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REVIEWS

Review

Kinetochore Function from the Bottom Up

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During a single human lifetime, nearly one quintillion chromosomes separate from their sisters and transit to their destinations in daughter cells. Unlike DNA replication, chromosome segregation has no template, and, unlike transcription, errors frequently lead to a total loss of cell viability. Rapid progress in recent years has shown how kinetochores enable faithful execution of this process by connecting chromosomal DNA to microtubules. These findings have transformed our idea of kinetochores from cytological features to immense molecular machines and now allow molecular interpretation of many long-appreciated kinetochore functions. In this review we trace kinetochore protein connectivity from chromosomal DNA to microtubules, relating new findings to important points of regulation and function.

Kinetochore Organization and the Generation of Force at the Centromere

Eukaryotic chromosome segregation, or the distribution of genetic material to progeny, is an astonishingly complex cellular task. Protein assemblies called kinetochores (see Glossary), which occupy chromosomal regions called centromeres and maintain connections between chromosomal DNA and **spindle microtubules**, are central to the completion of this task. In doing so they serve at least five functions required for faithful chromosome segregation: (i) they couple chromosome movement to microtubule dynamics; (ii) they monitor microtubule connections and respond appropriately, allowing incorrect attachments to reset and preventing anaphase until all connections are securely established; (iii) in most eukaryotes, kinetochores propagate during successive cell divisions through an epigenetic mechanism; (iv) although not the case for **budding yeast**, which make a single microtubule connection per chromatid, the kinetochores of most eukaryotes involve many such connections along a single chromatid, all of which must orient towards the same cell pole. In meiosis I, **co-orientation** also encompasses sister chromatids; and (v) kinetochores enhance the connection between sister chromatids, which counteracts until anaphase the pulling force exerted by microtubules. Phosphorylation regulates these varied functions by activating distinct kinetochore assembly states. Progress in understanding kinetochore architecture now allows us to consider the mechanisms that enable fulfillment of these five functions.

The mechanistic questions discussed here carry with them major implications for human health. Cancer cells display severe defects in chromosome segregation fidelity, and meiotic chromosome mis-segregation causes birth defects and infertility. Roughly one third of somatic cells display whole-chromosome imbalances in mice expressing a mutant allele of a kinetochore component (*BUB1B*$^{H/H}$) [1]. This cellular defect manifests at the organismal level as an elevated incidence of cancer, decreased fertility, and progeria [2]. Explanation of these defects requires a detailed understanding of how the kinetochore organizes and responds to cellular events during cell division.

Trends

Biochemical reconstitution of kinetochore activities has shown how a catch-bond connection is established and maintained, how kinetochore proteins assemble onto a CENP-A nucleosome template, and how individual subcomplexes come together to mediate centromere–microtubule connections.

Phosphoregulation of kinetochore architecture has begun to explain how microtubule attachment is regulated during the cell cycle.

Kinetochore mechanisms for establishing centromere cohesion, propagating centromere identity during cell divisions, and regulating DNA replication timing are now understood in molecular detail.

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An Overview of the Chromosome–Microtubule Connection

Fascination with the interface between chromosomes and the mitotic spindle dates to the late 19th century [3]. Recent research, enabled by decades of work to identify the molecules that make up these features, now called kinetochores, has focused on a conserved set of factors (schematic shown in Figure 1, Key Figure). The mammalian kinetochore is made from an array of kinetochore units, each built upon a single nucleosome-like particle. The budding yeast kinetochore consists of a single such unit (Figure 1). The core machinery is essentially identical in yeast and humans, and we discuss these organisms together, giving both names where appropriate. To provide a conceptual foundation for the kinetochore functions listed above, we trace the link between chromosomal DNA and microtubules, starting with the so-called '**inner kinetochore**' proteins that associate with centromeric DNA.

The anchor point of the **kinetochore** is a nucleosome defined by a histone H3 variant, CENP-A in humans and Cse4 in budding yeast (Figure 1, purple). In addition to deposition and removal factors [4–7], two kinetochore proteins, CENP-N/Chl4 and CENP-C/Mif2 (Figure 1, green), interact with the CENP-A/Cse4 histone fold domain [8,9]. Correspondingly, two unique features distinguish CENP-A/Cse4 from histone H3. One is a surface on the central helix of the CENP-A/Cse4 histone fold, called the CENP-A targeting domain (CATD), which is sufficient for interaction with its chaperone HJURP/Scm3 [5]. Formation of a histone octamer is incompatible with HJURP/Scm3 binding [10,11], suggesting that, although Scm3 remains associated with the kinetochore throughout the cell cycle [12], it maintains its localization by binding kinetochore proteins other than Cse4 [13]. CENP-N/Chl4 also contacts the CATD [9], presumably after eviction of the CENP-A/Cse4 chaperone, but the specific features of this interaction are not yet resolved (see Outstanding Questions). The second distinguishing feature of CENP-A/Cse4 is a cluster of hydrophobic residues near its C-terminus that interact with CENP-C/Mif2 [14]. Finally, an interaction between the yeast Ctf19 protein complex and the Cse4 N-terminal tail [15] suggests additional contact between inner kinetochore proteins and the CENP-A/Cse4 nucleosome, a possibility consistent with kinetochore assembly defects observed in **fission yeast** and human CENP-A/Cse4 N-terminal tail mutants [16,17].

