Published in final edited form as:

Trends Genet. 2012 May ; 28(5): 204–212. doi:10.1016/j.tig.2012.02.003.

Flexibility of centromere and kinetochore structures

Laura S. Burrack and Judith Berman

Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN 55405

Abstract

Centromeres, and the kinetochores that assemble on them, are essential for accurate chromosome segregation. Diverse centromere organization patterns and kinetochore structures have evolved in eukaryotes ranging from yeast to humans. In addition, centromere DNA and kinetochore position can vary even within individual cells. This flexibility manifests in several ways: centromere DNA sequences evolve rapidly, kinetochore positions shift in response to altered chromosome structure, and kinetochore complex numbers change in response to fluctuations in kinetochore protein levels. Despite their differences, all of these diverse structures promote efficient chromosome segregation. This robustness is inherent to chromosome segregation mechanisms and balances genome stability with adaptability. In this review, we explore the mechanisms and consequences of centromere and kinetochore flexibility as well as the benefits and limitations of different experimental model systems for studying them.

Keywords

centromere; neocentromere; kinetochore; aneuploidy; genome stability

Centromere/kinetochore dynamics: balancing genome stability and adaptability

During growth and cell division, cells must balance the requirement for faithful chromosome replication and segregation with the need to adapt to changing conditions. Failure of centromere/kinetochore function can result in aneuploidy, a change in chromosome number [1]. Aneuploidy is often detrimental [2, 3], but it has the potential to be adaptive under some conditions [4, 5]. For example, defects in genomic stability are implicated in cancer and can cause miscarriages and birth defects, while moderate aneuploidy may contribute to increased proliferation of cancer cells and to resistance to antifungals in yeast [4, 6, 7]. Chromosome segregation errors can lead to DNA damage and chromosome rearrangements [8, 9].

The kinetochore, a complex of ~100 proteins, must assemble on centromere DNA to allow spindle microtubule attachment to the chromosome during mitosis. Genome stability requires efficient and accurate kinetochore assembly, ensuring that each chromatid attaches to a single pole and that sister chromatids attach to microtubules from opposite poles. Thus, centromeres, and the kinetochores assembled on them, must participate in an exquisitely

© 2012 Elsevier Ltd. All rights reserved.

Corresponding author: Berman, J. (berman@umn.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

regulated process to ensure proper chromosome segregation. However, kinetochore assembly must be robust enough to handle physiological changes, such as overexpression of kinetochore proteins in cancer cells [10], that could perturb the balance of kinetochore components available for assembly.

Recent work suggests that centromeres/kinetochores tolerate significant deviations from the canonical structural features. These deviations include: variations in centromere DNA length, frequent alteration of the underlying DNA sequences, shifts in kinetochore position on the DNA and changes in kinetochore complex number (Box 1). This review discusses mechanisms cells use to maintain accurate chromosome segregation despite alterations to centromere/kinetochore structure. We suggest that centromere/kinetochore flexibility provides a buffering mechanism that facilitates successful evolution by maintaining chromosome segregation fidelity.

Box 1

Types of centromere flexibility

Centromere DNA length—The length of DNA associated with CENP-A and kinetochore protein complexes varies significantly from yeast to humans.

Centromere DNA sequence – The DNA sequences where centromeres are found vary greatly between organisms and also within a single species. Additionally, studies of closely related species indicated that the DNA associated with kinetochores might undergo accelerated mutation, further enhancing centromere sequence diversity.

Kinetochore position – The position of the kinetochore on the chromosome is flexible in a wide variety of organisms ranging from the yeast *C. albicans* to humans. One of the characteristics of primate speciation is the appearance of evolutionary new centromeres: kinetochores that associate with chromosomal regions that differ from the position of the ancestral centromere. Additionally, acentric chromosome fragments can be rescued by neocentromeres, kinetochores that form at new chromosomal positions.

Kinetochore complex number – The kinetochore complex number is defined as the number of kinetochore protein complexes assembled per centromere DNA region. The kinetochore complex number varies with changes in centromere DNA length and kinetochore protein concentrations in the cell.

Diversity of centromere structure

Eukaryotes exhibit diverse centromere organization patterns and kinetochore structures [11, 12] (Table 1). Many proteins that make up the kinetochore are conserved from yeast to humans. Centromeres are defined as chromosomal regions that bind CENP-A (CenH3), a histone H3 variant that replaces canonical H3 at centromeres, and assemble a functional kinetochore. Centromeres vary in length as well as inheritance mechanisms. *Saccharomyces cerevisiae* has point centromeres in which a DNA binding sequence is necessary and sufficient to drive kinetochore assembly [12]; in contrast, other fungi, plants and mammals form regional centromeres that usually occupy repetitive DNA and are inherited epigenetically. Many different models have been proposed to explain the mechanism(s) of epigenetic regional centromere inheritance [13]. Centromere regions vary in length from 125 bp in *Saccharomyces spp.* [12], to megabases of repetitive DNA found in mammalian and plant centromeres [11], and to entire chromosomes found in organisms that have holocentric centromeres, such as *Caenorhabditis elegans* [14]. As centromere structures have diverged, centromeric DNA sequences have also evolved rapidly.

DNA sequence evolution

Not only does centromere DNA differ dramatically between organisms [12], it differs between centromeres within a given organism [15, 16]. For example, different chicken centromeres vary in length from 30 kb to 500 kb, and contain either non-repetitive or highly repetitive DNA [15]. In contrast, human centromeres are all associated with alpha-satellite repeat DNA, although its length can vary from several hundred kb to 10 Mb [17, 18]. Diversity in the degree of repetitiveness indicates centromere “age” in evolutionary terms, as centromeres accumulate repeats that are maintained over time [19, 20]. The dog and horse genomes include high sequence divergence in centromeric satellite repeats on different chromosomes [21]. In the fungal pathogen *Candida albicans*, which has regional centromeres of ~ 3 kb, each centromere contains a unique, non-repetitive core sequence [22].

