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Centromere dynamics

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At the foundation of all eukaryotic kinetochores is a unique histone variant, known as CenH3 (centromere histone H3). We are starting to identify the histone chaperones responsible for CenH3 deposition at centromere DNA, and the mechanisms that restrict CenH3 from chromosome arms. The specialized nucleosome that contains CenH3 in place of canonical histone H3 lies at the interface between microtubules and chromosomes and directs kinetochore protein assembly. By contrast, pericentric chromatin is highly elastic and can stretch or recoil in response to microtubule shortening or growth in mitosis. The variety in histone modification is likely to play a key role in regulating the behavior of these distinct chromatin domains.

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Introduction

During mitosis, the eukaryotic cell constructs a bipolar array of microtubules that serves as the machinery to segregate duplicated chromosomes. The centromere on each sister chromatid specifies the assembly of the kinetochore, a DNA–protein complex that interacts with the plus-ends of kinetochore microtubules. The kinetochore is assembled in chromatin with a centromere-specific histone H3 variant. This histone H3 variant, CenH3, is deposited at all eukaryote centromeres studied to date (CENP-A in mammalian cells, Cse4 in *Saccharomyces cerevisiae*, Cnp1 [also known as Sim2] in *Schizosaccharomyces pombe*, CID in *Drosophila melanogaster*, and in HCP-3 *Caenorhabditis elegans*). CenH3 is unique in its timing of deposition [1], post-translational regulation [2] and thermodynamic stability [3]. In addition, this nucleosome and flanking chromatin (pericentric chromatin) are subject to forces from spindle microtubules in mitosis, as evidenced by the separation of kinetochores and elasticity of pericentric chromatin. This review explores recent studies that extend our understanding of how the site of CenH3 is specified, how the pericentric chromatin is organized and how this chromatin resists mitotic forces.

Centromere DNA and the ubiquitous centromere histone H3 variant

Centromere DNA is specified by 125 bp in *S. cerevisiae* and *Kluyveromyces lactis*, 3–4kb in *Candida albicans*, 40–60kb in *S. pombe* and 1–4 Mb in mammalian cells. The 125 bp centromeres are referred to as point centromeres, versus regional centromeres that are characteristic of most other eukaryotes [4]. Interestingly, all yeasts with point centromeres have a CenH3 END (essential N-terminal domain) homology in the N terminus [5•]. Centromere DNA does not cross species boundaries, even when sequences from species with similar organization, such as *S. cerevisiae* and *K. lactis*, are swapped [6]. Thus, unlike many genetic control elements, centromere DNA is highly species-specific.

All centromeres are characterized by the incorporation of a unique centromeric histone, CenH3. CenH3 is related to Histone H3 but is not a typical histone variant. Unlike histone variants that are conserved throughout their length relative to the canonical histone, CenH3 is only 50% identical to histone H3 over the shared histone-fold domain (HFD; ~100 amino acids) [7]. At the N terminus, there is a divergent helix (N-helix; ~50aa) that in budding yeasts (*Saccharomyces* and *Kluyveromyces*) is essential for function (i.e. END). END contributes to site-specific deposition unique to *S. cerevisiae*. The other distinguishing hallmark is that loop 1, flanked by two α-helices in the HFD, is considerably longer [5•,7]. Loop 1 directly contacts the nucleosomal DNA, a feature that might facilitate binding specificity between CenH3 and centromere DNA. However, there is no recognizable sequence or secondary structure that reveals the centromere- or species-specificity of CenH3. An alternative approach to gain insight into CenH3 function employs homology modeling and molecular dynamics simulation [8•]. The highly charged N-terminal tails in *S. cerevisiae* are predicted to be largely unstructured random coils that cluster to the exit and entry sites of the nucleosome. The conservation of the END domain among *Saccharomyces* and *Kluyveromyces* might be indicative of a key role for this motif in the organization of point centromeres. The CenH3-containing nucleosome has been shown to make more a compact tetramer with Histone H4, relative to H3 and H4 tetramers [9]. These structural aspects might contribute to the DNA binding specificity and/or thermodynamic stability of the centromere.

