ANNUAL REVIEWS

Annual Review of Genetics

The Four Causes: The Functional Architecture of Centromeres and Kinetochores

Andrew D. McAinsh[1,2] and Adele L. Marston[3]

[1]Centre for Mechanochemical Cell Biology, University of Warwick, Coventry, United Kingdom; email: A.D.McAinsh@warwick.ac.uk

[2]Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, United Kingdom

[3]The Wellcome Centre for Cell Biology, Institute of Cell Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom; email: adele.marston@ed.ac.uk

Annu. Rev. Genet. 2022. 56:279–314

First published as a Review in Advance on September 2, 2022

The *Annual Review of Genetics* is online at genet.annualreviews.org

https://doi.org/10.1146/annurev-genet-072820-034559

Copyright © 2022 by Annual Reviews. All rights reserved

ANNUAL REVIEWS CONNECT

www.annualreviews.org

- Download figures
- Navigate cited references
- Keyword search
- Explore related articles
- Share via email or social media

Keywords

kinetochore, centromere, mitosis, meiosis, microtubule, chromosome, cell division, mitotic spindle, chromatin

Abstract

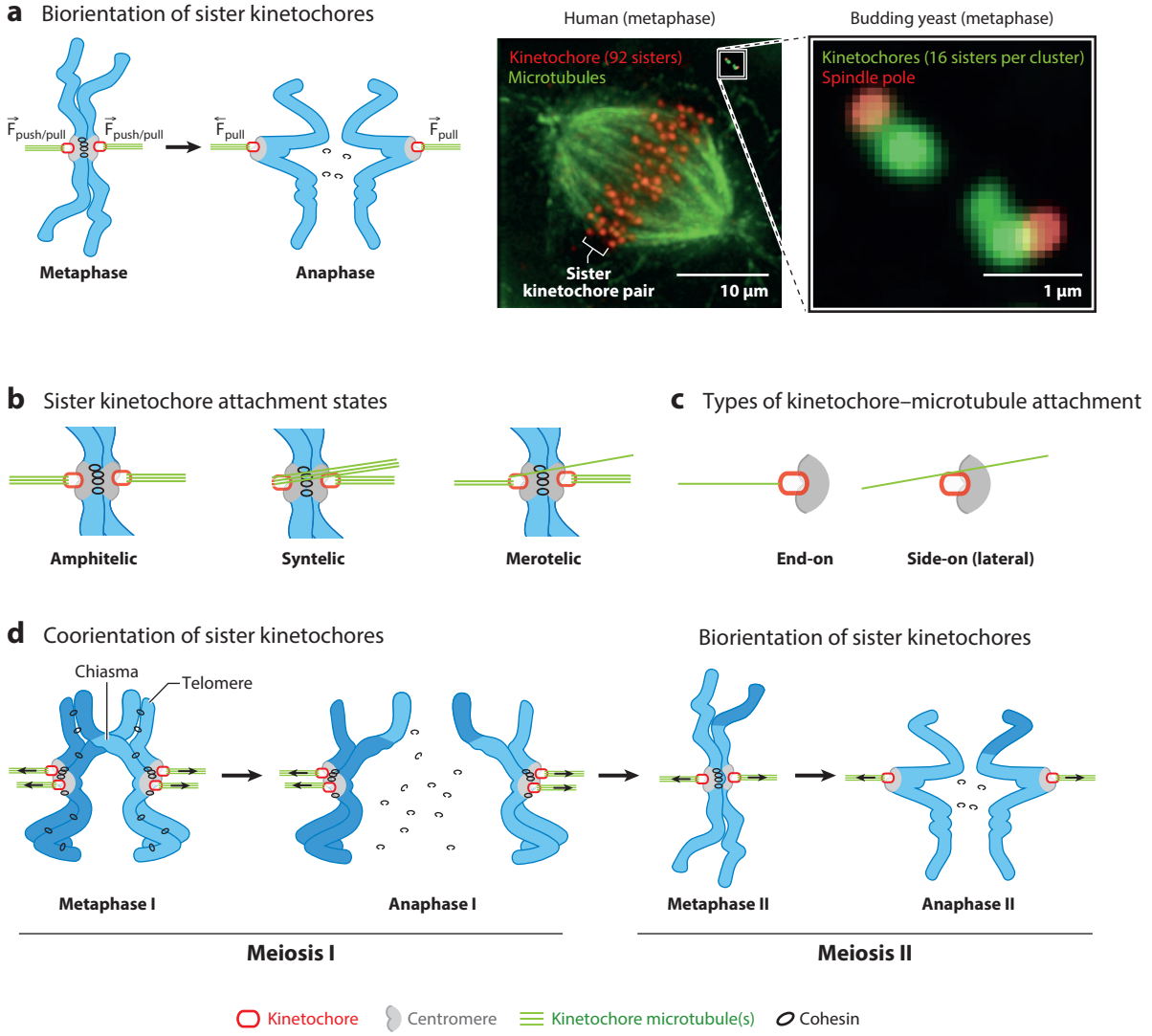
Kinetochores are molecular machines that power chromosome segregation during the mitotic and meiotic cell divisions of all eukaryotes. Aristotle explains how we think we have knowledge of a thing only when we have grasped its cause. In our case, to gain understanding of the kinetochore, the four causes correspond to questions that we must ask: (*a*) What are the constituent parts, (*b*) how does it assemble, (*c*) what is the structure and arrangement, and (*d*) what is the function? Here we outline the current blueprint for the assembly of a kinetochore, how functions are mapped onto this architecture, and how this is shaped by the underlying pericentromeric chromatin. The view of the kinetochore that we present is possible because an almost complete parts list of the kinetochore is now available alongside recent advances using in vitro reconstitution, structural biology, and genomics. In many organisms, each kinetochore binds to multiple microtubules, and we propose a model for how this ensemble-level architecture is organized, drawing on key insights from the simple one microtubule–one kinetochore setup in budding yeast and innovations that enable meiotic chromosome segregation.