CENP-C/Mif2 anchors the kinetochore by linking centromere-defining nucleosomes with distal kinetochore components [18–21]. All CENP-C/Mif2 homologs contain at least one CENP-C signature motif, and this interacts with the hydrophobic residues near the C-terminus of CENP-A [14]. CENP-C/Mif2 dimerization through a C-terminal cupin-fold domain [22] suggests that a single such dimer might assemble across the nucleosome dyad. While likely true at budding yeast centromeres, which have a single Cse4 nucleosome per chromatid [23], the arrangement could be more complex in organisms with multiple CENP-A nucleosomes per **centromere**. For example, the ratio of CENP-A to H3 in a reconstituted nucleosome array determines the efficiency of kinetochore formation in a *Xenopus laevis* egg extract system [24], hinting at the possibility that CENP-C crosslinks adjacent CENP-A particles. Regardless, targeting vertebrate CENP-C, which has tandem nucleosome recognition motifs [14], to a defined chromosomal locus drives kinetochore assembly in cells [25,26].

Three protein complexes assemble directly onto CENP-C/Mif2 [18,19,21,27–29]. The first of these, the MIND complex (Figure 1, grey), contains MIS12/Mtw1, PMF1/Nnf1, Nsl1, and Dsn1. The second, known as the COMA complex in budding yeast, contains CENP-P/Ctf19, CENP-Q/Okp1, CENP-O/Mcm21, and CENP-U/Ame1 [27]. The third, the Ctf3 complex in budding yeast, contains CENP-I/Ctf3, CENP-H/Mcm16, and CENP-K/Mcm22 [30]. MIND is the structural backbone of the kinetochore. The connection between CENP-C/Mif2 and MIND depends on an N-terminal fragment of CENP-C/Mif2 [20,21] and is the target of kinase regulation [31,32].

