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Down the Rabbit Hole of Centromere Assembly and Dynamics

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Abstract

The centromere is perhaps the most iconic feature on a eukaryotic chromosome. An amateur enthusiast equipped with a light microscope can easily identify the center of each metacentric chromosome, marking the spot responsible for accurate genome segregation. This review will highlight findings which provide novel insights into how centromeres are assembled and disassembled, the role centromeric proteins play in repair, epigenetic features uniquely found at the centromere, and the three dimensional organization of centromeres caught in the act of mitosis. These advances have unveiled a veritable wonderland of non-canonical features that drive centromere function.

Introduction

In eukaryotes, centromeres are organized into three discrete strata. The inner most unit is the centromeric chromatin, which serves as the platform to recruit inner and outer kinetochore proteins, which in turn facilitate binding to spindle microtubules at mitosis [1]. The centromeric chromatin is composed of AT-rich centromeric DNA repeats, packaged by specialized nucleosomes containing a centromere-specific histone H3 variant (CENH3). CENH3 homologs include CENP-A in humans, Cse4/Cnp1 in yeast, CENH3 in *Arabidopsis*, Cid in flies, and HCP-3 in worms [2] (Table 1). CENH3 depletion results in mitotic arrest, and overexpressed CENH3s can seed centromeres at ectopic locations [3–5]. Thus, it is thought that key inner kinetochore proteins recognize some features of centromeric chromatin. However, CENH3s are only ~50% conserved [6], and CENH3 chromatin domains vary widely, ranging from a single centromeric nucleosome per chromosome in budding yeast, to large arrays of CENH3/H2A nucleosomes alternating with arrays of H3/H2A.Z nucleosomes in metazoan chromosomes.

Despite the diversity noted above, centromere organization across most species is similar (Table 1), and centromere ultra-structure probably derives from a common ancestral identity. Swap experiments support the view that the *leitmotif* lies within the CENH3 nucleosome itself. When introduced into fly cells, yeast Cse4, human CENP-A, and worm HCP-3 localize to *Drosophila* centromeres [7]; Cse4 can localize to and functionally complement human CENP-A depletion [8]; residues of CENH3 swapped into H3 allow the chimera to localize to centromeres [9–10] and, avian CENP-A can preferentially associate with mouse 120bp centromeric repeats [11]. Consequently, studies dissecting CENH3 assembly, localization, and structure are of considerable interest to achieve a unitary model for centromere function [12–13].

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Assembly of CENH3 nucleosomes

Chaperones guiding CENH3 assembly to centromeres have been pursued for over a decade. The prevalent model posits that specific complexes target CENH3 precisely to the centromere [14], while others have argued that CENH3 can occupy gaps in chromatin [15]. The earliest experimental indication of how CENH3 assembles into chromatin comes from genetic dissections in yeast. Deletions of two proteins, Mis16 and Mis18, results in loss of centromeric features and mis-segregation [16]. Mis18 occupies centromeric locations ahead of newly incorporated Cse4 [16], and recruits the fungal protein Scm3, which associates with Sim3 to facilitate Cse4 incorporation [17]. Synthetic hyper-acetylation rescues Mis18 deletion [18], suggesting that Mis18 may drive acetylation and mobilization of H3 nucleosomes, providing gaps for CENH3 assembly. In worms, a Mis18 binding protein, KLN-2, escorts HCP-3 to chromatin, indicating a conserved role for Mis18 in holocentric centromeres [19]. Similarly, in fly cells, a Mis16 homolog, RbAp48 co-purifies with the pre-nucleosomal Cid/H4 complex. RbAp48 is found in all H3 assembly complexes, and in vitro, chaperones Cid/H2A/H2B/H4 into chromatin [20]. Thus, its identification as a Cid chaperone supports non-specific CENH3 assembly.