CenH3 specificity (or lack thereof)

Like centromere DNA, CenH3 for the most part does not cross species boundaries. In a detailed domain-swapping analysis, Baker and Rogers [5•] found that only CenH3 from highly related yeasts were able to functionally complement in a heterologous environment. By contrast, it has been reported that Cse4 from budding yeast complements RNAi-depleted cell cycle arrest of human cells [10]. Whether the latter study reflects bona fide complementation and incorporation of Cse4 into human centromere, or that Cse4 can bind residual CENP-A in the depleted cells, remains to be seen.

If budding yeast CenH3 can recognize CENP-A and/or mammalian CEN DNA and incorporate into a human centromere, then what is the specificity determinant? There is no indication that histones or their variants exhibit strict DNA sequence specificity. There are sequence-based cues for nucleosome positioning [11••] that might predispose the shape of centromere DNA that contributes to centromere DNA binding specificity. Remarkably, CenH3 is not restricted to centromere loci in budding yeast. 2 μ circle is a parasitic DNA invader of budding yeast (~6 kb), with a unique origin of replication and recombination mechanism that functions to maintain a steady state of about 60 copies per cell [12]. There is no centromere DNA on 2 μ circle, yet Cse4 binds and moreover directs 2 μ to the mitotic spindle [13,14••]. An alternative hypothesis that the centromere adopts a unique shape and/or structure that is conserved throughout phylogeny (see below). This structure could be the basis for epigenetic specification of centromere. A study from the pathogenic yeast reveals that pre-existing centromeres remain functional, but if they are isolated as naked DNA and reintroduced back into the cell, functional centromeres do not form [15••]. These and other studies might force us to consider that three-dimensional architecture dictated by specific chromatin configurations contributes to pattern recognition in the nucleus, and these patterns are heritable. In this regard, it is noteworthy that different genetic requirements are necessary for the propagation of established versus *de novo* kinetochores [16].

Histone variants in pericentric chromatin

The packaging of pericentric chromatin is another important determinant of centromere function. This chromatin is populated with 'conventional' histone variants and/or specific histone modifications. In budding yeast, the histone H2A variant Htz1 is recruited to pericentric chromatin by the Swr1 chromatin remodeling complex [17]. Histone H3K4me2 (dimethylation at lysine 4) is interspersed with CENP-A in human satellite chromatin [18]. Methylated K9H3 is required for centromere silencing in *S. pombe* [19], and mutations in conserved non-helical regions of histone H2B result in decreased CenH3 binding and loss of segregation and silencing function [20•]. These modifications might be important for either recruiting non-histone kinetochore proteins or marking the centromere for the next cycle. Alternatively, they might contribute to the physical stability of centromere. Using a competitive nucleosome reconstitution assay with chicken erythrocyte histone, Mattei *et al.* [3] evaluated the thermodynamic stability of *Kluyveromyces* centromeric chromatin. They found that centromeric nucleosomes are more stable than bulk chromatin. The finding that centromere chromatin was more stable in a heterologous reconstitution assay is quite remarkable. What is the stability of this chromatin with native histone modifications and variants inserted instead? In addition, Mattei *et al.* [3] found multiple isoenergetic positions with respect to the nucleosome dyad axis. Perhaps the mobility of the H3 nucleosome at the centromere aids in the transition to the specialized nucleosome before mitosis (see below).

Centromere histone dynamics

Core histones are typically dispersed to daughter strands during replication. By contrast, CenH3 is completely replaced in budding yeast [21] or inherited semi-conservatively in *Drosophila* and human [1,22]. These diverse strategies might reflect how the centromere is identified. In organisms with a sequence-specific centromere DNA sequence (e.g. *Saccharomyces*), CenH3 is completely replaced at each cell division [21]. CenH3 deposition and complete replacement might be dependent upon the unique END domain found in *Saccharomyces* and *Kluyveromyces*. By contrast, in organisms in which centromere identity is inherited epigenetically, CenH3 remains bound to the daughter strands, thereby marking the domain for histone replacement later in the cell cycle. New CenH3 is loaded in G_2 in a replication-independent mechanism [1,22,23•]. The question becomes how are these strategies are executed, and what is the mechanism that directs new CenH3 to the centromere? It is likely that histone chaperones are involved in this process. In budding yeast, chromatin assembly factors Cac1 and Hir1 contribute to centromere function [24]. In vertebrates, an even larger complex of proteins, denoted Nucleosome-associated complex (NAC), can be affinity purified by tandem affinity purification [25•].