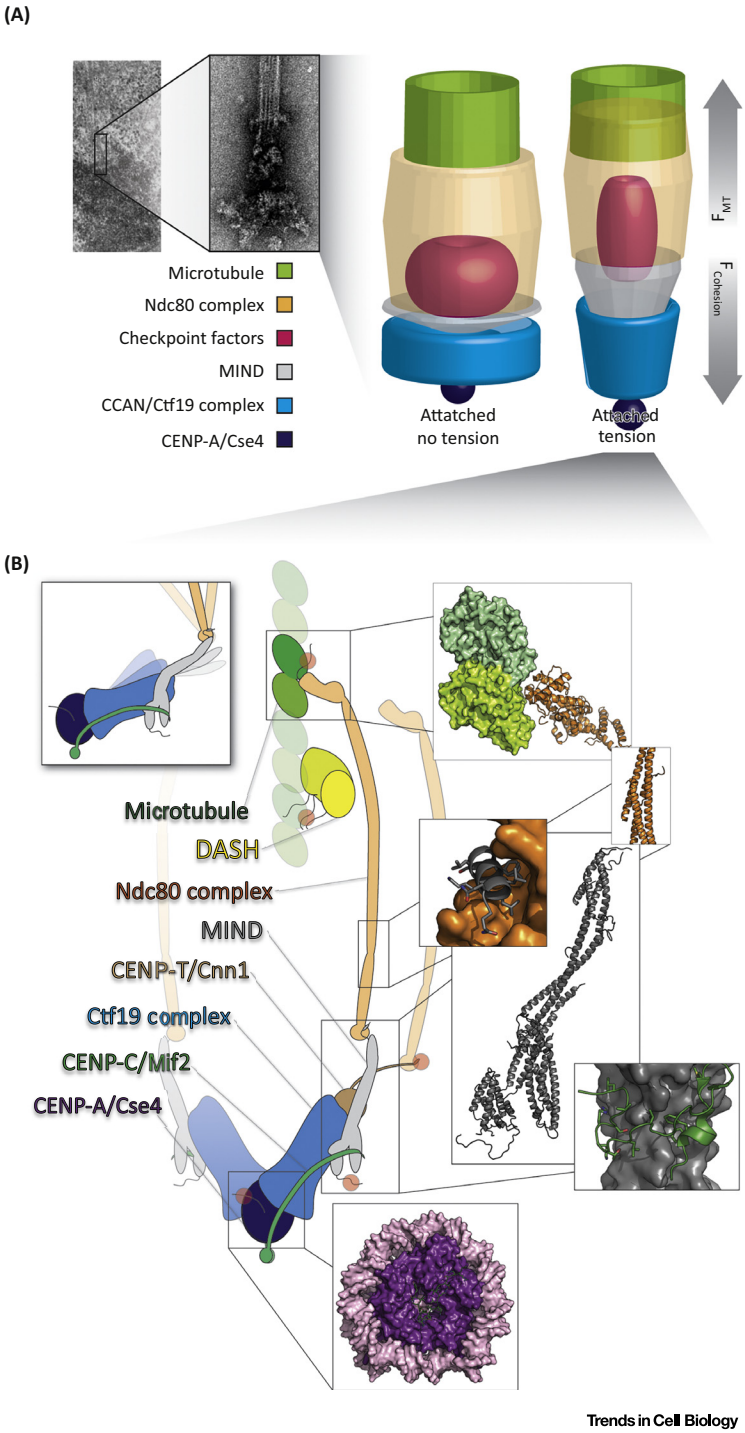
Glossary

Budding yeast/fission yeast: budding yeast is used here to refer to *Saccharomyces cerevisiae*, while fission yeast is used to refer to *Schizosaccharomyces pombe*. The two differ in the structure of their kinetochores: budding yeast have a single CENP-A/Cse4 nucleosome per chromatid, and fission yeast have several per chromatid.
Centromere: the DNA element on which a kinetochore assembles.
Co-/bi-orientation: when a pair of microtubule attachment points (typically distinct kinetochores) connects to spindle microtubules emanating from the same (co-orientation) or opposite (bi-orientation) cell pole(s).
Ctf19 complex/CCAN: group of conserved inner kinetochore proteins with shared and interdependent functions.
Inner kinetochore: a subgroup of kinetochore proteins located within ~30 nm of centromere DNA. Many of these proteins interact with DNA.
Kinetochore: a protein assembly that connects centromeric DNA to spindle microtubules and enables chromosome segregation.
Microtubule lattice: the side of a microtubule.
Microtubule plus end: the tip of a microtubule that faces the kinetochore during end-on attachment.
Outer kinetochore: a subgroup of kinetochore proteins that interacts with microtubules, either directly or indirectly. This group includes Ndc80 and Ska1, for example.
Sister chromatids: the pair of double-stranded DNA molecules generated after a round of DNA replication.
Spindle assembly checkpoint (SAC): a collection of factors and their associated activities that prevent mitotic cells from proceeding to anaphase until all kinetochores are properly attached to microtubules.
Spindle microtubules: protein filaments that grow from organizing centers at the cell poles and converge at the cell equator in mitosis, a subset of which connects to kinetochores.
Tension: a characteristic of kinetochore-spindle microtubule attachment when force exerted by microtubule depolymerization is balanced by force in the opposite

Key Figure

Model of a Single Kinetochore Unit

direction, typically due to biorientation of sister kinetochores on the mitotic spindle.



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Once installed at the kinetochore, MIND establishes microtubule contact by recruiting the conserved Ndc80 tetramer (Spc24, Spc25, Ndc80, and Nuf2; Figure 1, orange) [33]. Spc24 and Spc25 bind to a C-terminal peptide of Dsn1 and connect to the Ndc80 and Nuf2 proteins through a four-helix bundle that joins the extended coiled-coil regions of both dimers [28,34–37]. A calponin homology domain in Ndc80 and its flexible, N-terminal extension contact the **microtubule lattice** [38]. While the Ndc80 complex is sufficient to track depolymerizing microtubule tips *in vitro* [39], interactions between Ndc80, microtubule-associated proteins, and microtubules are required for the establishment and maintenance of microtubule attachment *in vivo* (Figure 2) [40–43].

Aside from Ndc80, the kinetochore–microtubule interface is surprisingly divergent among eukaryotes. In yeast the key feature is a 10-protein assembly called the DASH complex (Figure 1, yellow) [44] which oligomerizes to form a sliding clamp around a kinetochore microtubule [45,46]. Kinetochores initially contact the microtubule lattice (Figure 2, state 2), and only upon conversion of this connection to a so-called 'end-on' attachment, a multistep process that involves active transport along the microtubule, does the DASH complex become essential [47,48]. Vertebrates use the Ska complex, which evolved independently of DASH [49], to track depolymerizing microtubule ends [50,51]. Dependence on DASH in yeast may reflect reliance on a single microtubule per chromatid [52]. Indeed, increasing the number of kinetochore microtubules in *Candida albicans* relaxes the dependence of this organism on DASH proteins [53].

