

Plant centromeres: structure and control

Eric J Richards* and R Kelly Dawe†

Recent work has led to a better understanding of the molecular components of plant centromeres. Conservation of at least some centromere protein constituents between plant and non-plant systems has been demonstrated. The identity and organization of plant centromeric DNA sequences are also beginning to yield to analysis. While there is little primary DNA sequence conservation among the characterized plant centromeres and their non-plant counterparts, some parallels in centromere genomic organisation can be seen across species. Finally, the emerging idea that centromere activity is controlled epigenetically finds support in an examination of the plant centromere literature.

Addresses

*Department of Biology, Washington University, One Brookings Drive, St. Louis, MO 63130, USA; e-mail: richards@biodec.wustl.edu

†Department of Botany and Department of Genetics, Miller Plant Sciences Building, University of Georgia, Athens, GA 30602, USA; e-mail: kelly@dogwood.botany.uga.edu

Current Opinion in Plant Biology 1998, 1:130–135

<http://biomednet.com/elecref/1369526600100130>

© Current Biology Ltd ISSN 1369-5266

Introduction

Centromeres are nucleoprotein complexes that orchestrate proper chromosome partitioning at mitosis and meiosis by controlling sister chromatid adhesion and driving chromosome movement. Despite their importance, centromeres remain poorly understood, especially in multicellular eukaryotes with large chromosomes. One of the themes developing from work on centromeres in humans and *Drosophila* is that the centromeric repetitive DNA, often viewed with some suspicion as genomic flotsam, may play an important role in centromere function. Also emerging is the idea that assembly of an active centromere is not strictly tied to particular DNA sequences but can be controlled epigenetically by processes that operate above the level of the primary nucleotide sequence. We would like to relate recent findings on the molecular composition of centromeres in higher plants, as well as a growing body of work on developmentally controlled neocentromeres in maize, to the emerging idea that centromeres are epigenetic constructions built upon a foundation of evolutionarily-divergent repeat arrays.

Protein components of plant centromeres

Cytologically, the centromere is a constriction in the chromosome flanked on either side by deeply-staining heterochromatin. In the center of the constriction lies the proteinaceous kinetochore. The major functions of both plant and animal kinetochores are to align the chromosomes on the metaphase plate and mobilize

the chromosomes poleward in anaphase. In addition, kinetochores appear to provide a signal for the spindle assembly checkpoint that determines when anaphase begins [1]. The animal kinetochore has four plate-like layers that may have unique functions in chromosome movement. The outermost layer, known as the fibrous corona, carries molecular motors that are involved in chromosome movement [2]. Inside of the fibrous corona is an outer plate which appears to interact with microtubules, a non-staining spacer region, and a diffuse inner plate which may contain centromeric DNA. In contrast, plant kinetochores are ball-shaped structures that lack defined ultrastructural domains. At metaphase plant kinetochores usually appear as granular structures that are embedded in a cup of chromatin (as in the case of *Lilium longiflorum*; see [3]). The reproducible absence of substructure implies that the plant kinetochores are less organized than their animal counterparts, but does not rule out the existence of more subtle subdomains that will only be identifiable using specific antisera [4].

Currently, the most promising strategy for identifying and characterizing the proteins that make up plant kinetochores is to make use of the large amount of information available from mammalian systems. Much of the information on mammalian kinetochores has been derived either directly or indirectly from human patients with the CREST variant of scleroderma, an autoimmune disease that is characterized by the production of anti-kinetochore antibodies [2]. The majority of CREST sera do not recognize plant kinetochores [5]. Nevertheless, at least two CREST sera (EK and S174) do react with plant kinetochores, demonstrating that plant and animal kinetochores share common epitopes [5–9]. The best studied CREST serum (EK) has been used to identify *Haemanthus*, *Tradescantia*, and maize kinetochores [7–9]. In *Haemanthus*, the EK serum revealed that the kinetochores can be resolved into two distinct subregions, consistent with the observation in animals that the kinetochore is composed of multiple reiterated subunits [10]. Another antiserum that recognizes plant kinetochores, called C6C [11], was originally raised against calf centrosomes. Centrosomes are the microtubule organising centers (MTOCs) at animal spindle poles (ultrastructurally, centrosomes appear to be absent in plants). Instead of identifying the poles of plant spindles, the C6C antibody recognised the plant nuclear envelope in prophase and the kinetochores in metaphase and anaphase. These data are consistent with the idea that kinetochores organize microtubules, either by nucleating (starting) tubulin polymerization or by capturing already polymerized microtubules (see [11]). The transient kinetochore localization of the C6C epitope, and other uncharacterised phosphoproteins [12], suggests that the constitution of the kinetochore changes as a

function of the cell cycle. Whereas the cross-reactivities of animal anti-kinetochore sera provide strong evidence for the evolutionary conservation of kinetochore proteins, it has not been possible to determine the identity of the proteins that are conserved.

