The plant kinetochore

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Kinetochores are large protein complexes that bind to centromeres. By interacting with microtubules and their associated motor proteins, kinetochores both generate and regulate chromosome movement. Kinetochores also function in the spindle checkpoint; a surveillance mechanism that ensures that metaphase is complete before anaphase begins. Although the ultrastructure of plant kinetochores has been known for many years, only recently have specific kinetochore proteins been identified. The recent data indicate that plant kinetochores contain homologs of many of the proteins implicated in animal and fungal kinetochore function, and that the plant kinetochore is a redundant structure with distinct biochemical subdomains.

Kinetochores have several roles in cell division, including the control of chromosome alignment at the metaphase plate, the regulation of anaphase onset via the spindle checkpoint and the control of anaphase chromosome movement[1]. Among the proteins that make up the kinetochore are DNA-binding proteins that interact with the centromere to form a structure known as the centromere–kinetochore complex. In mammalian cells, the centromere–kinetochore complex has a distinct ultrastructure, with inner and outer plates as well as a diffuse external layer known as the fibrous corona[2,3]. Many mammalian kinetochore proteins have been identified and characterized, and several have been localized to the distinct ultrastructural domains of the kinetochore[4–6]. Kinetochores are also well characterized in budding yeast, where there are at least 12 centromere-associated proteins, some of which have homologs in other organisms[5].

In plants, the centromere–kinetochore complex has been referred to as a 'ball in the cup', owing to its uniform ultrastructure and tendency to be embedded in chromatin[7,8]. Although the structure and behavior of plant kinetochores were documented many years ago[9], it is only recently that the tools have become available to analyze the plant centromere–kinetochore complex at the molecular level. The role of this article is to highlight recent advances in identifying the protein components of plant kinetochores. More general perspectives on centromeric DNA and the mammalian and fungal centromere–kinetochore complexes have been published elsewhere[1,6,10,11].

Molecular composition of the plant kinetochore

A major breakthrough in mammalian kinetochore research occurred in 1980, when it was discovered that patients with an autoimmune disease known as CREST (calcinosis, Raynaud phenomenon, esophageal dismotility, sclerodactyly, telangiectasia) frequently produce antibodies to kinetochore proteins[12]. Many of the now well studied kinetochore proteins in animals were initially discovered as antigens in CREST patients. Sera from CREST patients were also the first reagents used to identify plant kinetochores specifically. The kinetochores from *Haemanthus*, *Tradescantia* and maize were labeled with one CREST serum[13–15], and a second CREST serum was found to recognize the kinetochores of field bean (*Vicia faba*)[16]. In addition, several monoclonal antibodies raised against mammalian proteins were shown to recognize the kinetochores of plants[17–19].

These antibody cross-reactivity data provided strong evidence that a subset of plant kinetochore proteins is evolutionarily conserved. More recently, modern genomics resources have provided a direct and effective way to exploit this protein sequence conservation. Combinations of EST and genomic sequence databases, and PCR experiments using these data, have now been used to identify several plant kinetochore genes. A catalog of the known plant kinetochore proteins identified by immunolocalization and protein sequence homology is shown in Table 1.

Constitutive and structural components

A small group of kinetochore proteins (CENP-A, CENP-B and CENP-C) are distinguished by their localization to kinetochores throughout the cell cycle and interaction with DNA. Each is thought to participate in kinetochore assembly and/or maturation[6]. CENP-A is a histone H3 variant that is thought to have a crucial role in positioning and maintaining the kinetochore[1]. As a highly conserved protein[20], CENP-A is probably present in plants, although a true plant homolog has yet to be confirmed. CENP-B binds to and might organize the satellite DNA of several mammalian species but does not appear to have a significant role in kinetochore function[21]. One report suggests that CENP-B is present in *Phaseolus vulgaris*[22].