A prediction from the gap-filling model is that excess CENH3 should enrich in regions of high nucleosome turnover. Results from a seminal study in budding yeast suggest this may be the case. Chromatin immunoprecipitation followed by deep sequencing (ChIPSeq) has revealed that in addition to 16 centromeric locations, Cse4 occupies 132 promoters of highly expressed genes [21]. How do kinetochore proteins distinguish between Cse4 at centromeric versus ectopic locations? In yeast, the key yeast inner kinetochore complex, CBF3, binds directly to centromeric DNA, and is thus anchored to a single location [22]. Lack of kinetochore specificity in metazoans may explain why these organisms restrict CENH3 expression to late G2, and incorporation to M/early G1 [23–24], where M-phase specific chromosome compaction serves as a natural barrier to CENH3 mis-incorporation. However, G2/M timing of CENH3 expression/incorporation leads to dilution of CENH3 during DNA replication. Equal inheritance of centromere location would then require old CENH3 to be divided equally between daughter chromatids (Figure 1). Another problem is that during early M phase, unincorporated CENH3 could diffuse away. How is this prevented? Using a genome-wide RNAi screen in fly cells, researchers determined that Cal-1 co-localizes with CENH3 at interphase and mitosis [25]. When Cal-1 is depleted, new CENH3 is unable to deposit. Conversely, Cal-1 binding is disrupted if CENH3 or CENP-C is depleted, suggesting that Cal-1 itself requires a pre-existing platform of centromeric chromatin. Cal-1 may serve as a mitosis-specific bridge between centromeric chromatin and soluble CENH3/H4/RbAp48 complexes, pointing to an attractive convergence of gap-filling and targeting mechanisms.

An exception to the mechanisms of CENH3 deposition discussed above comes from plants, which deposit CENH3 during G2 [26], leaving open the potential for mis-incorporation within euchromatin. Therefore, it is likely that plants have evolved additional epigenetic mechanisms to restrict CENH3 assembly [27]. As discussed later, one such trick might be to couple DNA methylation to inner kinetochore assembly.

A key tenet of the targeted assembly model requires a specific complex to deliver CenH3 to centromeres exclusively. Three recent studies have identified a CENP-A-specific assembly chaperone that satisfies the first requirement [28–30]. CENP-A pre-assembly complexes were found to contain one copy of histones H4 and CENP-A (endogenous and ectopic CENP-A do not mix in this complex), **H**olliday **J**unction **R**ecognition **P**rotein (HJURP), **N**ucleophosmin (NPM), and the histone chaperone RbAp48. One of the groups also identified H2a and H2b within CENP-A- and H3- assembly complexes [30], leading to the tantalizing possibility that histones deposit as half-nucleosomal intermediates.

HJURP satisfies the requirement for specificity because GST-fusion assays show that it can specifically discriminate loop 1/helix 2 regions of CENP-A, previously identified as the CATD region, necessary for CENP-A localization to centromeres. Reciprocal experiments show that ectopically expressed HJURP associates with endogenous CENP-A and H4 from human cells. HJURP co-localizes with GFP-CENP-A when the latter is incorporated into the centromere. Concurrently, HJURP depletion results in loss of GFP-CENP-A from the centromere [28]. These studies support the view that HJURP is indeed a CENP-A-specific chaperone.

A notable finding is that 80 amino acids within HJURP's N-terminus are necessary for its association with CENP-A [30]. This is an exciting discovery because HJURP and the fungal protein Scm3 contain evolutionarily conserved TYTL motifs spanning this region [31]. Scm3 is required for Cse4 assembly and retention on yeast centromeric DNA [17,32–34], and in the absence of deep sequence homology, Cse4 can functionally rescue CENP-A depletion in human cells [8]. Furthermore, HJURP is present in all mammals and Scm3 is present in all fungi, implying similar CENH3 assembly pathways exist in evolutionary distant groups.