Recent studies using GFP-tagged histones and fluorescence recovery after photobleaching have revealed that histones are indeed dynamic [26•,27••]. Thus, the turnover of histones and potential histone variants is unlikely to be unique to the centromere. The mechanism of assembly, however, could be different from that reported for histone H3 and H3.3 variants [28]. Furuyama *et al.* [29••] have recently reconstituted CenH3 assembly with just one chaperone, RpAp48. It remains to be seen whether the key feature for centromere assembly is exclusion of canonical assembly factors, histone replacement, or recognition of a unique architecture.

The levels of CenH3 must be critically controlled. Limiting CenH3 can compromise centromere function, whereas excess CenH3 can lead to centromere formation at ectopic sites and chromosome catastrophes [30•]. In budding yeast and *Drosophila*, CenH3 is regulated by ubiquitin-proteasome-mediated proteolysis [2,31•]. Using a genetic screen to identify the key regulatory sites, Collins *et al.* [2] found that a single dominant–lethal mutant containing 14 amino acid mutations was required for Cse4 to escape the proteolysis machinery. Thus, another function of the kinetochore is to protect Cse4 from protein degradation.

Centromere elasticity

Sister kinetochores can be separated by several microns when attached to opposite spindle poles in a variety of cell types. Analysis in live cells revealed that sister centromeres and/or kinetochores in budding yeast are also separated before anaphase. Repeated arrays of the lac operator (*Escherichia coli lacO*) inserted into the yeast genome can be visualized upon introduction of Lac repressor fused to GFP [32]. Placement of the *lacO* array at varying distance from the centromere revealed that chromosome arms were closely apposed, whereas about 10 kb of pericentric chromatin is stretched pole-ward in mitosis, before anaphase onset [33–36]. Sister centromeres on a single chromosome oscillate relative to each other, and are often separated by distances of up to 800 nm [36]. The oscillation separation distance suggests that the pericentromeric regions of the chromosome are elastic, stretching in response to their dynamic kinetochore microtubule attachments. Pearson *et al.* [21] also marked centromeres of all chromosomes with the centromeric histone H3 variant Cse4, fused to GFP. Cse4–GFP at sister kinetochores is organized into two lobes on either side of the equator of the metaphase spindle. This bipolar alignment is indicative of sister centromere separation before anaphase. Subsequent visualization of several kinetochore proteins and examination of their behavior after photobleaching [21] has substantiated the finding that sister centromeres are pulled apart by sister kinetochore pulling forces in metaphase. What is the basis for centromere elasticity and what is the structure of chromatin as it extends and contracts in metaphase?

Cohesin

The physical linkage of sister chromatids is the mechanism for generation of tension between sister centromeres. This linkage is mediated by a multisubunit complex cohesin, composed of two members of the Smc (structural maintenance of chromosomes) family of ATPases, Smc1 and Smc3, and two non-Smc subunits, Mcd1 (also know as Scc1) and Scc3 [37•,38•]. Cohesin is associated with chromosomes from G_1 until the onset of anaphase. Cohesin promotes association between sister chromatids (intermolecular linkage) and is the basis for tension when sister chromatids are oriented to opposite spindle-pole bodies. Scc1 is cleaved by separase upon anaphase onset and disappears from chromosomes when sisters separate at the metaphase–anaphase transition. The key experiment demonstrating that loss of cohesin is sufficient for sister chromatid separation was artificial cleavage of a modified form of Scc1 by a foreign protease (TEV; tobacco etch virus) [39]. Activation of TEV protease promotes sister chromatid separation in budding yeast when arrested in metaphase. The discovery of cohesin dispelled the view that sister chromatids might be held by intercatenation of sister DNAs that was resolved at anaphase owing to microtubule pulling forces.