CENP-C is one of the most actively studied kinetochore proteins and is well characterized in a variety of species, including plants. Mammalian CENP-C binds to DNA, is present at centromeres throughout the cell cycle and localizes to the inner layer of the trilaminar kinetochore[1]. There is strong evidence that CENP-C is necessary for kinetochore function, although it does not appear to be sufficient to induce kinetochore assembly at non-centromeric sites[23,24]. The CENP-C homolog in budding yeast (Mif2p) is an essential protein that interacts with the centromeric DNA and, like CENP-C, is thought to have a structural role early in kinetochore assembly[25].

In maize, CENPC is a constitutive component of the kinetochore[26], where it occupies an inner domain of the ball-shaped organelle[19]. Recent data indicate that CENP-C is also present at the kinetochores of field bean and barley[27]. A full-length cDNA from one of the maize *cenpc* homologs (GenBank AF129857) has recently been sequenced (E.N. Hiatt and R.K. Dawe., unpublished). A comparison of the full-length maize CENPC sequence with the predicted protein sequence from an *Arabidopsis* CENP-C gene (GenBank AAF71990) indicates an overall similarity of ~37%. The highest sequence homology between maize and *Arabidopsis* CENP-C, and the only significant region of homology among all plant, animal and fungal CENP-C proteins, is confined to a 23 amino acid region known as region I (Fig. 1) (Refs 28,29). The function of region I is not clear, although a *mif2* mutation with a temperature-sensitive phenotype lies within this region[28].

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Table 1. Plant kinetochore components

Kinetochore component	Apparent function	Plant species	Kinetochore localization[a]	Gene cloned	Refs
CBF5	Unknown	*Vicia faba*, *Hordeum vulgare*	Yes	Yes (*Hordeum vulgare*)	27
CENPC	Structural	*Zea mays*, *Vicia faba*, *Hordeum vulgare*	Yes	Yes (*Zea mays*)	26,27
CENPE	Chromosome motility	*Vicia faba*, *Hordeum vulgare*	Yes	No	27
CENPF	Unknown	*Hordeum vulgare*	Yes	No	27
MAD2	Spindle checkpoint	*Zea mays*	Yes	Yes	19
Meiotic Histone	Unknown	*Lilium longiflorum*	Yes	Yes	48,49
MPM2 antigen(s)	Unknown	*Vicia faba*	Yes	No	18
SKP1	Unknown	*Vicia faba*, *Hordeum vulgare*	Yes	Yes	27
ZW10	Spindle checkpoint	*Arabidopsis*	NA[b]	Yes	45
3F3/2 antigen	Spindle checkpoint	*Zea mays*	Yes	No	19
6C6 antigen	MTOC	*Allium sativum*, *Tulbaghia violacea*	Yes	No	17
γ-tubulin	MTOC	*Vicia faba*	Yes	No[c]	35

[a]The protein has been localized to the kinetochore by immunofluorescence.
[b]NA, information not available.
[c]γ-tubulin DNA sequences from other plant species are available in GenBank.

Putative microtubule-organizing proteins

Angiosperms seem to lack centrosomes, which are the organelles used in most animal cells to organize spindles. Instead, flowering plants form mitotic and meiotic spindles using specialized microtubule-organization centers (MTOCs) that nucleate and stabilize the ends of microtubules. The most prominent MTOC in plants is the nuclear envelope[30,31] but several lines of evidence suggest that kinetochores might also serve as MTOCs during the assembly of plant spindles. Microtubules appear to accumulate around kinetochores early in spindle formation[19,32,33] and kinetochores are one of the first sites in the cell to reacquire microtubules after treatment with microtubule depolymerizing drugs[34].

Further supporting a MTOC role for the kinetochore is the fact that at least two plant kinetochore proteins have homology to animal centrosome components. One of these, γ tubulin, an important component of centrosomes in animals (and spindle pole bodies in fungi), has been found in *V. faba* kinetochores[35]. This protein is thought to function primarily in microtubule nucleation but might also be involved in the regulation of microtubule dynamics and organization[36]. Similarly, the monoclonal antibody 6C6, which recognizes a calf centrosomal antigen, localizes to plant kinetochores just after nuclear envelope breakdown[17].