Paradoxically, HJURP also localizes to Holliday junctions at breakpoints across the human genome [35], which contradicts the second requirement for targeted assembly: exclusive location. Indeed, GFP-CENP-A localizes to sequence-specific double strand breaks (DSB), presumably through its association with HJURP [36]. HJURP guiding CENP-A to both centromeric and ectopic locations presents an obvious conundrum. How are metazoan centromere domains distinguished from ectopic CENH3 nucleosomes? A speculative hypothesis is that there are two modes of CENH3 assembly, resulting in two types of CENH3 nucleosomes. One CENH3 form predominates at centromeres, reinforced by key kinetochore partners, while the other is an outcome of promiscuous assembly in euchromatin. The latter mode would have self-imposed constraints, such as blocking domains recognized by kinetochore proteins.

Bi-stability in CENH3 nucleosome structure

Evidence suggests that the CENH3 nucleosome can exist in stable non-canonical forms (Figure 2). In *S. cerevisiae*, the non-histone protein Scm3 associates with the Cse4/H4 nucleosome via Cse4's CATD region [37], and in vitro, Scm3 substitutes one copy of H2A/H2B in the Cse4 octamer, resulting in a hexameric nucleosome [33]. However, Cse4/H4 also co-purifies with H2A/H2B from CEN DNA [38], thus it is unclear if Scm3/Cse4 is mutually exclusive with H2A/H2B in vivo. In *S. pombe*, Cnp1 co-purifies with reduced amounts of H2A, H2B, and its association with Scm3 appears cell-cycle dependent [17,32], and with the Swi/Snf2h chromatin remodeling complex [39]. In contrast to in vitro, where fly Cid easily salt-assembles into cross-linkable octamers, native Cid nucleosomes in *Drosophila* cells co-IP with equal amounts of H2A, H2B and H4, cross-link as tetramers, and have half the height of canonical octamers [40]. In-solution recognition imaging by atomic force microscopy pinpoints a single N-terminal epitope per native Cid nucleosome [41], supporting the concept of "hemisome" tetramers [42].

Arguing against non-canonical models, a recent study reports that while Scm3 deletions are lethal as previously documented [33,37], over-expressed Cse4 rescues Scm3 deletions, and, Cse4 co-IPs with H2A, H2B, and H4, leading the authors to conclude that Cse4 makes conventional nucleosomes [34]. However, it should be noted that rescued yeast cells have mitotic defects, suggesting Scm3-depleted Cse4 nucleosomes are compromised in function. The study also maps Cse4 lethal mutations in vivo, most of which impact Cse4:DNA and Cse4:H4 interactions, and concurrently, fail to assemble in vitro as well. One critical mutation targets His207, required for CENH3:CENH3 dimer interface in octamers [43]. Concurrently, this mutant yields <10% octamers in vitro. In sharp disagreement, the same His207 mutant co-

IPs efficiently with another tagged Cse4 molecule from whole cell extracts. Further, a mutation in Leu220, which does not affect reconstitution efficiency *in vitro*, inhibits co-IPs *in vivo*. One possibility is that co-IPs reflect non-nucleosomal Cse4 interactions. Alternatively, salt-mediated *in vitro* assembly may not adequately reflect CENH3 behavior at centromeres. Indeed, a recent study has reported that salt-mediated Cse4 octamers cannot occupy centromere-like DNA, whereas Cse4 assembled by Scm3 localizes to centromere-like DNA exclusively [44].

What is the biological significance of structural duality in Cse4 nucleosomes? Genome-wide Cse4 occupancy data reveal that Cse4 is present at centromeric DNA and at euchromatic locations [21]. Thus, an exciting possibility is that a distinctive CENH3 structural form specifically marks centric loci. Evidence in support of this idea comes from analyzing CENH3 nucleosomal topology *in vitro* and *in vivo*. Using RbAp48-mediated assembly, fly CENH3/H2A/H2B/H4 wrap 120bp of DNA, forming stable nucleosomes with a normal twist [20]. However, the topological state of DNA in these CENH3 nucleosomes is unique- instead of 2 left handed turns, they wrap DNA in a single right-handed super-helix [45]. The relevance of *in vitro* topological non-conformity was tested directly in yeast, using mini-plasmids with 0, 1, or 2 copies of the yeast 120bp centromeric DNA known to recruit Cse4. Remarkably, echoing an earlier finding [46], Cse4 nucleosomes on these circles wrap DNA in the right-handed topological state ***in vivo***. Importantly, the topological changes are quantized: for every 0, 1, or 2 Cse4 nucleosomes added to the circle, precisely 0, 1, or 2 linking number changes in topology occur. Genetic deletions rule out mitotic torque and other kinetochore proteins involvement in the altered topology. Because right-handed wrapping is structurally incompatible with canonical octamers, previously described CENH3 tetramers provide a sound structural basis for the topology [47] (Figure 2).