An alternative approach to the immunoscreening of proteins, that of searching expressed sequence tag (EST) databases, was used to identify an *Arabidopsis* homolog of the *Drosophila* ZW10 gene [13••]. ZW10 is one of several kinetochore proteins thought to be required for the spindle assembly checkpoint: an important stage of the cell cycle that involves sensing tension between the kinetochore and the kinetochore microtubules [1]. ZW10 is thought to be involved in ensuring that metaphase, as detected by the presence of tension on both opposing kinetochores, is complete before anaphase begins. Consistent with this interpretation is the observation that, in animals, ZW10 is found primarily on chromosomes that have not yet aligned on the metaphase plate [13••]. Immunolocalization studies will be required to assess whether the *Arabidopsis* ZW10 protein is also preferentially associated with unaligned chromosomes.

DNA components of plant centromeres

Much of the work on eukaryotic centromeres has been framed by the successful isolation, almost two decades ago, of a short (approximately 125 bp) DNA sequence, lacking flanking repeats, that specifies centromere function in the budding yeast, *Saccharomyces cerevisiae* [14]. This finding fueled the hope that well-conserved, short DNA sequence motifs would be found to control centromere function in other organisms. Such a view might have gained support from work on another essential chromosome element—the telomere—the structure of which was found to be very similar among widely diverged eukaryotic organisms [15]. The picture for centromeres, however, has turned out to be considerably more complicated.

A great deal of effort has focused on characterization of DNA sequences present in eukaryotic centromeric regions outside of budding yeast and in almost all instances, the definition of functional centromeric DNA has been elusive. The major exception is the centromere of the unicellular fungus *Schizosaccharomyces pombe* (fission yeast), in which the DNA sequences necessary and sufficient for specification of centromere function have been identified. The *Sc. pombe* centromere regions are 50–150 kb, consisting of a 4–7 kb central core of low-copy sequences surrounded by complex tandem repeats organized into a large palindrome [16]. The minimal functional *Sc. pombe* centromere consists of the central core and at least a portion of the flanking repeats [17]. Hence, functional centromeric sequences (CEN DNA) from fission yeast and budding yeast are strikingly different in primary nucleotide sequence, size and genomic organization.

In other well-studied systems, the general rule is that centromeric DNA is organized into a large block of repet-

itive DNA [18]. It is likely that this block corresponds, on the cytological level, to the heterochromatin that encompasses the region associated with the kinetochore. This pericentric heterochromatin appears to have a role in controlling and/or mediating sister chromatid cohesion in metaphase (of mitosis and meiosis II) [19,20]. A number of highly reiterated repeats that reside in centromere regions in plant species have been described [21–24,25•,26•,27,28] (see Table 1 for a partial list). Because most of these DNA repeats were localized to the centromere by chromosome *in situ* hybridization it is not possible to determine the position of the repeats relative to the functional centromere with certainty. Some corroborative evidence is available in *Arabidopsis* where the 180 bp repeat arrays map genetically to the intervals expressing meiotic centromere function delimited by tetrad analysis [29•,30•]. A higher resolution mapping of centromere function relative to centromeric repeat arrays was accomplished by physical mapping in maize. Kaszás and Birchler, using chromosome breakage to progressively subdivide the maize B (supernumerary) chromosome centromere, demonstrated that the B centric repeat was distributed throughout the functional centromere [31•]. In addition to the highly reiterated repeats, there is a variety of other sequences, including low-copy sequences [22,32–35], transposable elements [36] and telomere-similar repeats [22,28,37], found specifically or primarily in centromere regions in various plant species.