Proteins involved in chromosome motility

Mammalian kinetochores are actively involved in moving chromosomes towards the metaphase plate in prometaphase (the stage immediately before metaphase) as well as moving chromosomes away from the metaphase plate in anaphase[37,38]. The available data suggest that the work required for chromosome movement is generated by the polymerization and depolymerization of microtubules and the action of microtubule-based motor proteins[39]. Among the motor proteins that act at kinetochores is CENP-E, a protein in the kinesin superfamily of motors. CENP-E is thought to mediate the movement of chromosome towards the metaphase plate by tethering the kinetochores to the dynamic plus ends of microtubules[40]. The demonstration that antisera against human CENP-E recognize *V. faba* and *Hordeum vulgare* kinetochores provides the first indication that a similar mechanism is operating in plant cells[27].

Spindle checkpoint proteins

Kinetochores have an important function in regulating the onset of anaphase[41]. In this capacity, kinetochores sense the attachment of microtubules and the resulting tension applied by the spindle apparatus[38]. When a sufficient number of microtubules attach and/or sufficient tension has been applied, a host of proteins known as spindle checkpoint proteins relay the information to the anaphase-promoting complex (APC), which initiates anaphase[4,42]. The biochemistry of the process is so sensitive that a single unaligned chromosome can delay anaphase for hours or even stop the cell cycle[41]. The spindle checkpoint is thought to be the primary mechanism for avoiding aneuploidy.

Many of the proteins involved in the spindle checkpoint are highly conserved and several have been found in plants[19,43]. Preliminary analysis of the maize homolog of MAD2, a well characterized checkpoint protein originally discovered in budding yeast, suggests that MAD2 functions in plants essentially as it does in animals[19]. During mitosis, MAD2 is abundant at kinetochores in early prometaphase but is barely detectable once the microtubules have attached. By contrast, microtubule attachment does not have a significant effect on MAD2 staining in maize meiosis; instead, it appears that the tension applied by the maturing spindle correlates better with the loss of MAD2 staining at metaphase[19]. One indicator of tension at animal kinetochores is the dephosphorylation of a phosphoepitope recognized by the 3F3/2 antibody[41,44]. In support

Origin	Accession number	Total aa length
Human[a]	M95724	943
Sheep[b]	P49453	402
Chicken[a]	BAA24110	853
Mouse[a]	U03113	906
Maize, CenpcA[a]	AF129857	702
Maize, CenpcC[b]	AF129859	451
Maize, CenpcB[b]	AF129858	440
Arabidopsis	AAF71990	711
Tomato[b]	AI485238	173
Mif2[a], *Saccharomyces cerevisiae*	P35201	549
Schizosaccharomyces pombe	CAB52737	604
Caenorhabditis elegans	AAB42237	866
Neurospora crassa	CAB91393	765

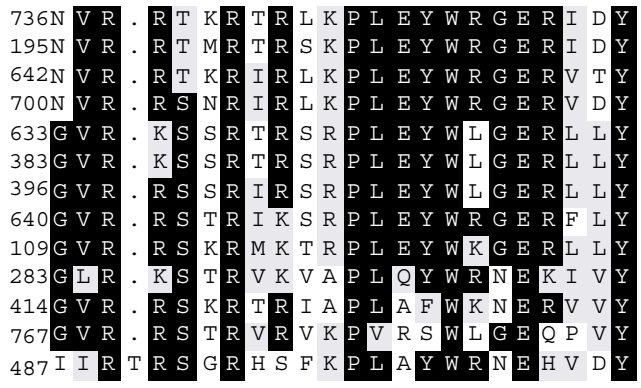

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Fig. 1. Alignment of the CENPC region I sequence from various organisms. In each case, region I was identified using the Block Maker Server (http://www.blocks.fhcrc.org/)[64]. A black box indicates either an invariant amino acid or the consensus amino acid at that position (column); if there are no black boxes, there is no consensus. Gray boxes indicate amino acids that are similar to the consensus or other amino acids in the same column. Numbers immediately to the left of the sequence data indicate the amino acid start position for region I. [a]Demonstrated experimentally to localize to kinetochores; [b]partial cDNAs.