Yeast and fly CENH3s are 70% dissimilar in sequence, and derive from organisms whose last common ancestor lies at the root of the eukaryotic tree. Common structural motifs within CENH3 nucleosomes, such as topology, probably originate from a shared ancestry, and should persist in other species. Indirect evidence comes from studies in human cells, where CENP-A occupies 80% AT-rich satellites, and co-IPs with H2A, H2B and H4 [48]. A recent human study has found that two distinct populations of CENP-A nucleosomes exist: a salt-sensitive and mitosis-capable form during G2/M; and, a salt-resistant RSF-associated form that prevails during mid-G1 [49]. In *Drosophila*, the chromatin remodeler RSF substitutes the fly H2A variant into nucleosomes [50], which could strongly influence nucleosome stability [51]. Consequently, dependent on location, chromatin remodelers could drive CENH3 to pair with different H2A variants to modulate stability, and kinetochore binding.

Kinetochore protein recognition of CENH3 chromatin

An ongoing quest in centromere biology is to uncover how kinetochore proteins recognize centromeric regions. Most members of the centromere-associated network (CAN) proteins have been identified [52–54]. Key CAN members such as CENP-C have homologs across all species, and participate with CENH3 to create the distinctive centromeric domain (Table 1). Concurrently, CENP-C genetic deletions are lethal. CENP-C co-localizes with CENH3 in both metazoan centromeres [55], and at ectopic locations [3,56,57].

How does CENP-C recognize CENH3 chromatin? CENP-C has three domains of significant homology across eukaryotes. Of these, the Mif2/CENP-C domain binds human alpha satellite DNA directly, and intriguing evidence from bi-fluorescence complementation (biFC) experiments suggests that the same domain interacts with the C-terminus of CENP-A [58]. However, biochemical experiments show that CENP-A/CENP-C co-IPs yield smeary DNA ladders ranging from 100bp-1000bp, [59–60], not single nucleosomes. In extensively

nucleasedigested chromatin, CENP-C instead co-purifies with H3, which may have been deposited at centromeres during replication because of CENH3 dilution [60]. A potential concern is that intrinsic instability in the CENP-A nucleosome [61] could be exacerbated during some chromatin preparation procedures [62], resulting in kinetochore protein re-localization to H3 chromatin.

To investigate the reversible nature of centromere establishment, researchers created an alpha-satellite based human artificial chromosome, whose centromere can be synthetically inactivated [56]. Surprisingly, they observed rapid loss of CENP-C, even when CENP-A-domains are intact. Centromere re-establishment was also examined in maize, wherein an inactive smaller second centromere was reactivated by excision [27]. This system reveals that CENP-C is weakly loaded during late anaphase at reactivated centromeres, in the absence of any known epigenetic mark. Thus, CENP-C recognizes multiple epigenetic features at centromeres. Indeed, the CENP-C domain which binds alpha satellite DNA also interacts with the DNA methyltransferase DNMT3b at metaphase [63], targeting de novo DNA methylation to flanking chromatin. Because CENH3 nucleosomes themselves are anti-correlated with DNA methylation in *A. thaliana* and *Z. mays* [64], DNA methylation likely targets pericentric heterochromatin. Concurrently, when CENP-C or DNMT3b are depleted, pericentric and centric transcription increases, even though CENP-A amounts are constant [65]. Concurrently, in fission yeast, CENP-C depletion results in loss of H2A.Z restriction, and increased transcription at centromeres, also without affecting Cnp-1 [66–67]. Studies in plants and fission yeast indicate that CENP-C is involved in chromosome orientation at mitosis and meiosis as well [68]. Thus, this fascinating protein plays myriad roles in regional centromeres.