The growing collection of plant centromeric DNA sequences provides an opportunity to look for conserved structural patterns or primary nucleotide sequence motifs that may contribute to centromere function. Table 1 outlines the structural features of five centromere repeats that are among the best candidates for plant DNA sequences with a role in centromere function. The repeats share little primary nucleotide similarity, with the exception of the previously noted similarity between the 180 bp repeat found at maize neocentromeres (described below) [27] and a larger repeat element present in the centromere region of the maize B chromosome (B centric repeat) [28]. Only the maize 180 bp knob repeat has a significant match (8 of 9 bp) to the DNA binding site of CENP-B [38], a protein which may contribute to centromere function in mammals (but see [39]). Considering structural features beyond the primary nucleotide sequence, it is difficult to discern a convincing connection between the repeats. It should be noted, however, that the cereal centromeric repeats, CSS1 [25•] and Sau3A9 [26•], were recently shown to cross-hybridize to centromeres from a variety of monocots. Whether such repeats represent a well-conserved plant centromere DNA sequence under functional constraint, or simply a more slowly evolving centromeric repeat family, remains to be determined.

If no clear consensus is emerging regarding the DNA sequence of centromeric DNA in plants, some common themes are seen in the genomic organization of the

Table 1

Plant centromeric repeats.

Repeat (source)	%AT	Unit length	Organization	Localization	Mammalian CENP-B box*	Similarities to other plant centromeric DNA?	References
At180 (<i>Arabidopsis</i>)	63%	180 bp	Tandem	<i>In situ</i> , and genetic mapping	No		[21–24,29*,30*]
B centric (maize)	63%	1.4 kb	Degenerate tandem	<i>In situ</i> , and physical mapping	No	Similarity to Zm180knob (72% over 90 kb)	[28,31*]
CSS1 (<i>Brachypodium</i>)	53%	250 bp	Interspersed?	<i>In situ</i>	No	Cross-hybridization with centromeres from other monocots; related elements isolated from maize, barley and rice Low similarity to Sau3A9 (~50% over 60 bp)	[25*]
Sau3A9 (sorghum)	56%	745 bp	Interspersed?	<i>In situ</i>	No	Cross-hybridizes with centromeres from other monocots	[26*]
Zm180knob (maize)	56%	180 bp	Tandem	<i>In situ</i>	8/9 bp	Similarity to maize B centric repeat	[27]

*Mammalian CENP-B box: TTCGNNNNANCGGG [38].

few plant centromere regions which have been at least partially physically mapped. The size of the regions encompassing the centromere-associated repeats is large. For example, the maize B centromere region is estimated to be at least 9 Mbp in size [31*]. Even *Arabidopsis*, with its relatively small genome (100–150 Mbp/haploid), has centromere repeat arrays that occupy approximately 1 Mbp per centromere [30*]. While it is not clear how much of these regions are involved in centromere function, the maize B centromere work [31*] and classical cytogenetic experiments on other plant species [40,41] argue that some large centromeric regions can be subdivided into smaller nonoverlapping functional units.

The picture of the suborganization of the centromere regions is still crude, but it appears that a range of architectures can be discerned. On one end of the spectrum is the apparently simple organization of the facultative centromeres known as neocentromeres. Neocentromeres are best understood in maize [42**] but have been documented in at least sixteen other plant species [43–45]. Neocentromeres are often heterochromatic (see [44,46] for examples) and in maize they are composed of long tandem arrays of a 180 bp repeat [47] which form heterochromatic knobs [27]. Most of the available data indicate that the arrays at maize knobs are homogenous [47,48]; however, maize genomic clones containing retrotransposable elements inserted into 180-bp repeats have been recovered (E Ananiev and R Phillips, personal communication). These clones may represent sequences at the border of uninterrupted 180 bp repeat arrays, rare retrotransposon islands embedded within the arrays, or 180 bp repeats at non-knob sites. In any case, the relatively homogenous organization of the maize knob neocentromeres most

closely resembles that seen at human centromeres [49]. *Arabidopsis* centromere regions appear to be a more complex mosaic of long uninterrupted arrays of 180 bp repeats and interspersed regions containing middle- or low-copy sequences [22,30*,32–35]. A more complicated genomic organization is seen in the maize B centromere region where the 1.4 kb B centric repeat element is arranged in a degenerate tandem array with a minimum of interspersed sequences [28,31*]. Physical mapping suggests that the elemental B centric repeat may be organized into higher-order repeat units [31*]. Even at this early stage of characterization, it is clear that several genomic architectures are able to support the assembly of a functional centromere.