A second protein complex containing CENP-T/Cnn1 (Figure 1, tan) recruits Ndc80 to the kinetochore [54]. CENP-T/Cnn1 depends on its binding partners CENP-W/Wip1 and the CENP-I/Ctf3 complex for kinetochore recruitment [55,56]. An N-terminal extension of CENP-T/Cnn1 connects directly to Spc24/25, mimicking the Dsn1–Spc24/25 connection [34,57]. This extension, when artificially tethered to a minichromosome lacking a true centromere, enables the minichromosome to segregate on the mitotic spindle [56]. This and similar observations in human cells [25] pose the question: to what extent does CENP-T/Cnn1 represent a connection between DNA and microtubules that is both distinct from and functionally redundant with the CENP-C/Mif2-dependent connection? That *cnn1Δ* strains are viable while *mif2Δ* strains are not suggests that this is not, strictly speaking, the case in yeast (Table S1 in the supplemental information online). Do more complex centromeres fail to make sufficient microtubule connections in the absence of CENP-T? Are the remaining connections insufficiently buttressed, or is there a so far unappreciated function of CENP-T that makes it indispensable?

Regulation of Kinetochore Assembly and Function

Kinetochore structure is not monolithic but changes during the cell cycle to meet changing demands (e.g., [58,59]). Kinases regulate kinetochore assembly in response to the cell cycle

Figure 1. (A) (From left to right) Micrograph showing a vertebrate kinetochore, micrograph showing a single purified yeast kinetochore, and schematics showing single kinetochore units in the absence or presence of tension (labels below). Electron micrographs have been adapted from published sources [80,131]. Kinetochore features are not drawn to scale and are only intended to suggest overall architecture. (B) Schematic showing the connection between CENP-A and a microtubule. The inset at upper left suggests likely flexibility in the absence of tension. Kinetochore components are colored as in (A) with the exception that centromeric DNA is colored pink, CENP-C/Mif2 is green, and CENP-T/Cnn1 is tan. The hydrophobic C-terminal tail of CENP-A, which contacts CENP-C [8], is in the center of the histone octamer and is also colored pink. Only a cutout of the DASH ring is drawn (yellow). High-resolution structures were taken from published sources [14,36,57,132]. Red circles indicate kinase-regulated interfaces (Table S2) [57,61,62,66,69,70,133]. Observed competition between CENP-T and CENP-C for MIND interaction [68] is not shown. Abbreviations: F, force; MT, microtubule.