Another key mammalian inner kinetochore protein, CENP-N, immuno-precipitates with CENP-A nucleosomes. Consequently, CENP-A depletion alters CENP-N localization. In vitro, CENP-N directly recognizes CENP-A nucleosomes through the latter's CATD domain [69]. In conventional CENP-A octamers, the two CATD domains are supposed to be inaccessible [10] (Figure 3), making it difficult to envision two molecules of CENP-N binding CATD efficiently. However, CATD domains should be accessible in both non-canonical tetramer models (Figure 3). Thus, future experiments to dissect precisely which CENP-A domain is bound by CENP-N in vivo will be very informative.

Other kinetochore proteins like CENP-W and CENP-T, originally identified as part of the human CAN proteins, also disrupt CENH3 chromatin when depleted [54], but these two proteins associate with H3 and DNA, not CENP-A, [60]. Thus, their interactions present a new mechanism by which kinetochore proteins bind centromeres. Over 40 kinetochore proteins have been identified in multiple species [1]. Because of the large size of the kinetochore complex, and the surprising fragility of CENH3 domains, teasing apart function by biochemical or genetic approaches seems a Sisyphean endeavor. Two new methods, lifetime-FRET and biFC, offer the distinct advantage of coupling fluorescently tagged proteins with live imaging to track kinetochore dynamics in situ. Such experiments independently confirm interactions between CENP-A and CENP-B/C/N, support the view that CENP-W/T bind H3 not CENP-A [58,70–71], and unveil a new role for H1 at the centromere [72]. Thus, these kinds of studies will allow visualization of kinetochore protein binding to centromeric domains in situ.

Functional and structural dichotomy in pericentric domains

Over the past two years, pericentric heterochromatin has been found to be essential for centromere establishment [12]. A study in fission yeast has found that RNAi and pericentric repeats can be bypassed by tethering the histone methyltransferase Clr4 to create heterochromatin in an ectopic plasmid, which in turn, recruits CENH3/CENP-C [39]. Surprisingly, in fission yeast, native pericentric regions are also permissive to RNA polymerase

III, while centric regions contain both RNA polymerase II and the active chromatin mark, H3K4Me2 [73–74]. In the human X centromere, repressive histone modifications such as H3K9Me/K20Me/K27Me co-exist with permissive states like H3K4Me2, indicating a bivalent state [65], correlating with the finding that pericentric gamma satellite DNA has an open chromatin structure [75]. Open chromatin structure is also supported by the observation that the kinetochore complex CENP-H recruits the chromatin remodeler FACT (FAcilitates Chromatin Transcription), which in turn promotes CENP-A localization [52]. Several groups have also reported non-coding RNA originating from centromeres, whose role is currently under-appreciated [77–80]. Furthermore, open chromatin at centromeres has strong implications for centromere higher order structure.

3D view of the centromere fiber at interphase and mitosis

Scanning electron microscopy (SEM) studies of intact chromosomes show that parallel fibers encompass the primary constriction, leading to an intuitive model [91] where CENH3 domains are presented to the outside, while intervening H3 domains are buried within. However, recent immuno-SEM data from HeLa chromosomes suggest that CENH3 chromatin occupies $2/3^{\text{rd}}$ the length, but only $1/3^{\text{rd}}$ the height and width of adjacent compacted H3 domains. These data have been interpreted to support 30nm fibers at the centromere [81]. 30nm chromatin fibers have a controversial history, and recent cryo-EM studies of unfixed mitotic chromosomes have challenged their very existence [82–83]. Thus, while these latest results present a serious challenge in envisioning how the kinetochore interacts with a buried centromeric fiber, they await validation by native-imaging techniques.