A critical unresolved question centers around the role of the highly reiterated centromere repeats in non-yeast systems. Are these repeats excess baggage surrounding rare functional centromeric sequence motifs or are the repeats an integral part of the centromere, perhaps the CEN DNA itself? Work in mammals has provided evidence for both points of view. A conservative reading of these results, primarily based on re-introduction of mammalian centromere repeat arrays into cultured mammalian cells, suggests that homogenous arrays of the short repeats can specify at least some properties of the centromere, including chromosome constriction and sister chromatid attachment [50–53]. Similar experiments have not been done in plant systems but this conservative view is consistent with cytological observations of plant sister chromatid cohesion via pericentric heterochromatin domains [19]. The larger question of whether the repeats contribute to the assembly or specification of the kinetochore has been brought to the fore by the recent demonstration

that microinjection of arrays of the alphoid class of human centromeric repeats (plus telomeres and a selectable marker) into cultured cells can lead to the formation of centromere-containing microchromosomes [54]. These data strongly suggest that simple centromere repeats are sufficient for chromosome segregation in humans, however, the authors did not rule out the possibility that the newly-formed microchromosomes contain rearranged centromeres from an endogenous chromosome. Support for the role of simple DNA repeats in specification of centromere function also comes from structural dissection of human and *Drosophila* centromeres. Analysis of structurally rearranged human Y chromosomes indicates that the alphoid repeat array is an important functional component of the centromere [55,56]. In *Drosophila*, detailed structural analysis has narrowed a functional centromere down to a genomic region containing simple-sequence satellite arrays and a handful of interspersed retrotransposable elements [57,58,59]. In plants, the evidence that small centromere repeats contribute to chromosome segregation comes primarily from the analysis of knobs/neocentromeres in maize. In their active neocentromere form, knobs are preferentially segregated to progeny [60]. The degree of preferential segregation is positively correlated with knob size [61], which is in turn correlated with the number of 180 bp knob repeats [27]. Larger knobs also migrate faster to the spindle poles than smaller knobs in anaphase II [42•]. Although it is likely that the 180 bp knob repeats are required for neocentromere motility, the characteristics of neocentromeres differ from those of normal centromeres. Instead of the end-on interactions observed between the microtubules and normal centromeres/kinetochores, microtubules were found to interact laterally with neocentromeres [42•]. These and other data suggest that maize neocentromeres utilize only a subset of the functions of a normal kinetochore: they are probably incapable of aligning chromosomes on the metaphase plate and lack the ability to regulate their rates of movement. Nevertheless, maize neocentromeres are capable of pronounced poleward movement in the presence of necessary *trans*-acting gene products [62•], perhaps through the interaction with microtubule-based motor proteins [42•].

Epigenetic control of centromere activity

Accumulating evidence from a number of systems indicates that centromere DNA sequences are not always sufficient, nor are they always necessary, for specification of centromere activity [63,64••,65,66•,67•]. The observation that there is little sequence conservation among the known centromere repeats, including those characterized to date in plants, is consistent with the idea that primary DNA sequence may provide only part of the information necessary to specify centromere function. The remaining information is thought to be epigenetic, superimposed on the primary nucleotide information, in the form of stable, self-propagating chromatin states.

The maize neocentromere system is one of the best understood epigenetic centromeres. The DNA sequence at the knobs fails to organize a functioning centromere except in a particular genetic and developmental context: during meiosis I and II in the presence of *trans*-activating loci on abnormal chromosome 10 [62•,68]. Even in this developmental and genetic background there is considerable variability in the strength of neocentromeric activity expressed by the knobs [69]. Similar variability is seen in neocentromeric activity of chromosomes in other plant species (e.g. [44,45]). These observations suggest that the *cis*-acting determinants at the auxiliary sites are not always sufficient to organize a functional neocentromere, even under conditions appropriate for neocentromere formation.

Variable activation of a plant centromere has also been documented in early cytogenetic work on a wheat dicentric chromosome [70]. In this case, one of the centromeres, described as 'weak,' was active in unpaired univalents at meiosis I. When the dicentric was paired, however, the weak centromere was active in only a small minority of the bivalents. Additional evidence for epigenetic regulation of plant centromeres comes from the behavior of maize chromosomes introduced into an oat background by sexual hybridization [48,71]. In most cases, the maize chromosomes are lost following an oat–maize hybridization, which has been interpreted as a consequence of maize centromere dysfunction. It is possible to isolate lines from the oat–maize cross, however, which contain a mitotically stabilized maize chromosome. The stabilization appears to be a stochastic process leading to the recovery of one of at least eight maize chromosomes. The mechanism of mitotic stabilization is not known, but the absence of detectable chromosome rearrangement points toward the epigenetic model of acquisition and propagation of a functional state at the maize centromere.