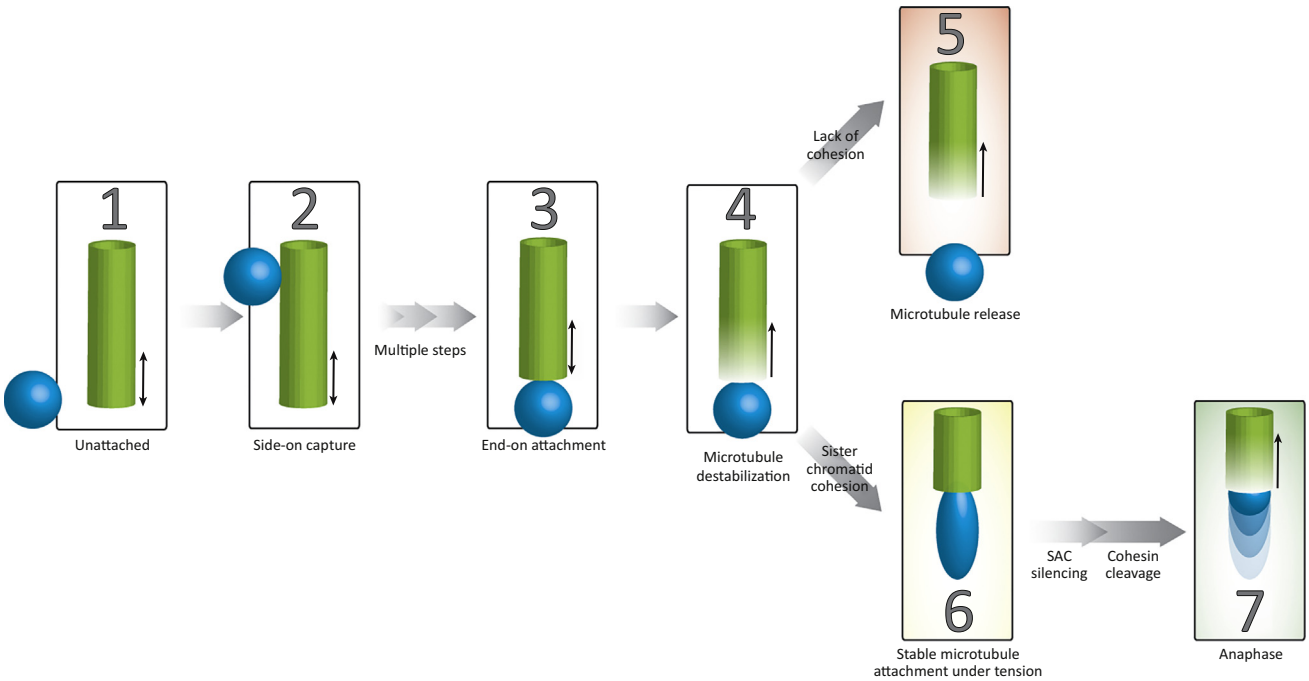

Figure 2. Diagram of Kinetochore Attachment States. Kinetochore capture converts unattached kinetochores (1) to side-on attachments (2). Side-on attachments must be converted to end-on attachments (3) [47,48]. In yeast, this follows a multistep process involving both active transport of kinetochores along microtubules and depolymerization of the microtubule plus-end. End-on attachment destabilizes the microtubule plus-end (4) [82]. Kinetochores not under tension release depolymerizing microtubules (5) [76]. Failure to do so would result in both sisters being dragged to the same spindle pole. Conversely, kinetochores experiencing tension maintain a tight connection with the microtubule plus-end (6). Once all kinetochore pairs are properly attached, cohesin cleavage triggers anaphase, and kinetochores track with depolymerizing microtubule tips (7). Abbreviation: SAC, spindle assembly checkpoint.

and microtubule attachment states (Table S2). Evidence for kinase regulation at the inner kinetochore includes the findings that human PLK1 and CDK1 kinases restrict CENP-A deposition to early stages of the cell cycle [60] and that, in yeast, phosphomimetic mutations in Cse4 partially bypass an Ipl1 kinase temperature-sensitive allele [61]. Aurora B/Ipl1 kinase also enables kinetochore assembly by phosphorylating Dsn1 [62,63]. A peptide close to the Dsn1 N-terminus competes with CENP-C/Mif2 for MIND binding, and Aurora B/Ipl1 phosphorylation of Dsn1 at serine residues within this peptide stimulates **outer kinetochore** assembly by tilting the balance of this competition in favor of CENP-C/Mif2 [57]. Inactivation of a key Cdk1 target site (Dsn1-S264) negates the requirement for Ipl1-mediated Dsn1 phosphorylation [62], implying that the preceding pathway does not fully describe MIND recruitment by the inner kinetochore. A related Ipl1-dependent mechanism is active early in meiosis when kinetochore–microtubule connections must be broken and re-established for meiosis II [59,64].

Kinases also regulate the interface between kinetochores and microtubules. For instance, Mps1 kinase phosphorylates Cnn1 to prevent Ndc80 binding [34,65]. Cdk1 and Ipl1 also phosphorylate Cnn1, and total Cnn1 phosphorylation correlates with its kinetochore recruitment [65–67]. In vertebrates, CDK1 phosphorylation of CENP-T promotes Ndc80 complex recruitment [68]. A crystal structure of Dsn1 bound to Spc24/25 suggests phosphorylation may similarly regulate the MIND–Ndc80 interaction [57]. In an additional regulatory step, Aurora B phosphorylates Ndc80, which allows Ndc80 to bind to Mps1 instead of to the microtubule, and ultimately leads to KNL1/Spc105 phosphorylation and activation of the **spindle assembly checkpoint (SAC)** [69,70]. Finally, Mps1, CDK1, and Aurora B regulate Ska complex

recruitment to kinetochores [71–73]. Other kinase activities associated with the SAC are beyond the scope of this review [74].

The regulated kinetochore assembly steps presented above cannot reconcile a set of conflicting observations. In yeast, deletion of the N-terminal fragment of Mif2, which is necessary and sufficient for MIND binding, is not lethal *in vivo* [18]. Why then should the regulation of the Mif2–MIND interaction be essential [62]? That a defined fragment of the Ame1 subunit of the COMA complex binds to MIND, and that deletion of this fragment is in fact lethal [18], further complicates this situation. A leading proposal to explain these observations holds that cooperative assembly of inner kinetochore proteins is required for stable microtubule connection [18], but, unless there are substantial architectural differences between yeast and vertebrate kinetochores, the absence of a viability effect upon deletion of vertebrate CENP-U/Ame1 frustrates this interpretation (Table S1) [54,75]. Identification of genetic suppressors of *AME1* deletion and reconstitution of active kinetochores [29,42,76] in the presence and absence of the COMA complex will therefore be important steps towards connecting kinetochore architecture with function.

Kinetochore Assembly States

Kinetochores have a complex subunit stoichiometry that is subject to the kinase regulation discussed earlier. The number of Ndc80 molecules at each kinetochore has been used as a measure of kinetochore assembly state; one yeast Cse4 nucleosome corresponds to a single kinetochore microtubule and approximately eight Ndc80 complexes at metaphase [23,52,77,78]. It is not known whether vertebrate CENP-A nucleosomes and kinetochore microtubules are paired, but a similar Ndc80-to-microtubule ratio has been reported [79]. How the copy-number mismatch between CENP-A/Cse4 and Ndc80 arises is not yet fully understood, but a crystal structure of the yeast MIND complex shows conserved oligomerization interfaces that, in principle, would enable about six MIND complexes to assemble into a ring with the Spc24/25-binding peptides projecting from its periphery [57]. This geometry could explain features seen in micrographs of purified yeast kinetochore particles (Figure 1) [80]. It could also account for up to six Ndc80 molecules per CENP-A/Cse4 nucleosome, leaving the remainder to be recruited by CENP-T/Cnn1 [34,56,66].