In situ imaging advances like super high-resolution light microscopy uses point-spread functions to observe pairs of fluorescently tagged proteins, achieving ~20nm resolution in vivo [84–85]. Applying this revolutionary approach to mitotic chromosomes physically attached to microtubules, CENP-A and CENP-C were reported to be most compliant of 16 inner kinetochore proteins studied, while cohesins form a ring around the centric domain. These data suggest that CENP-A/CENP-C chromatin adopts a spring-like state encircled by cohesin (Figure 4). Another study has found that condensin regulates the spring constant of the human CENP-A fiber, and, in condensin mutants, weak centromeric fibers fail to facilitate mitosis [85]. Indeed, spring-like behavior in centromeres is readily apparent in immuno-SEM images of human mitotic chromosomes, which reveal an extended CENP-A array when the lowest concentration of fixative is used, and conversely, which collapse into a coiled state deep within the primary constriction when exposed to high concentration of fixative [81,87] (Figure 4).

Could features within individual CENH3 nucleosomes promote this intriguing spring-like behavior? Indeed, structural implications of arrays of right-handed tetramers are profound. Topological changes at the level of toroidal wrapping should also induce change in intervening linker DNA, thus disrupting the chromatin fiber's plectonemic packing. A speculative outcome of altered wrap of DNA driven by CENH3 tetramers is entire CENH3 domains springing out from the flanking quasi-crystalline H3 chromatin, with each pliable loop tethered by cohesins at its base (Figure 4). Together, these features may afford resilience to the CENH3 fiber, thus reducing chromatin breakage upon application of mitotic torque.

Future prospects

The data discussed above have exposed surprising adaptability in CENH3 chromatin, strongly dependent upon location, timing, transcription, and interacting partners. These factors may assist in demarcating CENH3 centric domains from ectopic CENH3 nucleosomes during mitosis. Biochemical experiments designed to uncouple these forms will provide much-needed insight into the very dynamic centromeric nucleosome. In vivo imaging approaches, combined with classical genetic analyses, will address outstanding questions such as how stepwise

centromeric assembly occurs, how remodeling events target centromeres in a cell-cycle dependent manner, and how these events reinforce variant occupancy. Deep sequencing technologies applied to regional centromeres will close the current genomic information gap to assess whether all CENH3s are indeed able to occupy two distinct nuclear domains.

New findings reviewed here might lead an observer, much like Alice in Wonderland [88], to exclaim that centromeres get “curiouser and curiouser”. However, an overarching theme that emerges is that centromeres, long cloaked in Bohemianism, are actually subject to same plebian forces shaping much of the genome’s epigenetic landscape. It will be exciting to decipher how CENH3 dynamic instability, in assembly and in structure, drive centromere function and dysfunction. While a singular theme that applies to all centromeres is not quite within our grasp, ongoing studies signal that escape from the black hole [90] is indisputably on the horizon.

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* Of special interest

** Of exceptional interest

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Figure 1.

A model for how HJURP/RbAp48-mediated CENH3 assembly is regulated by the cell cycle. Majority of CENH3 nucleosomes are in the tetrameric configuration during G2/M. At M phase, newly expressed CenH3 is weakly associated with centromeric chromatin. At early G1, HJURP/RbAp48 drive CENH3 incorporation into centromeres, and there is a greater propensity to equilibrate towards the octameric form. During replication, CENH3 nucleosomes split into the stable tetrameric configuration allowing equal segregation. Color Legend: magenta/dark blue: CenH3; orange/light blue: H2A; tan/terra cotta: H2B; grey/green: H4; red: DNA; green propeller: RbAp48.

Figure 2.