Conclusions

The data available to date suggest that plant centromeres conform to the pattern seen in non-plant centromeres: they exhibit conservation of at least some kinetochore protein components; presence of a large block of repetitive DNA near the centromere; relatively poor conservation of primary repeat sequence; and epigenetic modulation of centromere activity. In addition to the long-term goal of understanding the mechanisms of chromosome segregation in plants, a potentially important application of centromere research is in the production of artificial chromosomes for use as plant transformation vectors. One of the largest technical obstacles hindering the development of plant artificial chromosomes is the lack of functional assays to define CEN DNA. Development of such functional assays could be significantly complicated by epigenetic modulation of centromere activity. Little is known about epigenetics in plants and efforts to control epigenetic regulation are in their infancy. The fact that kinetochore components appear to be more conserved

than CEN DNA sequences suggests that efforts may be profitably directed toward the identification of functional kinetochore proteins and how those proteins interact with DNA.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Allshire RC: **Centromeres, checkpoints, and chromatid cohesion.** *Curr Opin Genet Dev* 1997, **7**:264-273.
 2. Earnshaw WC: **Structure and molecular biology of the kinetochore.** In *Microtubules*. Edited by Hyams JS, Lloyd CW. New York: Wiley-Liss; 1994:393-412.
 3. Braselton JP, Bowen CC: **The ultrastructure of the kinetochores of *Lilium longiflorum* during the first meiotic division.** *Caryologia* 1971, **24**:49-58.
 4. Wordeman L, Steur ER, Sheetz MP, Mitchison T: **Chemical subdomains within the kinetochore domain of isolated mitotic chromosomes.** *J Cell Biol* 1991, **114**:285-294.
 5. Houben A, Guttenbach M, Kreh W, Pich U, Schubert I, Schmid M: **Immunostaining and interphase arrangement of field bean kinetochores.** *Chromosoma Res* 1995, **3**:27-31.
 6. Houben A, Brandes A, Pich U, Manteuffel R, Schubert I: **Molecular-cytogenetic characterization of a higher plant centromere/kinetochore complex.** *Theor Appl Genet* 1996, **93**:477-484.
 7. Mole-Bajer J, Bajer AS, Zinkowski RP, Balczon RD, Brinkley BR: **Autoantibodies from a patient with scleroderma CREST recognized kinetochores from the higher plant *Haemanthus*.** *Proc Natl Acad Sci USA* 1990, **87**:3359-3603.
 8. Dawe RK: **Meiotic chromosome organization and segregation in plants.** *Annu Rev Plant Phys Plant Mol Biol* 1998, **49**:371-395.
 9. Palevitz BA: **Kinetochore behavior during generative cell division in *Tradescantia virginiana*.** *Protoplasma* 1990, **157**:120-127.
 10. Zinkowski RP, Meyne J, Brinkley BR: **The centromere-kinetochore complex: a repeat subunit model.** *J Cell Biol* 1991, **113**:1091-1110.
 11. Schmit AC, Stoppin V, Chevrier V, Job D, Lambert AM: **Cell cycle dependent distribution of a centrosomal antigen at the perinuclear MTOC or at the kinetochores of higher plant cells.** *Chromosoma* 1994, **103**:343-351.
 12. Binarova P, Chalikova J, Dolezel J: **Localization of MPM-2 recognized phosphoproteins and tubulin during cell cycle progression in synchronized *Vicia faba* root meristem cells.** *Cell Biol Int* 1993, **17**:847-856.
 13. Starr DA, Williams BC, Li Z, Etemad-Moghadam B, Dawe RK, Goldberg ML: **Conservation of the centromere/kinetochore protein ZW10.** *J Cell Biol* 1997, **138**:1289-1301.
- Reports the identification of an *Arabidopsis* homolog of a *Drosophila* protein that is thought to monitor tension between the spindle and the kinetochore. This is the first use of an expressed sequence tag database to identify a putative plant kinetochore protein.
14. Jiang W, Carbon J: **Molecular analysis of the budding yeast centromere/kinetochore.** *Cold Spring Harbor Symp Quant Biol* 1993, **58**:669-676.
 15. Blackburn EH: **Structure and function of telomeres.** *Nature* 1991, **350**:569-573.
 16. Clarke L, Baum M, Marschall LG, Ngan VG, Staehner NC: **The structure and function of *Schizosaccharomyces pombe* centromeres.** *Cold Spring Harbor Symp Quant Biol* 1993, **58**:687-695.
 17. Baum M, Ngan VK, Clarke L: **The centromeric K-type repeat and the central core are together sufficient to establish a functional *Schizosaccharomyces pombe* centromere.** *Mol Biol Cell* 1994, **5**:747-761.
 18. Sunkel CE, Coelho PA: **The elusive centromere: sequence divergence and functional conservation.** *Curr Opin Genet Dev* 1995, **5**:756-767.