Consistent with the sequence diversity found between centromeres, centromere DNA evolves at rates much higher than rates at other non-coding regions. For example, *C. albicans* and *Candida dubliniensis* diverged more than 20 million years ago, and while the centromeric synteny in these two organisms has been maintained, the centromere DNA sequences diverged faster than other orthologous intergenic regions [23]. Similarly, maize centromere sequences evolve rapidly via high rates of gene conversion [24], and mouse Y centromere repeat DNA sequences have diverged from repeat sequences found at other chromosomes and in closely related mouse species [25]. The mechanisms directing rapid evolution of centromere DNA sequences are a topic of active research. Meiotic drive, an evolutionary force in which paired chromosomes compete for access to the egg during reproduction, has been proposed to promote centromere sequence divergence [26]. In *S. cerevisiae*, where meiotic drive would not be expected to be a substantial evolutionary force, as meiosis results in four equal spores rather than a single egg, rapid evolution of centromere DNA is consistent with an elevated mutation rate at centromere DNA [27], perhaps due to features of the centromeric chromatin that enhance mutational frequencies or inhibit DNA repair mechanisms.

Evolutionary new centromeres

In addition to having diverse primary DNA sequences, centromeres have evolved by moving to new positions in the genome. Different, evolutionary new centromere positions are a defining characteristic of primate species. Ectopic kinetochore assembly is a key step in their formation. For example, human centromere 6 was repositioned from an ancestral

location to its current location in a common ancestor of hominids [28]. Indeed, approximately half of the human chromosomes have evolutionary new centromeres, based upon comparison with macaques [20].

Evolutionary new centromeres often arise in gene deserts [29] and accumulate repetitive sequences [20]. Similar events have been seen in other mammals [30] as well as in plants (e.g., rice [19] and cucurbits [31]). These evolutionary events, detected via altered synteny of centromere-associated satellite DNA as well as through karyotype analysis, require that kinetochore proteins assemble at a new locus and no longer assemble at the old locus. A similar process, termed neocentromere formation, occurs in modern humans as well as in model organisms.

Neocentromeres

Neocentromeres are new kinetochores that assemble at DNA loci not previously associated with kinetochore proteins. Neocentromere formation can occur in the absence of chromosome rearrangements (repositioned neocentromeres) or it can rescue chromosome fragments, amplifications or rearrangements on either linear or ring chromosomes (rescue neocentromeres) [32]. Importantly, neocentromeres can be inherited from one generation to the next. In two documented cases of repositioned neocentromeres, the new centromere position has been maintained through at least three generations [28, 33]. While repositioned neocentromeres are rare [32], it is likely that their incidence is underreported, because they are only identified by cytogenetic screening and cause no obvious phenotypes, as the chromosome is still segregated during mitosis and the genome remains essentially intact. The majority of the ~100 reported neocentromeres are rescue neocentromeres [32] and the accompanying amplifications, deletions, or gene disruptions cause phenotypes including developmental disabilities as well as cancers such as non-Hodgkin's lymphoma [34], acute myeloid leukemia [35], and liposarcomas [36].

Neocentromeres map to at least 21 different human chromosomes, including X and Y, although they are particularly common on specific chromosome domains including 3q, 13q and 15q [32, 37]. Little is known about how neocentromeres form or why they form at particular positions. Human centromeres are always found in highly repetitive regions; the same is true for some neocentromeres [38], but not for others [39]. Chromosome regions that maintain neocentromere function show little similarity, and none of them are associated with alpha-satellite DNA, making it difficult to ascertain the sequence elements that might contribute to a good site of neocentromere formation. Interestingly, even among neocentromeres found in the same chromosomal band, no two neocentromeres have formed at exactly the same underlying DNA sequences [32, 38, 40]. LINE retrotransposon sequences appear to be important for establishing neocentric chromatin at some neocentromeres, as knockdown of transposon transcript levels impaired neocentromere function during mitosis [41]. ChIP-chip analyses failed to define common features of human neocentromeres [30], but higher resolution analysis in the future with ChIP-seq or ChIP-exo on CENP-A may provide additional insight. To date, the only sequence data available does not reveal any obvious patterns, supporting the idea that centromere position is remarkably flexible in humans.

Unlike evolutionary new centromeres that have persisted and that always formed in regions that were gene deserts [29], human neocentromeres form either in gene deserts or in regions that include actively transcribed genes [32, 39]. Of course, it is unknown which recently formed neocentromeres will or will not persist to become evolutionary new centromeres. It is tempting to speculate that those formed in gene deserts are more likely to persist, while those formed on actively transcribed regions may cause phenotypes that interfere with their

selection and evolutionary persistence. Indeed, some neocentromeres are more prone to chromosome missegregation than native centromeres. Human neocentromeres are more defective in recruiting Aurora B kinase and in correcting chromosome segregation errors than native centromeres [42]. Additionally, some patients exhibit neocentromere mosaicism (presence of the neocentromere in a fraction of somatic cells), suggesting that the chromosome carrying the neocentromere was lost in a subpopulation of the cells and implying that, *in vivo*, some neocentromeres are less stable than native centromeres [32]. Determinants of the chromosome segregation efficiency of different neocentromere positions remain to be identified.

Kinetochore position flexibility occurs in diverse species (Table 2), with some neocentromeres near the native centromere [43], others at the boundaries of euchromatin and heterochromatin [44, 45] and yet others within subtelomeric regions in a manner dependent upon heterochromatin formation [46]. In contrast, human neocentromeres exhibit no apparent association with heterochromatin [39]. In plants there are two types of neocentromeres: neocentromere knobs' and rescue' neocentromeres that, like human and *C. albicans* neocentromeres, appear following centromeric deletions or rearrangements [16, 48]. Neocentromere knobs' drive selection of specific chromosomes during meiosis [49]. Thus, they are likely to involve different mechanisms of formation and/or maintenance [50].