of a role for tension, MAD2 staining on maize meiotic kinetochores was tightly correlated with the presence of the 3F3/2 phosphoepitope[19]. These data are consistent with the idea that both microtubule attachment and the resulting tension are important factors in the destruction or dislocation of MAD2 at maize kinetochores.

Proteins with unknown kinetochore functions

The functions of five of the proteins and antibody cross-reactivities listed in Table 1 are as yet difficult to interpret. Cbf5p was originally described as a centromere binding protein that interacts genetically with a yeast kinetochore protein known as Ndc10p (Ref. 45). However, Cbf5p is now thought to be a nucleolar protein that is required for rRNA synthesis in both yeast and mammalian cells[46]. The recent demonstration that a *V. faba* CBF5 homolog localizes to kinetochores[27] seems to support the initial interpretation but the conflicting data make it difficult to determine the kinetochore function of CBF5.

CENP-F is a nuclear matrix protein during G2 of interphase but relocates to the kinetochores during prophase, metaphase and early anaphase, and is then lost from kinetochores. CENP-F is thought to be involved in the early stages of kinetochore maturation[47], although how it is involved in this process is still poorly understood. Antibodies to human CENP-F cross-react strongly with plant kinetochores[27].

Meiotic histone of *Lilium* is a centromeric histone H1 homolog that is present only at meiosis[48]. Although most antibodies to meiotic histone [also known as meiotin-1 (Ref. 49)] stain meiotic chromosomes throughout their length, one particular serum stained only the centromeric regions[48]. These data might indicate that a unique histone H1 epitope is present at meiotic centromeres.

The MPM2 antibody identifies a large family of mammalian phosphorylated epitopes on mitotic structures such as centrosomes, kinetochores, spindles and chromosome scaffolds. The antibody cross-reacts with other species and, in *V. faba*, kinetochores are among the structures identified[18]. Because neither the kinase responsible for these phosphorylation events nor all the protein targets are known[50], it is not yet possible to determine which plant kinetochore protein(s) is recognized by the MPM2 antibody.

A final protein in this category is Skp1p, a component of the Skp1, cullin and F-box (SCF) protein complex. The SCF is a ubiquitin-ligase complex that targets a variety of proteins for proteolysis, including a non-conserved budding yeast kinetochore protein known as Ctf13p (Ref. 51). Skp1p is also involved in the initiation of DNA replication in budding yeast[51], centrosome separation in mammals[52] and the separation of homologous chromosomes during male meiosis in *Arabidopsis*[53]. Both *H. vulgare* and *V. faba* kinetochores contain putative homologs of yeast Skp1p (Ref. 27), suggesting that one or more plant kinetochore proteins are targeted for ubiquitin-dependent proteolysis. Further research will be required to determine the nature of these targets and their role in regulating kinetochore function.

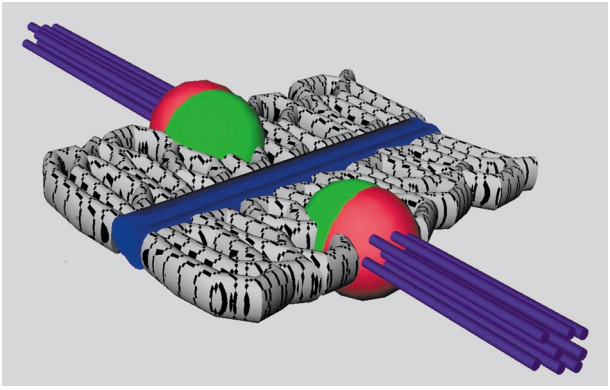

Fig. 2. A model of the maize meiotic kinetochore showing the centromeric region of a meiosis II chromosome. The kinetochore is depicted as a spherical structure with two subdomains. The inner (green) domain contains the maize protein CENP-C and the outer (red) domain contains the MAD2 protein and the 3F3/2 antigen. The chromatids, indicated by wavy lines, are attached by chromosome cores (blue). Microtubules are shown in purple.