 19. Lima-de-Faria A: **The division cycle of the kinetochore.** *Hereditas* 1955, **41**:238-240.
 20. Miyazaki WY, Orr-Weaver TL: **Sister-chromatid cohesion in mitosis and meiosis.** *Annu Rev Genet* 1994, **28**:167-187.
 21. Martinez-Zapater J, Estelle MA, Somerville CR: **A highly repeated DNA sequence in *Arabidopsis thaliana*.** *Mol Gen Genet* 1986, **204**:417-423.
 22. Richards EJ, Goodman HM, Ausubel FM: **The centromere region of *Arabidopsis thaliana* chromosome 1 contains telomere-similar sequences.** *Nucleic Acids Res* 1991, **19**:3351-3357.
 23. Simoens CR, Gielen J, Montagu MV, Inze D: **Characterization of highly repetitive sequences of *Arabidopsis thaliana*.** *Nucleic Acids Res* 1988, **16**:6753-6766.
 24. Maluszynska J, Heslop-Harrison JS: **Localization of tandemly repeated DNA sequences in *Arabidopsis thaliana*.** *Plant J* 1991, **1**:159-166.
 25. Aragon-Alcaide L, Miller T, Schwarzacher T, Reader S, Moore G: **A cereal centromeric sequence.** *Chromosoma* 1996, **105**:261-268. Demonstrates a conserved monocot centromere sequence. A subclone (CCS1) of a previously characterized *Brachypodium* genomic clone is shown to hybridize specifically to centromere regions from a number of monocots. Related sequences from rice, maize and barley are amplified, cloned, sequenced and shown to hybridize to centromere regions.
 26. Jiang J, Nasuda S, Dong F, Scherrer CW, Woo SS, Wing RA, Gill BS, Ward DC: **A conserved repetitive DNA element located in the centromeres of cereal chromosomes.** *Proc Natl Acad Sci USA* 1996, **93**:14210-14213. Provides more evidence for conserved plant centromeric sequences. The characterization of a sorghum sequence, designated Sau3A9, that hybridizes with centromeres from a variety of monocots is described.
 27. Peacock WJ, Dennis ES, Rhoades MM, Pryor AJ: **Highly repeated DNA sequence limited to knob heterochromatin in maize.** *Proc Natl Acad Sci USA* 1981, **78**: 4490-4494.
 28. Alfenito MR, Birchler JA: **Molecular characterization of a maize B chromosome centric sequence.** *Genetics* 1993, **135**:589-597.
 29. Copenhaver GP, Browne WE, Preuss D: **Assaying genome-wide recombination and centromere functions with *Arabidopsis* tetrads.** *Proc Natl Acad Sci USA* 1998, **95**:247-252. This elegant genetic study applies tetrad analysis to *Arabidopsis* to monitor recombination events throughout the genome. More importantly for the purpose at hand, this analysis was used to narrow down the genetic locations of the functional meiotic centromeres.
 30. Round EK, Flowers SK, Richards EJ: ***Arabidopsis thaliana* centromere regions: genetic map positions and repetitive DNA structure.** *Genome Res* 1997, **7**:1045-1053. Describes the initial physical and genetic mapping of centromeric repetitive DNA arrays in *Arabidopsis*. Despite its small genome size, *Arabidopsis* contains megabase-sized centromere repeat arrays, underscoring the technical difficulties which must be overcome to isolate and characterize centromeres from even streamlined genomes.
 31. Kaszás E, Birchler JA: **Misdivision analysis of centromere structure in maize.** *EMBO J* 1996, **15**:5246-5255. The most detailed structural dissection of a plant centromere published to date. Successive rounds of centromere misdivision are used to whittle down the maize B chromosome centromere region from 9Mbp to less than 1 Mbp. Physical mapping of chromosomal derivatives carrying the deleted centromeres indicates that the B centric repeat is found in the functional centromere region.
 32. Brandes A, Thompson H, Dean C, Heslop-Harrison JS: **Multiple repetitive DNA sequences in the paracentromeric regions of *Arabidopsis thaliana* L.** *Chromosome Res* 1997, **5**:238-246.
 33. Schmidt R, West J, Love K, Lenehan Z, Lister C, Thompson H, Bouchez D, Dean C: **Physical map and organization of *Arabidopsis thaliana* chromosome 4.** *Science* 1995, **270**:480-483.
 34. Thompson H, Schmidt R, Brandes A, Heslop-Harrison JS, Dean C: **A novel repetitive sequence associated with the centromeric regions of *Arabidopsis thaliana* chromosomes.** *Mol Gen Genet* 1996, **253**:247-252.
 35. Thompson HL, Schmidt R, Dean C: **Identification and distribution of seven classes of middle-repetitive DNA in the *Arabidopsis thaliana* genome.** *Nucleic Acids Res* 1996, **24**:3017-3022.
 36. Pelissier T, Tutois S, Tourmente S, Deragon JM, Picard G: **DNA regions flanking the major *Arabidopsis thaliana* satellite are**