Genetic model organisms can be used to test hypotheses based on the analysis of clinical neocentromeres. For example, neocentromere mosaicism suggests that some neocentromeres are more functional than others [32]. Neocentromeres in *C. albicans* resemble those in humans in that they are flexible in their position and not associated with heterochromatin [47]. Furthermore, as a unicellular eukaryote, *C. albicans* is highly amenable to selection of low frequency events such as chromosome loss. Thus, it can be used to compare the efficiency of chromosome segregation of different neocentromeres.

Features shared by neocentromeres with high chromosome segregation accuracy and native centromeres are candidates for properties necessary for kinetochore function. In *C. albicans*, centromeres co-localize with the earliest, most efficient replication origins, and DNA regions that acquire a neocentromere become the earliest, most efficient replication origins on the chromosome and associate with the origin recognition complex [51]. *S. pombe CEN1* also replicates earliest of all loci on chromosome 1 [51], and in several other fungi the sequence features (DNA skew [52]) suggest that replication and centromere function are linked [51]. Additionally, in *S. cerevisiae*, cohesin binding is enriched at pericentromere regions, and cohesin binding to pericentric DNA requires a functional kinetochore [53]. Neocentromeres with different levels of stability may be useful for examining the roles of DNA replication, cohesin binding, and chromatin structures in promoting accurate chromosome segregation.

In vitro, telomere-telomere fusions produce dicentric chromosomes that subsequently delete alpha-satellite repeats to inactivate one of the centromeres [54]; this process has also been detected in clinical specimens [55]. This is particularly interesting because it may be analogous to events that initiate evolutionary new centromeres [56]. A similar mechanism has been documented in plants: during the evolution of maize and grass genomes, chromosomal rearrangements that produced dicentric chromosomes were resolved by inactivation of one centromere [57, 58]. *Drosophila* may be a particularly interesting model for examining repositioned neocentromeres that resemble the evolutionary new centromeres in primates [20, 28]. *Drosophila* cells form dicentric chromosomes following overexpression of CENP-A [45], and mechanisms that determine which centromere remains functional may provide insights into which human neocentromeres are destined to become evolutionary new centromeres.

Kinetochores composition

The overall structure of each kinetochore-microtubule complex is remarkably conserved from yeast to humans, although the number of complexes per centromere varies substantially, and some kinetochore proteins have specialized roles. *S. cerevisiae* and other organisms with point centromeres canonically contain one kinetochore-microtubule complex per centromere [59, 60], and organisms with larger kinetochores, such as fission yeast, have multiple iterations of essentially the same kinetochore-microtubule complex [59]. There are some kinetochore proteins found specifically in organisms such as *S. cerevisiae* with point centromeres (e.g., Ndc10) and others found specifically in organisms such as *S. pombe* and humans with regional centromeres (e.g., CENP-H/Fta3) [12], but overall the sub-complexes that comprise the kinetochore are conserved in composition and architecture.

Recently, tethering experiments with kinetochore proteins have defined the minimal requirements for centromere function, elucidating multiple mechanisms that can result in kinetochore assembly and, by implication, neocentromere formation. Interestingly, these requirements appear to differ in different organisms. For example, in *S. cerevisiae*, tethering Ask1, a member of the fungal-specific Dam1 complex, forms a synthetic kinetochore and improves plasmid segregation [61, 62]. In the *Xenopus* egg extract system, CENP-A nucleosomes recruit other kinetochore proteins, resulting in kinetochore-microtubule formation [63]. In fact, the 6 C-terminal amino acids of CENP-A when fused to the rest of conventional H3, are sufficient to bind CENP-C and to recruit the other kinetochore components [63], suggesting that CENP-C may be sufficient to initiate *Xenopus* kinetochore assembly. Similarly, the *Drosophila* CENP-A protein, CID, is sufficient for kinetochore formation when CID is tethered to LacO operator sequences and thereby incorporated (together with other histone proteins) into previously non-centromeric chromatin [64].

In humans, overexpression of CENP-A alone is not sufficient to form complete kinetochores [65], although implications of this negative result are unclear. Interestingly, several different routes to kinetochore assembly in human cells have been demonstrated using elegant tethering experiments to induce synthetic kinetochores. First, alpha-satellite DNA, presumably via binding of CENP-B can recruit kinetochores to human artificial chromosomes (HACs) [66]. However, HAC formation is inefficient, occurring only in a small number of cell lines [67]. Second, tethering of HJURP, a CENP-A chaperone initially identified as a Holiday Junction Recognition Protein, to DNA promotes CENP-A incorporation and the specification of centromeric chromatin in human cells [68]. Third, tethering both CENP-C and CENP-T to the same DNA nucleates kinetochore-microtubule attachments and partially rescues an acentric chromosome [65]. Such tethering experiments provide insights into the order of assembly events and the dependencies involved. For example, tethering of both CENP-C and CENP-T bypasses the requirement for CENP-A, supporting the idea that a normal function of CENP-A is to recruit CENP-C and CENP-T, which together direct kinetochore assembly. Accordingly, such experiments may reveal the proteins most important for neocentromere formation and tethering or engineering the levels of critical kinetochore components may facilitate the efficient production of human artificial chromosomes.

Kinetochore complex number

Centromere flexibility also manifests through variation in the amount of CENP-A bound per centromere and in the number of kinetochore-microtubule protein complexes assembled per centromere DNA region, defined here as the kinetochore complex number. Kinetochore complex number varies greatly on evolutionary time scales. Kinetochore complex number

can also change rapidly as a physiological response to alterations in the centromere DNA or levels of kinetochore proteins.

The amount of CENP-A bound to the centromere DNA defines the region with the potential to assemble kinetochore complexes and to bind microtubules. Centromere regions in most organisms contain a mix of CENP-A and histone H3 nucleosomes [69]. Even organisms with small point centromeres, may contain a mix of CENP-A and H3 as recent evidence suggests that there may be 2–4 CENP-A nucleosomes per centromere in *S. cerevisiae* [70, 71], although this is controversial as there appears to be one major CENP-A nucleosome [72, 73], and other CENP-A nucleosomes may be randomly interspersed with H3 nucleosomes in the surrounding region [70].