Structural and functional conservation at the plant kinetochore

Recent immunofluorescence analyses of meiotic maize kinetochores have challenged the conventional 'ball and cup' view of the plant kinetochore[19,26]. Immunolocalization data indicate that there are clear substructural domains in the maize kinetochore: an inner domain containing CENPC and an outer domain containing MAD2 (Ref. 19; Fig. 2). The inner CENPC domain overlaps but does not perfectly colocalize with a conserved centromeric DNA element known as Sau3a (Refs 26,54). Staining for the 3F3/2 antigen essentially overlaps with staining for MAD2 in the outer domain[19], which is consistent with the fact that both antibodies report the activity of the spindle checkpoint. The domain structure of the mature plant kinetochore is reminiscent of the trilaminar organization in the mammalian kinetochore. An important next step in the analysis of plant kinetochore subdomains will be to carry out high resolution immuno-ultrastructural studies using the collection of antisera that are now available.

Although the kinetochores of both animals and plants have a clear substructure and inherent polarity with respect to the spindle axis (Fig. 2), a variety of data suggest that the kinetochore is actually redundant in structure and remarkably plastic. The first demonstration of redundancy in the centromere–kinetochore complex was the finding that a single maize centromere can be divided into two functional parts[55]. Other studies have verified that plant centromeres can be reduced in size considerably[56] and that they contain an extreme level of DNA sequence redundancy[57]. An analysis of single-kinetochore chromosomes produced by the maize meiotic mutation *afd1* (*absence of first division 1*)[58] provided further evidence of redundancy in the kinetochore[59]. Immunofluorescence and *in situ* hybridization analyses showed that a portion of the single kinetochore chromosomes aligned at the metaphase plate, where they split into subunits and interacted with both spindle poles in a tension sensitive manner[59].

These and previous data from animals[60,61] suggest that the kinetochores from plants and other higher eukaryotes are composed of multiple independent modules (a similar model was described recently[62]). The individual modules not only possess the ability to acquire spindle microtubules independently but can also carry out chromosome movement and spindle checkpoint control functions that are usually assigned to the kinetochore as a whole[59]. The minimal size of a kinetochore module might correspond to a single nucleosome, as found at the budding yeast kinetochore[63].

Perspective

During the past few years, at least a dozen plant kinetochore proteins have been identified by either protein sequence homology or their cross-reactivities with antibodies to animal proteins (Table 1). Although kinetochore research is much more advanced in other organisms, there are compelling reasons to continue studying the kinetochores of plants. Among these are the large size of the kinetochores in plants such as maize and the fact that both the mitotic and meiotic chromosomes are easily studied[19]. The ease of genetic analysis in *Arabidopsis* should also make it possible to begin functional analyses of kinetochore proteins. Another major application of plant kinetochore research will be in the development of artificial chromosomes as transformation vectors[11]. A clear understanding of the protein components of the centromere–kinetochore complex and of the generation of good reagents for their detection will be an important part of developing the technology for artificial chromosomes.

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Since the writing of this review, new data were published demonstrating that CENP-E functions in the spindle checkpoint[65].

Acknowledgements

Our work was supported by a grant to R.K.D. from the National Science Foundation (MCB9513556). We thank Michael Muszynski and Pioneer Hi-Bred International for the full length maize CENPC cDNA and Rogier ten Hoopen for allowing us to cite unpublished data.

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