- principally enriched in *Athila* retroelement sequences. *Genetica* 1996, **97**:141-151.
37. Presting GG, Frary A, Pillen K, Tanksley SD: **Telomere-homologous sequences occur near the centromeres of many tomato chromosomes.** *Mol Gen Genet* 1996, **251**:526-531.
 38. Kipling D, Mitchell AR, Masumoto H, Wilson HE, Nicol L, Cooke HJ: **CENP-B binds a novel centromeric sequence in the asian mouse *Mus caroli*.** *Mol Cell Biol* 1995, **15**:4009-4020.
 39. Kipling D, Warburton PE: **Centromeres, CENP-B and *Tigger* too.** *Trends Genet* 1997, **13**:141-145.
 40. Darlington CD: **Misdivision and the genetics of the centromere.** *J Genet* 1939, **37**:341-364.
 41. Lima-de-Faria A: **The structure of the centromere of the chromosomes of rye.** *Hereditas* 1949, **35**:77-85.
 42. Yu H-G, Hiatt EN, Chan A, Sweeney M, Dawe RK: **•• Neocentromere-mediated chromosome movement in maize.** *J Cell Biol* 1997, **139**:831-840.
- By combining the analysis of fixed and living meiocytes, the authors demonstrate that neocentromere activity differs from centromere activity both with respect to the rate of poleward movement and the mode of interaction with spindle fibers.
43. Bajaj A, Ostergren G: **Centromere-like behaviour of non-centromeric bodies.** *Hereditas* 1961, **47**:563-598.
 44. Kavander T, Viinikka Y: **Neocentric activity in open-pollinated cultivars of rye.** *Hereditas* 1987, **107**:229-233.
 45. Viinikka Y: **Identification of the chromosomes showing neocentric activity in rye.** *Theor Appl Genet* 1985, **70**:66-71.
 46. Vardhan ZV, Lakshmi N: **A new triploid race of *Pennisetum orientale* Rich exhibiting chromosomal numerical mosaicism and neo-centric activity.** *Proc Indian Acad Sci* 1983, **92**:259-264.
 47. Dennis ES, Peacock WJ: **Knob heterochromatin homology in maize and its relatives.** *J Mol Evol* 1984, **20**:341-350.
 48. Birchler JA: **Do these sequences make CENs yet?** *Genome Res* 1997, **7**: 1035-1037.
 49. Tyler-Smith C, Willard HF: **Mammalian chromosome structure.** *Curr Opin Genet Dev* 1993, **3**:390-397.
 50. Haaf T, Warburton PE, Willard HF: **Integration of human a-satellite DNA into simian chromosomes: Centromere protein binding and disruption of normal chromosome segregation.** *Cell* 1992, **70**:681-696.
 51. Taylor SS, Larin Z, Tyler-Smith C: **Analysis of extrachromosomal structures containing human centromeric alphoid satellite DNA sequences in mouse cells.** *Chromosoma* 1996, **105**:70-81.
 52. Larin Z, Fricker MD, Tyler-Smith C: **De novo formation of several features of a centromere by introduction of a Y alphoid YAC into mammalian cells.** *Hum Mol Genet* 1994, **3**:689-695.
 53. Heartlein MW, Knoll JHM, Latt SA: **Chromosome instability associated with human alphoid DNA transfected into the Chinese hamster genome.** *Mol Cell Biol* 1988, **8**:3611-3618.
 54. Harrington JJ, Bokkelen GV, Mays RW, Gustashaw K, Willard HF: **Formation of de novo centromeres and construction of first-generation human artificial chromosomes.** *Nat Genet* 1997, **15**:345-355.
 55. Tyler-Smith C, Oakey RJ, Larin Z, Fisher RB, Crocker M, Affara NA, Ferguson-Smith MA, Muenke M, Zuffardi O, Jobling MA: **Localization of DNA sequences required for human centromere function through an analysis of rearranged Y chromosomes.** *Nat Genet* 1993, **5**:368-375.
 56. Brown KE, Barnett MA, Burgdorf C, Shaw P, Buckle VJ, Brown WRA: **Dissecting the centromere of the human Y chromosome with cloned telomeric DNA.** *Hum Mol Genet* 1994, **3**:1227-1237.