In humans, CENP-A binds to alpha-satellite tracts, the length of which varies between chromosomes and between individuals. The amount of CENP-A bound to centromere DNA also changes in proportion to alpha-satellite tract length: longer repeats are associated with more CENP-A (Fig. 1a) [18]. Intriguingly, CENP-A and kinetochore protein levels are higher in cancer cells than in non-transformed somatic cells [10, 74, 75]. The amount of CENP-A bound to centromere DNA increases with increasing cellular CENP-A levels, generated either by transformation of a tissue culture cell line with oncogenes or by direct overproduction of CENP-A (Fig. 1b) [18]. Importantly, the CENP-A binding remains restricted to the alpha-satellite repeat tract.

In fungi, overproduction of CENP-A also alters kinetochore complex number, including increased numbers of kinetochore proteins and increased numbers of spindle microtubules at *C. albicans* centromeres (Fig. 1c) [76, 77]. Here, too, the larger kinetochore remains limited to within a core region of centromere DNA [76]. In *C. albicans*, as in fission yeast [59, 78], upon CENP-A overexpression [76], H3 binding to the centromere region decreases, suggesting that extra CENP-A nucleosomes can displace canonical nucleosomes. Because experiments with *C. albicans* CENP-A overexpression were performed on populations of cells [76], whereas experiments with human CENP-A overexpression analyzed individual cells [18], it is likely that the proportion of centromere DNA bound by CENP-A relative to H3-containing nucleosomes increases upon overproduction of CENP-A in humans as well. Flexibility in the precise positioning of CENP-A nucleosomes in individual cells would result in an increase in CENP-A binding per basepair in the population and a resulting increase in the number of kinetochore-microtubule attachments with potential accompanying changes to chromosome segregation.

Surprisingly, in humans and in *C. albicans*, cells engineered to express excess CENP-A recruit extra kinetochore complex proteins per centromere, yet in both cases chromosome segregation appears to be normal [18, 76]. Thus, the number of kinetochore complexes, and presumably microtubules bound, per centromere does not have an obvious effect on kinetochore function. However, the requirement for other kinetochore and cell cycle components may be altered by changes in kinetochore complex number. For example, increasing the kinetochore complex number from ~1 per centromere to >1 per centromere in *C. albicans* reduces the requirement for the Dam1 complex [76], consistent with the role of the Dam1 complex as a processivity factor for attachment of kinetochores to microtubules (Fig. 2a). Overexpression of CENP-A in transformed cancer cells may be a driving force for aneuploidy. Retinoblastoma protein (pRb)-depleted cells have elevated CENP-A, altered expression of many spindle checkpoint proteins, and high levels of aneuploidy. Interestingly, reduction of CENP-A to normal levels decreased the severity of mitotic defects in pRb-depleted cells [75]. Thus, altering CENP-A levels in combination with conditions permissive for cancer may have a profound effect on chromosome stability.

In organisms with multiple kinetochore-microtubule attachments per centromere, decreases in kinetochore complex number are also observed. Human kinetochores can function with just a small fraction of the normal CENP-A bound [79, 80]. Thus, human cells with multiple kinetochore-microtubule attachments per centromere have a buffering mechanism that permits chromosome segregation even when CENP-A levels are decreased. Interestingly, decreased levels of kinetochore proteins cause different responses in different cell types. For example, depletion of CENP-A protein in fibroblasts eliminates kinetochore function, while the same knockdown permits cell cycle progression in human pluripotent stem cells, presumably because they have stored CENP-A mRNA, further protecting them from CENP-A depletion [81].

An open question is whether increased kinetochore complex number alters the requirement for checkpoint pathways. In *C. albicans*, the reduced requirement for the Dam1 complex in cells with larger kinetochores suggests that multiple microtubule attachments per kinetochore increase the probability of a chromosome successfully attaching to a spindle pole. However, multiple microtubules per centromere also could increase the probability of merotelic attachments, where a single centromere attaches to both poles potentially causing chromosome missegregation. Accordingly, error-correction pathway components, such as Aurora-B-like kinases may have increased importance in cells with increased kinetochore complex number (Fig. 2b). Future studies combining altered levels of CENP-A and other kinetochore proteins should reveal the interactions between mechanisms regulating the number of kinetochore-microtubule attachments and checkpoint pathways.

Concluding remarks

We propose that centromere DNA, kinetochore position, and kinetochore complex number are more flexible than previously appreciated, because adaptation requires that chromosome segregation fidelity be maintained despite changes in cell physiology that alter kinetochore protein levels or disrupt native centromere DNA. The contribution of DNA sequence, if any, to regional kinetochore function is likely to become clearer as larger numbers of individuals are analyzed via high-throughput sequencing and as tools for the analysis of repetitive DNA emerge. In addition, model organisms with regional centromere sequences that are readily manipulated will reveal possible relationships between centromere sequence flexibility and kinetochore function [47, 76, 82].

DNA sequences or structures that support kinetochore assembly may also become evident as more neocentromeres are generated in model organisms. Identifying the underlying sequences of additional neocentromeres in clinical samples [38, 39], coupled with the development of techniques to manipulate human tissue culture cells to form neocentromeres, will provide a more complete picture of the mechanisms by which neocentromeres form. For example, overexpression of CENP-A alone is not sufficient to form ectopic centromeres in human cells when the native centromere is present [83]. However, whether extra CENP-A or other kinetochore proteins facilitate neocentromere formation in the absence of tethering remains to be determined.

Kinetochore complex number flexibility appears to be a common feature shared across diverse species. For example, when CENP-A is overproduced, kinetochore complex number increases in fungi and humans [18, 76]. Changes in kinetochore protein levels are associated with cancer cells [10, 74], which are particularly prone to alterations in kinetochore position [36] and to increased kinetochore complex number [18]. Whether chromosomes with altered kinetochore positions and/or complex numbers are more prone to segregation errors or are more dependent on specific checkpoint pathways also remains to be determined. Thus,

larger kinetochores, combined with secondary stresses that increase the numbers of merotelic attachments, may contribute to chromosome instability in cancer cells.