Biochemical reconstitutions have shown how a full complement of Ndc80 complexes could associate with each kinetochore. CENP-T can recruit MIND independently of CENP-C *in vivo* [25,67], and *in vitro* analysis of MIND–CENP-T–Ndc80 complexes has shown three Ndc80 extensions per particle, with two emanating from CENP-T and one from MIND [68]. Electron micrographs of a yeast Ctf3–Ndc80–Cnn1 complex have provided a related view [55]. Human CENP-C and phosphorylated CENP-T compete for MIND interaction *in vitro* [68], suggesting that CENP-C/Mif2 and CENP-T each recruit MIND independently. Another possibility is that CENP-T interacts with MIND subunits that are part of a multimeric assembly in which only two interact with CENP-C/Mif2. Biochemical data suggest that two CENP-T/Cnn1 molecules associate indirectly with each CENP-A/Cse4 nucleosome [29,55]. When considered along with possible MIND oligomerization, the final number of Ndc80 complexes per centromeric nucleosome would be 10 or 12. Without MIND oligomerization, this number is likely eight. Protein copy-number counting at isolated kinetochore pairs *in vivo* [77], with attention being paid to kinase dependencies and the cell cycle, provides one path towards evaluating these models.

Sensing and Sustaining Microtubule Attachment

An ideal kinetochore maintains a strong attachment to the microtubule tip only when its counterpart, located on a sister chromatid, is attached to an opposing microtubule. In the presence of **tension**, it must hold on for the duration of metaphase and must maintain this

connection during microtubule depolymerization at anaphase (Figure 2, state 7). Accordingly, pulling a kinetochore away from the microtubule tip to which it is attached stabilizes the kinetochore–microtubule connection, even in the absence of kinases [76]. Stu2, a spindle- and kinetochore-associated factor that binds the curved tubulin dimers at depolymerizing microtubule tips, stabilizes kinetochore–microtubule connections under tension [42], thereby providing a possible explanation for this activity. The vertebrate Ska complex also associates with curved tubulin [50], although whether Ska strengthens kinetochore–microtubule connections specifically in the presence of tension has not been explored.

Kinetochores are not merely responsive to microtubule fluctuations, and they also destabilize microtubules in the absence of tension and stabilize them in the presence of tension [81,82] (Figure 2, state 4). In human cells, tension-dependent microtubule stabilization depends on Ndc80 [81]. The overall kinetochore architecture discussed here suggests one way in which tension across sister kinetochores might help to silence the SAC [83]. Spindle tension could cause radially arranged MIND complexes to flex towards the microtubule along the kinetochore axis, drawing the proximal ends of Ndc80 complexes inward and separating checkpoint kinases from important substrates (Figure 1; 'Attached, Tension'). While these rearrangements might be part of the long-sought-after tensiometer [84], the fact that the inner kinetochore protein Sgo1 dissociates from centromeres in the presence of tension suggests that kinetochore stretching is at best only part of the mechanism [85].

Managing and Counteracting Spindle Forces

The ultimate function of the kinetochore is to coordinate the orderly separation of **sister chromatids**. Fulfilling this function depends both on a regulated pulling force and a resistance to this pulling force that keeps sister chromatids together until anaphase. Resistance depends on an association between sister centromeres, which in turn depends on the kinetochore [86,87]. Cells deficient in this activity mis-segregate chromosomes at elevated rates [88], and the defect becomes profound in meiosis [89]. Newly replicated sister chromatids are held together by the cohesin ring complex [90,91]. Chromosomal cohesin density peaks at centromeres and dissipates until it reaches baseline (arm) levels roughly 25 kb away [92–94]. Separation of sister centromeres on the mitotic spindle depletes the centromeric cohesin pool [88,95,96] (Figure 3A), suggesting that a subset of cohesins at centromeres connect sister chromatids before their separation, and that cohesin complexes that are not dispersed upon sister centromere separation do not [97]. Cohesin and related processes have been reviewed thoroughly elsewhere [90,91,98]. We address here the role of the kinetochore in this process, and we also consider implications for more complex centromeres and for meiosis.

The Ctf19 Complex Coordinates Sister Centromeres and Complex Kinetochores

Multiple approaches led to the identification of five kinetochore protein complexes with overlapping functions in mitotic fidelity, collectively referred to as the constitutive centromere-associated network (CCAN) in vertebrates and the Ctf19 complex in yeast [27,75,99–104]. These are the CENP-N/Chl4 complex (CENP-N/Chl4 and CENP-L/Iml3), the CENP-I/Ctf3 complex, the Nkp1/2 complex (Nkp1 and Nkp2, not found in vertebrates), the CENP-T/Cnn1 complex, and the COMA complex. Association of these factors with the kinetochore is cooperative and approximately hierarchical [55,105]. The COMA proteins lie upstream in the assembly pathway, followed by Chl4/Iml3, the Ctf3 complex, and the Cnn1 complex [30,55,106].