CENH3 structure intersects with dynamics at mitosis. Euchromatic CENH3 is depicted with DNA that wraps octameric nucleosomes ~1.7 times in a left-handed orientation. In *Drosophila* centromeric chromatin, tetramers of CENH3, H2A, H2B, and H4 wrap DNA in one right-handed turn. *Saccharomyces* spp. 'point' centromeres (boxed in grey) are depicted tetramers consisting of two each of CENH3 and H4 histones, wrapping DNA in one right-handed turn, and capped at either end with the nonhistone protein Scm3 (beige spheres). During mitosis, microtubules attach to centromeric chromatin via interactions with Ndc80 (brown), Cbf3, and Ndc10 (grey) in *Saccharomyces* spp. Similar dynamics are expected in other organisms. Color Legend: magenta/dark blue: CenH3; orange/light blue: H2A; yellow: H2B;

grey/green: H4; red: DNA. Structures were modeled in the program CN3D using the published crystallographic coordinates of the canonical nucleosome.

Figure 3.

Kinetochore access to CATD in CENH3 nucleosomal models. A hypothetical ~40 kD inner kinetochore protein (encapsulated in circle) has easy access to CENH3's exposed CATD (depicted in yellow) in the homo-tetrameric or hetero-tetrameric nucleosomal configuration. Color Legend: magenta/dark blue: CenH3; orange/light blue: H2A; tan/terra cotta: H2B; grey/green: H4; red: DNA.

Figure 4. Model for how centromeric chromatin behaves as a compliant spring. Left depicts tension-mediated spring behavior of centromeric domains which flex outward, tethered by cohesin. Middle depicts mitotic torque on the centromeric chromatin during chromosome movement. Right, collapse of the centric fiber upon microtubule loss, or cohesin/condensin depletion. Synthesized from [40,45,85,86,87].

Organization of Centromere Domains

Table 1

Point centromeres (budding yeast), Regional centromeres (fission yeast, insects, mammals, plants) and Holocentric centromeres (nematodes) have conserved organization. Homologs are indicated in italics. Ambiguity regarding function is denoted (?).

Organism	Centromeric DNA (>80% AT)	Centromere -specific histone H3	Assembly/Chaperone Complex	CENH3 Nucleosome Components	Inner Kinetochores Proteins	Outer Kinetochores Proteins	Chromatin Remodelers
<i>Saccharomyces cerevisiae</i>	120bp unique Centromere-Determining Element	CSE4	Mis16 (<i>RbAp48</i>) Mis18 Scm3	CSE4+H4 + Scm3; CSE4+H4 + H2AB	Mif2p (<i>CENP-C</i>) Ndc10/CBF3 CBF-1	KMN complex* Ctf19/COMA complex	Swi/Snf2 (<i>RSC</i>) GCN5p
<i>Schizosaccharomyces pombe</i>	1.5kb unique Central Core domain	CNP-1	Mis16 Mis18 Sim3 Scm3	CNP-1+H4 + Scm3; CNP-1+H4+H2AB	CNP-3 (<i>CENP-C</i>)	KMN complex	Swi/Snf2
<i>Drosophila melanogaster</i>	5bp repeats	CID	RbAp48	CID+H4+H2AB	CENP-C Cal-1 (?)	KMN complex	<i>unreported</i>
<i>Homo sapiens</i> <i>Note exceptions</i> i) <i>Neocentromeres</i> : no specific sequences ii) <i>Mouse</i> : 120bp minor B repeats	171bp α -satellite repeats	CENP-A	RbAp48 HJURP NPM	CENP-A+H4 +H2AB	CENP-C CENP-B CENP-N CENP-H	KMN complex CENP-E INCENP CENP-K CENP-M CENP-O CENP-T CENP-U CENP-V CENP-W	RSF FACT CHD1 Swi/Snf2B
<i>Arabidopsis thaliana</i> <i>Caenorhabditis elegans</i>	180bp repeats Holocentric: coats entire chromosome	CENH3 HCP-3	<i>unreported</i> KLN-2 (<i>Mis18BP</i>)	<i>unreported</i> <i>unreported</i>	CENP-C HCP-4 (<i>CENP-C</i>)	KMN complex KMN complex	<i>unreported</i> <i>unreported</i>

* KMN complex is KNL1/Spc105, Mtw1/Mis12 and Ndc80; see Refs [1,88]