The study of centromeres and kinetochores is advancing very rapidly, and models of structure and function are evolving much more quickly than the centromeres themselves. Initial mechanistic insights were based on studies of point centromeres, which have specific sequence requirements and were thought to adhere to a rigid structural model; yet recently, the composition, number and position of CENP-A-associated nucleosomes in *S. cerevisiae* has become controversial [70, 71, 73, 84, 85]. Flexibility in centromere sequence, kinetochore position and complex number is emerging as an inherent property of centromeres in virtually all eukaryotes. Thus, future models will need to account for this remarkable flexibility. The next few years will likely reveal many insights into mechanisms that segregate chromosomes under a range of conditions, including physiological and genetic stresses.

Acknowledgments

We apologize to authors whose work we did not have room to cite due to space limitations. We thank Berman lab members for helpful discussions and Simon Chan, Barbara Mellone, and Beth A. Sullivan for very useful comments on the manuscript. This work is supported by a Ruth L. Kirschstein NRSA Fellowship F32 AI800742 and a Postdoctoral Fellowship, Grant #PF-12-108-01-CCG from the American Cancer Society to L.S.B. and by NIH/NIAID AI075096 to J.B.

Glossary

Centromere	chromosomal DNA region where kinetochore proteins bind and assemble a functional kinetochore
CENP-A	histone 3 variant that replaces canonical H3 at centromeres (also known as CenH3, Cse4 in budding yeast, Cnp1 in fission yeast and CID in <i>Drosophila</i>)
Evolutionary new centromere	a DNA region not previously used as a centromere where a kinetochore has assembled and is maintained in the population
Kinetochore	a large complex of proteins, many of them conserved throughout eukaryotes, that assembles on centromere DNA to attach the chromosome to the microtubule during mitosis
Kinetochore complex number	the number of kinetochore protein complexes assembled per centromere DNA region as measured by the amount of a kinetochore protein, such as Ndc80, bound per centromere. For the purposes of this review, kinetochore complex number describes conditions in which the stoichiometry of kinetochore proteins per centromere is altered. In several experimental systems, the number of kinetochore protein complexes is proportional to the number of kinetochore-microtubule attachments
Neocentromere	a centromere that forms at ectopic loci, frequently following disruption of the native centromere or separation of a chromosome fragment from the native centromere
Synthetic kinetochore	assembly of a kinetochore based on manipulations such as tethering of a specific kinetochore protein to a targeted chromosomal region. These experiments provide insight into mechanisms of <i>de novo</i> kinetochore assembly

References

1. Thompson SL, et al. Mechanisms of chromosomal instability. *Curr Biol.* 2010; 20:R285–295. [PubMed: 20334839]
2. Torres EM, et al. Identification of aneuploidy-tolerating mutations. *Cell.* 2010; 143:71–83. [PubMed: 20850176]
3. Williams BR, et al. Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells. *Science.* 2008; 322:703–709. [PubMed: 18974345]
4. Pavelka N, et al. Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast. *Nature.* 2010; 468:321–325. [PubMed: 20962780]
5. Rancati G, et al. Aneuploidy underlies rapid adaptive evolution of yeast cells deprived of a conserved cytokinesis motor. *Cell.* 2008; 135:879–893. [PubMed: 19041751]
6. Weaver BA, et al. Aneuploidy acts both oncogenically and as a tumor suppressor. *Cancer Cell.* 2007; 11:25–36. [PubMed: 17189716]
7. Selmecki A, et al. Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. *Science.* 2006; 313:367–370. [PubMed: 16857942]
8. Janssen A, et al. Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. *Science.* 2011; 333:1895–1898. [PubMed: 21960636]
9. Sheltzer JM, et al. Aneuploidy drives genomic instability in yeast. *Science.* 2011; 333:1026–1030. [PubMed: 21852501]
10. Tomonaga T, et al. Overexpression and mistargeting of centromere protein-A in human primary colorectal cancer. *Cancer Res.* 2003; 63:3511–3516. [PubMed: 12839935]
11. Allshire RC, Karpen GH. Epigenetic regulation of centromeric chromatin: old dogs, new tricks? *Nat Rev Genet.* 2008; 9:923–937. [PubMed: 19002142]
12. Meraldi P, et al. Phylogenetic and structural analysis of centromeric DNA and kinetochore proteins. *Genome Biol.* 2006; 7:R23. [PubMed: 16563186]
13. Black BE, Cleveland DW. Epigenetic centromere propagation and the nature of CENP-a nucleosomes. *Cell.* 2011; 144:471–479. [PubMed: 21335232]
14. Maddox PS, et al. Functional genomics identifies a Myb domain-containing protein family required for assembly of CENP-A chromatin. *J Cell Biol.* 2007; 176:757–763. [PubMed: 17339379]
15. Shang WH, et al. Chickens possess centromeres with both extended tandem repeats and short non-tandem-repetitive sequences. *Genome Res.* 2010; 20:1219–1228. [PubMed: 20534883]
16. Nasuda S, et al. Stable barley chromosomes without centromeric repeats. *Proc Natl Acad Sci U S A.* 2005; 102:9842–9847. [PubMed: 15998740]
17. Black BE, et al. Centromere identity maintained by nucleosomes assembled with histone H3 containing the CENP-A targeting domain. *Molecular cell.* 2007; 25:309–322. [PubMed: 17244537]
18. Sullivan LL, et al. Genomic size of CENP-A domain is proportional to total alpha satellite array size at human centromeres and expands in cancer cells. *Chromosome Res.* 2011; 19:457–470. [PubMed: 21484447]
19. Nagaki K, et al. Sequencing of a rice centromere uncovers active genes. *Nat Genet.* 2004; 36:138–145. [PubMed: 14716315]
20. Ventura M, et al. Evolutionary formation of new centromeres in macaque. *Science.* 2007; 316:243–246. [PubMed: 17431171]
21. Alkan C, et al. Genome-wide characterization of centromeric satellites from multiple mammalian genomes. *Genome Res.* 2011; 21:137–145. [PubMed: 21081712]
22. Sanyal K, et al. Centromeric DNA sequences in the pathogenic yeast *Candida albicans* are all different and unique. *Proc Natl Acad Sci U S A.* 2004; 101:11374–11379. [PubMed: 15272074]
23. Padmanabhan S, et al. Rapid evolution of Cse4p-rich centromeric DNA sequences in closely related pathogenic yeasts, *Candida albicans* and *Candida dubliniensis*. *Proc Natl Acad Sci U S A.* 2008; 105:19797–19802. [PubMed: 19060206]