Cohesins can form ring-shaped structures *in vitro*, leading to several hypotheses that describe how these proteins connect sister chromatids [37•,38•]. These include the embrace model, in which the complex forms a ring around sister DNA helices, the snap model, in which each cohesin complex binds a single DNA helix and linkage occurs through the association of two complexes, and the bracelet model, in which cohesin complexes oligomerize to wrap around sister DNA helices. In each of these models, it is clear that transient dissociation of the hinge domain is crucial for their function [40••].

Genome-wide chromatin immunoprecipitation (ChIP) in budding yeast has revealed the predominant sites of cohesin binding [41,42]. Most notable is the finding that cohesin is enriched approximately threefold in a 20–50 kb domain flanking the centromere, relative to the concentration of cohesin on chromosome arms. The kinetochore (in yeast and Metazoa) functions as an enhancer of cohesin binding [41]. Although the location of cohesin along the length of the entire yeast chromosome has been established, little is known about how the concentration of cohesin within pericentric chromatin contributes to the fidelity of chromosome segregation. The accumulation and function of cohesin within the stretched pericentric chromatin reveals a major paradox in the field. What is the structure of cohesin at sites of separated sister centromeres, and how does cohesin respond to changes in centromere separation?

Behavior of chromatin under tension

The dynamics of microtubule growth and shortening have been well described. By contrast, we know little about chromatin dynamics that underlie kinetochore motility. There are several potential sources of chromatin extension and contraction. One is the elastic stretch of chromatin as described above. Alternatively, microtubule pulling forces could promote nucleosome release within the pericentric chromatin. Release of one nucleosome results in a 65 nm extension (from nucleosomal to B-form DNA). Loss of 20 nucleosomes at the centromere would increase sister centromere separation by ~650 nm. Based on estimates from centromere DNA dynamics in live cells, one can deduce that about 20 kb of DNA is separated at the centromere in metaphase [36]. This translates to ~100 nucleosomes (20 000/200bp of nucleosomal +

linker DNA). Loss of ~20% of the nucleosomes is enough to provide the full dynamic range of 800 nm average separation observed in live cells. There is sufficient force in the spindle to promote nucleosome release. Most of the biophysical studies on the strength of the nucleosome have come from *in vitro* reconstitution studies and deduce that 20pN of force will displace histones from DNA [43•,44]. This is within the measured forces that a single microtubule can generate [45,46]. A recent study indicates that at constant force and long measurement time, 2–3pN can favor nucleosome release [47•]. This nucleosome release model predicts the existence of a protein complex between separated centromeres that limits the amount of DNA under tension. Interestingly, loss of centromere elasticity has been found in cells lacking the centromere-associated protein Slk19 [48•]. Slk19 physically associates with the Scc1 subunit of cohesin and localizes between separated centromeres.

Conclusions and prospects

CenH3 provides a stable platform for building the kinetochore. Centromeric chromatin is thermodynamically more stable than bulk nucleosomes, a feature that is likely to contribute to kinetochore stability under load. By contrast, nucleosomes flanking the centromere can be constantly released and reassembled in response to mitotic forces, providing a mechanism for chromatin extensibility in mitosis.

One of the major goals for the future will be to determine the role of force in generating tension between sister chromatids, and the source of compliance and elasticity within the spindle, and to identify the macromolecules, including chromatin, that move and change shape in response to mechanical force. The fundamental conserved segregation unit might be the budding yeast attachment site (the kinetochore) that links pericentric chromatin to a single microtubule. This could be the unit of chromosome segregation that is conserved from budding yeast to mammalian cells. The chromosome segregation apparatus with microtubules under compression or tension and chromatin as an extensible element provides a rationale solution to the major paradoxes in the field, and offers significant insights into the function and evolution of mitotic mechanisms.

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