 57. Sun X, Wahlistrom J, Karpen G: **Molecular structure of a • functional *Drosophila* centromere.** *Cell* 1998, in press.
- The most detailed characterization to date of functional centromere DNA from a non-yeast system. The analysis shows the functional *Drosophila* centromere in question to be composed primarily of simple-sequence satellites and a cluster of transposable elements.
58. Murphy TD, Karpen GH: **Localization of centromere function in a *Drosophila* minichromosome.** *Cell* 1995, **82**:599-609.
 59. Le M-H, Duricka D, Karpen GH: **Islands of complex DNA are widespread in *Drosophila* centric heterochromatin.** *Genetics* 1995, **141**:283-303.
 60. Rhoades MM, Dempsey E: **The effect of abnormal chromosome 10 on preferential segregation and crossing over in maize.** *Genetics* 1966, **53**:989-1020.
 61. Kikudome GY: **Studies on the phenomenon of preferential segregation in maize.** *Genetics* 1959, **44**: 815-831.
 62. Dawe RK, Cande WZ: **Induction of centromeric activity in maize • by suppressor of meiotic drive 1.** *Proc Natl Acad Sci USA* 1996, **93**:8512-8517.
- Describes the isolation of a mutation that disrupts neocentromere formation at maize knobs and demonstrates the *trans*-acting genetic control of neocentromere activity.
63. Steiner NC, Clarke L: **A novel epigenetic effect can alter centromere function in fission yeast.** *Cell* 1994, **79**:865-874.
 64. Karpen GH, Allshire R: **The case for epigenetic effects on centromere identity and function.** *Trends Genet* 1997, **13**:489-496.
- The best synthesis of the evidence for epigenetic regulation of centromeres, which considers information from a number of eukaryotic species.
65. Brown W, Tyler-Smith C: **Centromere activation.** *Trends Genet* 1995, **11**:337-339.
 66. Du Sart D, Cancilla MR, Earle E, Mao J-I, Saffery R, Tainton KM, Kalitsis P, Martyn J, Barry AE, Choo KHA: **A functional neocentromere formed through activation of a latent human centromere and consisting of non-alpha-satellite DNA.** *Nat Genet* 1997, **16**:144-153.
- Describes one of the most striking examples of acquisition of centromere activity at an ectopic site. The functioning centromere of a rearranged human marker chromosome is localized to a small genomic interval (~80 kb) which is apparently structurally unrearranged and corresponds to an interstitial site in the long arm of chromosome 10. This region does not contain detectable alphoid repeats nor other known human centromeric DNA sequences.
67. Williams BC, Murphy TD, Goldberg ML, Karpen GH: **• Neocentromere activity of structurally acentric minichromosomes in *Drosophila*.** *Nat Genet* 1998, **18**:30-38.
- Describes the acquisition of functional centromeres on acentric chromosome fragments in *Drosophila*. These data highlight the importance of epigenetic regulation of centromere activity and demonstrate the flexibility that an organism may employ to build a centromere.
68. Rhoades MM: **Preferential segregation in maize.** In *Heterosis*, Edited by Gowen JW. Ames: Iowa State College Press; 1952:66-80.
 69. Emmerling MH: **Preferential segregation of structurally modified chromosomes in maize.** *Genetics* 1959, **44**:625-645.
 70. Sears ER, Camara A: **A transmissible dicentric chromosome.** *Genetics* 1952, **37**:125-135.
 71. Riera-Lizarazu O, Rines HW, Phillips RL: **Cytological and molecular characterization of oat x maize partial hybrids.** *Theor Appl Genet* 1996, **93**:123-135.