24. Shi J, et al. Widespread gene conversion in centromere cores. *PLoS Biol.* 2010; 8:e1000327. [PubMed: 20231874]
25. Pertile MD, et al. Rapid evolution of mouse Y centromere repeat DNA belies recent sequence stability. *Genome Res.* 2009; 19:2202–2213. [PubMed: 19737860]
26. Fishman L, Saunders A. Centromere-associated female meiotic drive entails male fitness costs in monkeyflowers. *Science.* 2008; 322:1559–1562. [PubMed: 19056989]
27. Bensasson D, et al. Rapid evolution of yeast centromeres in the absence of drive. *Genetics.* 2008; 178:2161–2167. [PubMed: 18430941]
28. Capozzi O, et al. Evolutionary descent of a human chromosome 6 neocentromere: a jump back to 17 million years ago. *Genome Res.* 2009; 19:778–784. [PubMed: 19411601]
29. Lomiento M, et al. Evolutionary-new centromeres preferentially emerge within gene deserts. *Genome Biol.* 2008; 9:R173. [PubMed: 19087244]
30. Rocchi M, et al. Centromere repositioning in mammals. *Heredity.* 2012; 108:59–67. [PubMed: 22045381]
31. Han Y, et al. Centromere repositioning in cucurbit species: implication of the genomic impact from centromere activation and inactivation. *Proc Natl Acad Sci U S A.* 2009; 106:14937–14941. [PubMed: 19706458]
32. Marshall OJ, et al. Neocentromeres: new insights into centromere structure, disease development, and karyotype evolution. *Am J Hum Genet.* 2008; 82:261–282. [PubMed: 18252209]
33. Tyler-Smith C, et al. Transmission of a fully functional human neocentromere through three generations. *Am J Hum Genet.* 1999; 64:1440–1444. [PubMed: 10205277]
34. Blom E, et al. A case of angioimmunoblastic T-cell non-Hodgkin lymphoma with a neocentric inv dup(1). *Cancer Genet Cytogenet.* 2010; 202:38–42. [PubMed: 20804919]
35. de Figueiredo AF, et al. A case of childhood acute myeloid leukemia AML (M5) with a neocentric chromosome neo(1)(qter-->q23 approximately 24::q23 approximately 24-->q43-->neo-->q43-->qter) and tetrasomy of chromosomes 8 and 21. *Cancer Genet Cytogenet.* 2009; 193:123–126. [PubMed: 19665076]
36. Sirvent N, et al. Characterization of centromere alterations in liposarcomas. *Genes Chromosomes Cancer.* 2000; 29:117–129. [PubMed: 10959091]
37. Liehr T, et al. First case of a neocentromere formation in an otherwise normal chromosome 7. *Cytogenet Genome Res.* 2010; 128:189–191. [PubMed: 20029167]
38. Hasson D, et al. Formation of novel CENP-A domains on tandem repetitive DNA and across chromosome breakpoints on human chromosome 8q21 neocentromeres. *Chromosoma.* 2011
39. Alonso A, et al. A paucity of heterochromatin at functional human neocentromeres. *Epigenetics Chromatin.* 2010; 3:6. [PubMed: 20210998]
40. Alonso A, et al. Genomic microarray analysis reveals distinct locations for the CENP-A binding domains in three human chromosome 13q32 neocentromeres. *Hum Mol Genet.* 2003; 12:2711–2721. [PubMed: 12928482]
41. Chueh AC, et al. LINE retrotransposon RNA is an essential structural and functional epigenetic component of a core neocentromeric chromatin. *PLoS Genet.* 2009; 5:e1000354. [PubMed: 19180186]
42. Bassett EA, et al. Epigenetic centromere specification directs aurora B accumulation but is insufficient to efficiently correct mitotic errors. *J Cell Biol.* 2010; 190:177–185. [PubMed: 20643881]
43. Maggert KA, Karpen GH. The activation of a neocentromere in *Drosophila* requires proximity to an endogenous centromere. *Genetics.* 2001; 158:1615–1628. [PubMed: 11514450]
44. Olszak AM, et al. Heterochromatin boundaries are hotspots for *de novo* kinetochore formation. *Nat Cell Biol.* 2011; 13:799–808. [PubMed: 21685892]
45. Heun P, et al. Mislocalization of the *Drosophila* centromere-specific histone CID promotes formation of functional ectopic kinetochores. *Dev Cell.* 2006; 10:303–315. [PubMed: 16516834]
46. Ishii K, et al. Heterochromatin integrity affects chromosome reorganization after centromere dysfunction. *Science.* 2008; 321:1088–1091. [PubMed: 18719285]