Ctf19 complex members work together to bring to the kinetochore the cohesin loading complex, a heterodimer of the Scc2 and Scc4 proteins (Scc2/4; NIPBL and Mau2 in vertebrates) [88,95,107] (Figure 3B). As part of this process, the Ctf19 complex recruits the Dbf4-dependent kinase (DDK; Cdc7-Dbf4 in yeast) to the kinetochore in G1, a step required both for

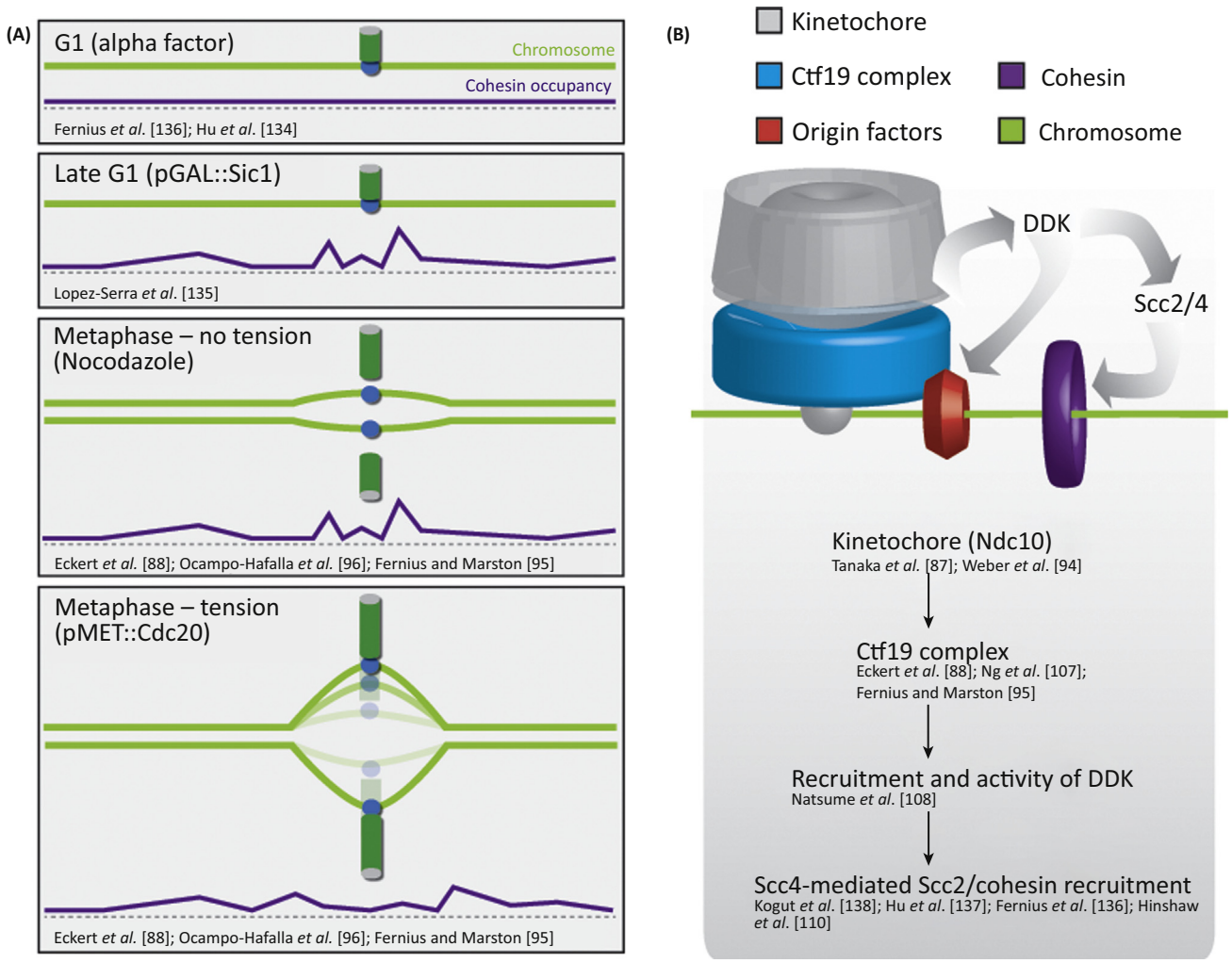

Figure 3. Cohesin Loading at the Centromere. (A) Schematic showing the idealized distribution of chromosomal cohesin (purple line) along the chromosome (green lines). Each grey box depicts a different cell-cycle arrest condition (top right) in which cohesin binding to chromatin has been measured genome-wide or by chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) in early G1 [95,134], late G1 [135], and metaphase with and without sister centromere separation [88,95,96]. Kinetochores are drawn as blue circles, and microtubules are drawn as dark-green tubes. (B) Diagram showing factors involved in centromeric cohesin loading [87,88,94,95,107,108,110,136–138]. The Ctf19 complex recruits DDK, and DDK activity is required both for early origin firing at the centromere and for enhanced centromeric cohesin loading through Scc2 recruitment.

