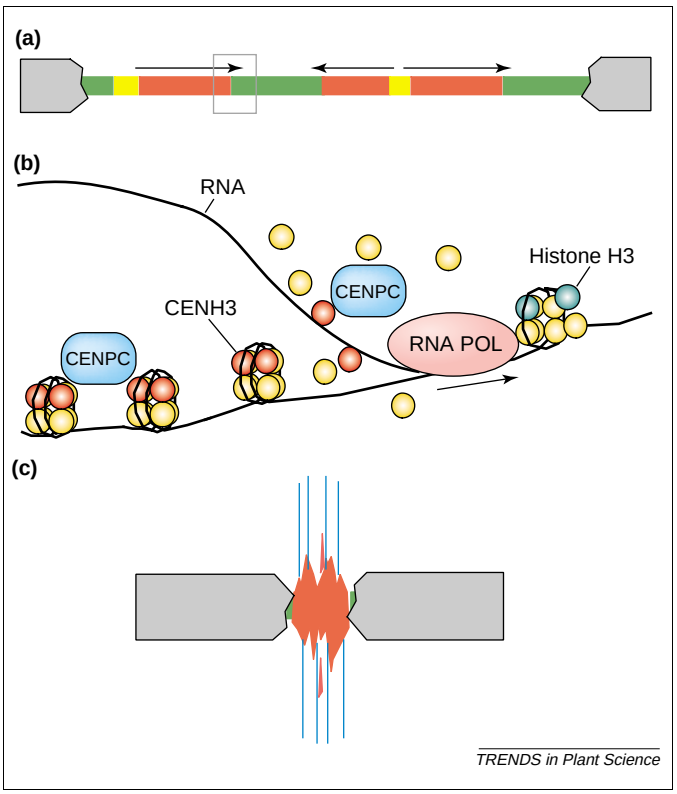

Figure 2. Model for centromere initiation and maintenance. **(a)** Embedded promoters might transcribe most of the centromere. Transcripts are probably initiated from the promoters within centromeric retrotransposon (CR) elements (yellow) and extend over flanking repeat arrays. Some of the RNA remains associated with the chromatin. We suggest that as transcription proceeds, histone H3-containing nucleosomes (green) are replaced with CENH3-containing nucleosomes (red). The heterochromatic state of the pericentromere (gray) might block the extension of centromeric transcripts. **(b)** A detailed view of the histone replacement process [the gray box in (a)]. As RNA polymerase (RNA POL) proceeds over the repeat arrays it destabilizes nucleosomes. Histones H2A, H2B and H4 are shown as yellow spheres. The RNA (depicted as the heavy black line extending behind RNA polymerase), might preferentially bind CENH3 and/or associated proteins such as centromere protein (CENPC [1]). By holding these key kinetochore proteins in close proximity to chromatin, the RNA could facilitate histone replacement. **(c)** Tension could reinforce and maintain the centromeric state (modified from [64]). The centromere–kinetochore complex (red) is distorted by the attached microtubules (blue). The chromosome arms (gray) do not interact with microtubules. Chromatin damage caused by excessive pulling forces might serve as the epigenetic mark of a successful centromere. If CENH3 is present in locally high concentrations, the damage is likely to be repaired using CENH3 instead of histone H3.

A transcription-mediated mechanism for CENH3 deposition might help to explain some aspects of centromere initiation, but it does not adequately explain the stability of the centromeres once they are established [63] or why centromeres often include non-satellite DNA [5]. One idea is that the centromeric state is reinforced and maintained by the tension applied during spindle attachment [64]. The excessive force applied during metaphase and anaphase (10 000 times what is needed [65]) might tear the histones from the DNA [11]. The damaged chromatin could then be marked for repair by a replication-independent mechanism similar to the one that originally installed CENH3 (Figure 2c). This model allows for considerable plasticity during centromere establishment, but favors successful centromeres by continually marking and reusing them.

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