47. Ketel C, et al. Neocentromeres form efficiently at multiple possible loci in *Candida albicans*. PLoS Genet. 2009; 5:e1000400. [PubMed: 19266018]
48. Topp CN, et al. Identification of a maize neocentromere in an oat-maize addition line. Cytogenet Genome Res. 2009; 124:228–238. [PubMed: 19556776]
49. Mroczek RJ, et al. The maize Ab10 meiotic drive system maps to supernumerary sequences in a large complex haplotype. Genetics. 2006; 174:145–154. [PubMed: 16849609]
50. Dawe RK, Cande WZ. Induction of centromeric activity in maize by suppressor of meiotic drive 1. Proc Natl Acad Sci U S A. 1996; 93:8512–8517. [PubMed: 8710901]
51. Koren A, et al. Epigenetically-inherited centromere and neocentromere DNA replicates earliest in S-phase. PLoS Genet. 2010; 6:e1001068. [PubMed: 20808889]
52. Sernova NV, Gelfand MS. Identification of replication origins in prokaryotic genomes. Brief Bioinform. 2008; 9:376–391. [PubMed: 18660512]
53. Eckert CA, et al. The enhancement of pericentromeric cohesin association by conserved kinetochore components promotes high-fidelity chromosome segregation and is sensitive to microtubule-based tension. Genes Dev. 2007; 21:278–291. [PubMed: 17242156]
54. Stimpson KM, et al. Telomere disruption results in non-random formation of de novo dicentric chromosomes involving acrocentric human chromosomes. PLoS Genet. 2010; 6.
55. MacKinnon RN, Campbell LJ. The Role of Dicentric Chromosome Formation and Secondary Centromere Deletion in the Evolution of Myeloid Malignancy. Genet Res International. 2011;2011.
56. Amor DJ, et al. Human centromere repositioning “in progress”. Proc Natl Acad Sci U S A. 2004; 101:6542–6547. [PubMed: 15084747]
57. Luo MC, et al. Genome comparisons reveal a dominant mechanism of chromosome number reduction in grasses and accelerated genome evolution in Triticeae. Proc Natl Acad Sci U S A. 2009; 106:15780–15785. [PubMed: 19717446]
58. Gao Z, et al. Inactivation of a centromere during the formation of a translocation in maize. Chromosome Res. 2011; 19:755–761. [PubMed: 21947957]
59. Joglekar AP, et al. Molecular architecture of the kinetochore-microtubule attachment site is conserved between point and regional centromeres. J Cell Biol. 2008; 181:587–594. [PubMed: 18474626]
60. Winey M, et al. Three-dimensional ultrastructural analysis of the *Saccharomyces cerevisiae* mitotic spindle. J Cell Biol. 1995; 129:1601–1615. [PubMed: 7790357]
61. Lacefield S, et al. Recruiting a microtubule-binding complex to DNA directs chromosome segregation in budding yeast. Nat Cell Biol. 2009; 11:1116–1120. [PubMed: 19684576]
62. Kiermaier E, et al. A Dam1-based artificial kinetochore is sufficient to promote chromosome segregation in budding yeast. Nat Cell Biol. 2009; 11:1109–1115. [PubMed: 19684577]
63. Guse A, et al. In vitro centromere and kinetochore assembly on defined chromatin templates. Nature. 2011; 477:354–358. [PubMed: 21874020]
64. Mendiburo MJ, et al. *Drosophila* CENH3 is sufficient for centromere formation. Science. 2011; 334:686–690. [PubMed: 22053052]
65. Gascoigne KE, et al. Induced Ectopic Kinetochore Assembly Bypasses the Requirement for CENP-A Nucleosomes. Cell. 2011; 145:410–422. [PubMed: 21529714]
66. Okada T, et al. CENP-B controls centromere formation depending on the chromatin context. Cell. 2007; 131:1287–1300. [PubMed: 18160038]
67. Mandegar MA, et al. Functional human artificial chromosomes are generated and stably maintained in human embryonic stem cells. Hum Mol Genet. 2011; 20:2905–2913. [PubMed: 21593218]
68. Barnhart MC, et al. HJURP is a CENP-A chromatin assembly factor sufficient to form a functional de novo kinetochore. J Cell Biol. 2011; 194:229–243. [PubMed: 21768289]
69. Blower MD, et al. Conserved organization of centromeric chromatin in flies and humans. Dev Cell. 2002; 2:319–330. [PubMed: 11879637]
70. Lawrimore J, et al. Point centromeres contain more than a single centromere-specific Cse4 (CENP-A) nucleosome. J Cell Biol. 2011; 195:573–582. [PubMed: 22084307]

71. Coffman VC, et al. CENP-A exceeds microtubule attachment sites in centromere clusters of both budding and fission yeast. *J Cell Biol.* 2011; 195:563–572. [PubMed: 22084306]
72. Henikoff S, Henikoff JG. ‘Point’ Centromeres of *Saccharomyces* Harbor Single CenH3 Nucleosomes. *Genetics.* 2012
73. Camahort R, et al. Cse4 is part of an octameric nucleosome in budding yeast. *Mol Cell.* 2009; 35:794–805. [PubMed: 19782029]
74. Tomonaga T, et al. Centromere protein H is up-regulated in primary human colorectal cancer and its overexpression induces aneuploidy. *Cancer Res.* 2005; 65:4683–4689. [PubMed: 15930286]
75. Amato A, et al. CENPA overexpression promotes genome instability in pRb-depleted human cells. *Mol Cancer.* 2009; 8:119. [PubMed: 20003272]
76. Burrack LS, et al. The requirement for the Dam1 complex is dependent upon the number of kinetochore proteins and microtubules. *Curr Biol.* 2011; 21:889–896. [PubMed: 21549601]
77. Roy B, et al. CaMtw1, a member of the evolutionarily conserved Mis12 kinetochore protein family, is required for efficient inner kinetochore assembly in the pathogenic yeast *Candida albicans*. *Mol Microbiol.* 2011; 80:14–32. [PubMed: 21276093]
78. Castillo AG, et al. Plasticity of fission yeast CENP-A chromatin driven by relative levels of histone H3 and H4. *PLoS Genet.* 2007; 3:e121. [PubMed: 17677001]
79. Liu ST, et al. Mapping the assembly pathways that specify formation of the trilaminar kinetochore plates in human cells. *J Cell Biol.* 2006; 175:41–53. [PubMed: 17030981]
80. Bergmann JH, et al. Epigenetic engineering shows H3K4me2 is required for HJURP targeting and CENP-A assembly on a synthetic human kinetochore. *EMBO J.* 2011; 30:328–340. [PubMed: 21157429]
81. Ambartsumyan G, et al. Centromere protein A dynamics in human pluripotent stem cell self-renewal, differentiation and DNA damage. *Hum Mol Genet.* 2010; 19:3970–3982. [PubMed: 20650959]
82. Kagansky A, et al. Synthetic heterochromatin bypasses RNAi and centromeric repeats to establish functional centromeres. *Science.* 2009; 324:1716–1719. [PubMed: 19556509]
83. Van Hooser AA, et al. Specification of kinetochore-forming chromatin by the histone H3 variant CENP-A. *J Cell Sci.* 2001; 114:3529–3542. [PubMed: 11682612]
84. Mizuguchi G, et al. Nonhistone Scm3 and histones CenH3-H4 assemble the core of centromere-specific nucleosomes. *Cell.* 2007; 129:1153–1164. [PubMed: 17574026]
85. Dalal Y, et al. Structure, dynamics, and evolution of centromeric nucleosomes. *Proc Natl Acad Sci U S A.* 2007; 104:15974–15981. [PubMed: 17893333]
86. Smith KM, et al. Heterochromatin is required for normal distribution of *Neurospora crassa* CenH3. *Mol Cell Biol.* 2011; 31:2528–2542. [PubMed: 21505064]
87. Sun X, et al. Sequence analysis of a functional *Drosophila* centromere. *Genome Res.* 2003; 13:182–194. [PubMed: 12566396]
88. Edwards NS, Murray AW. Identification of xenopus CENP-A and an associated centromeric DNA repeat. *Mol Biol Cell.* 2005; 16:1800–1810. [PubMed: 15673610]
89. Johnston K, et al. Vertebrate kinetochore protein architecture: protein copy number. *J Cell Biol.* 2010; 189:937–943. [PubMed: 20548100]
90. McEwen BF, et al. CENP-E is essential for reliable bioriented spindle attachment, but chromosome alignment can be achieved via redundant mechanisms in mammalian cells. *Mol Biol Cell.* 2001; 12:2776–2789. [PubMed: 11553716]