Scc2/4 recruitment and for early replication of all 16 yeast centromeres [108]. Once at centromeres, DDK phosphorylates Ctf19, which then interacts with a conserved surface of the Scc4 protein that is specifically required for targeting cohesin loading to centromeres in yeast [109,110]. The kinetochore therefore ensures robust cohesin loading early in the cell cycle. Cohesin translocation along DNA has now been observed *in vitro* [111,112], providing a likely explanation for the broad distribution of cohesin around centromeres. CENP-A-associated DNA also replicates early in fission yeast, flies, and mice [113–116], suggesting that kinetochore-mediated DDK recruitment, a limiting step in DNA replication initiation, might be common.

Like their homologs in budding yeast, fission yeast Ctf19 components were identified in genetic screens for mutants with chromosome segregation defects [117,118]. Ctf19 genes are essential for growth in fission yeast (Table S1) [117], and hypomorphic alleles of *fta2* and *mis15* (*CTF19* and *CHL4* in budding yeast) show elevated spindle checkpoint activity and unequal distribution of DNA to daughter cells [119,120]. The **CCAN/Ctf19 complex** is also essential in human cells, where knockdown or deletion of most subunits tested leads to anaphase arrest and aberrant spindle morphology (Table S1) [102,105,121]. The pattern of Ctf19 complex subunit essentiality across species – they are largely essential in mammals and dispensable in yeast – suggests that they may help to orient microtubule attachments along an individual chromatid.

Meiosis-Specific Kinetochore Functions and the Ctf19 Complex

Meiotic chromosome segregation, which entails the cosegregation of sister chromatids during the first division and the splitting of sister chromatids during the second division, requires adaptations of the kinetochore and its associated functions (reviewed in [90]). These adaptations include the co-orientation of sister kinetochores during meiosis I, the protection of sister centromere cohesion until its destruction at anaphase of meiosis II, and the resetting of kinetochore–microtubule connections without an intervening round of DNA replication. In yeast, kinetochore co-orientation depends on the Y-shaped monopolin complex [122–124] which is thought to clamp together MIND complexes from sister kinetochores [122,125,126]. Together, Cdc5 and monopolin are sufficient to direct cosegregation of sister centromeres in mitosis [127], but the Cdc5 substrates required for sister co-orientation in meiosis I have not been identified.

The Ctf19 complex serves at least two functions unique to meiosis. First, retention of centromeric cohesin during the first meiotic division depends on Sgo1 and the Ctf19 complex proteins Chl4 and Iml3 [89,128]. Second, Ctf19 complex proteins influence meiotic recombination by suppressing crossovers around centromeres in two steps [129]: Ctf19 complex-dependent cohesin recruitment biases double-strand break repair towards sister chromatids [129,130], and Ctf19 proteins suppress double-strand breaks at centromeres independently of cohesin recruitment [129]. Understanding these and additional meiotic functions of kinetochore proteins will be an essential step towards understanding chromosome segregation during gametogenesis.

Concluding Remarks

Centromeres, through their associated factors, organize and respond to opposing forces. The timing of cellular events, the details of which we have not explicitly addressed here, enables the orderly execution of these activities. We anticipate that advances in the coming years will address the coordination of regulated kinetochore assembly by the cell cycle with particular attention to the contributions of sequential waves of kinase activities as cells progress from G1 to metaphase. Fundamental questions remain: how are cellular decisions made, how are checks and balances on competing inputs encoded at the molecular level, and what are the long-term consequences of these decisions, both for individual cells and, where applicable, for whole organisms?

Outstanding Questions

What is the structure of an intact kinetochore, including the CCAN/Ctf19 complex? How is its assembly regulated during the cell cycle, and how does this regulation account for observed stoichiometric relationships between individual components *in vivo*?

What are the essential substrates of the mitotic kinases (Cdk1, Aurora B/Ipl1, PLK1/Cdc5), and how do they regulate kinetochore function?

To what extent are vertebrate kinetochores modular, and how do CENP-A nucleosomes cooperate along a single chromatid?

What prevents the kinetochore from disengaging from the microtubule during anaphase?

Why is regulation of Dsn1–CENP-C–Mif2 interaction essential while the MIND-binding fragment of Mif2 is not?

What explains the different requirements for CCAN/Ctf19 proteins in yeast and vertebrates, and how might the differences relate to centromere cohesion?

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Supplemental Information

Supplemental information associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tcb.2017.09.002.

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