Fig. 1. Determinants of kinetochore complex number in humans and fungi
CENP-A, a histone 3 variant that replaces canonical H3 at centromeres, is at or near the top of the kinetochore assembly hierarchy, and the amount of CENP-A bound to the centromere helps to determine the number of kinetochore protein complexes assembled at each centromere. (a) The length of centromere DNA determines the number of kinetochore protein complexes per centromere as the amount of CENP-A bound is proportional to alpha satellite array length in humans. (b) The expression level of kinetochore proteins also determines the kinetochore complex number as CENP-A binding increases with overexpression of *CENP-A* in humans. (c) Similarly, in *C. albicans*, overproduction of CENP-A results in increased kinetochore proteins and spindle microtubules bound to centromere DNA. Adapted from [76].

Fig. 2. Consequences of alterations in kinetochore complex number

Changes in the number of kinetochore protein complexes may have multiple effects on chromosome segregation. (a) Increased numbers of kinetochore protein complexes results in an increased probability of maintaining attachment between kinetochores and microtubules. Therefore, increased kinetochore proteins and spindle microtubules in *C. albicans* reduce the requirement for Dam1 complex members, fungal-specific proteins with roles in increasing the processivity of kinetochore-microtubule interactions. Republished from [76]. (b) Increased kinetochore complex number might increase the probability of merotelic attachments (attachments to both spindle poles). Therefore, increases in the kinetochore complex number may alter the requirements for error-correction mechanisms.

Table 1

Diversity of centromere length and structure

Organism	Centromere core CENP-A binding region	# kinetochore-microtubule attachments	Centromere inheritance	Centromere structure	Reference
<i>S. cerevisiae</i> (budding yeast)	~125 bp	1	DNA sequence (point)	3 part defined DNA sequence	[59, 60, 70, 71]
<i>C. albicans</i> (pathogenic yeast)	~3–5 kb	1	Epigenetic (regional)	Unique DNA sequences at each centromere	[22, 47]
<i>S. pombe</i> (fission yeast)	~4–7 kb	2–3	Epigenetic (regional)	Unique central core, flanked by inner and outer repeats	[59]
<i>N. crassa</i> (filamentous fungus)	~150–300 kb	ND	Epigenetic (regional)	Heterogeneous, AT-rich repeats	[86]
<i>D. melanogaster</i> (fruit fly)	~500 kb	ND	Epigenetic (regional)	Complex sequence islands in simple repeats	[87]
<i>C. elegans</i> (nematode)	Whole chromosome	ND	Epigenetic (regional)	Kinetochores not restricted to single region	[14]
<i>X. laevis</i> (frog)	ND	ND	Epigenetic (regional)	Arrays of repeats	[88]
<i>G. gallus</i> (chicken)	~30–500 kb	4–5	Epigenetic (regional)	Mix of repeats and unique sequences	[15, 89]
<i>O. sativa</i> (rice)	~0.75 – 2 Mb	ND	Epigenetic (regional)	Arrays of repeats, interspersed with active genes	[19]
<i>H. sapiens</i> (human)	~0.5–10 Mb	15–20	Epigenetic (regional)	Arrays of alpha-satellite repeats	[17, 18, 90]

ND – Not determined

Table 2

Summary of neocentromere formation in diverse organisms

Organism	Method to induce neocentromere formation	Positions where neocentromeres formed	Reference
<i>S. pombe</i>	Inducible deletion of centromere	Pericentric region, adjacent to telomere	[46]
<i>D. melanogaster</i>	γ -irradiation-induced chromosome breakage, overproduction of CENP-A	Pericentric, boundaries of heterochromatin and euchromatin	[43–45]
<i>C. albicans</i>	Deletion of native centromere	Pericentric, multiple locations on chromosome arms	[47]
Plants (barley, maize)	Inactivation of native centromere, chromosome rearrangements	Multiple locations on chromosome arms	[16, 48]
Humans	Inactivation of native centromere, chromosome rearrangements	Multiple locations on chromosome arms	[32]