1. INTRODUCTION

Kinetochore: the multiprotein complex that assembles on the centromere

Mitosis: the phase of the cell cycle where replicated chromosomes are equally partitioned into daughter cells

Kinetochores are macromolecular protein assemblies, the canonical function of which is to form load-bearing attachments to the plus ends of spindle microtubules on eukaryotic chromosomes. In mitosis, the identical sister chromatids, which are held together by cohesin, attach via their sister kinetochores to microtubules from opposite spindle poles (**Figure 1*a***). Kinetochores, together with cohesin, provide resistance and coupling to spindle microtubule–derived forces, generating tension and chromosome movement. Once this state of sister kinetochore biorientation has been achieved for all sister chromatid pairs, cohesin is abruptly lost, resulting in the equational segregation of sister chromatids. While not the focus of this review, these events are tightly regulated by (*a*) the spindle assembly checkpoint (SAC), which prevents anaphase onset when one or more kinetochores are unattached (138), and (*b*) error correction mechanisms that destabilize



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Geometries of chromosome segregation during mitosis and meiosis. (*a*, *top left*) In mitosis, the replicated chromosomes (sister chromatids, *blue*) are bioriented with sister kinetochores (*red*) in a back-to-back geometry and embedded in the pericentromeric chromatin domain (*gray*). Sister chromatids are physically held together by cohesin molecules, which trap the two DNA strands (*black circles*). The plus ends of spindle microtubules (*green*, either singular in budding yeast or multiple in animal cells) are embedded in the kinetochore with their minus ends projecting toward the centrosomes (human) or spindle pole bodies (budding yeast). Pulling forces generated by kinetochore–microtubule attachments pull sister chromatids apart in anaphase once cohesin is cleaved (on satisfaction of the spindle assembly checkpoint). (*a*, *top right*) Mitotic spindle in a human cell, with kinetochores (*red*) and microtubules (*green*), compared to budding yeast, with kinetochores (*green*) and spindle pole bodies (*red*); the latter is adapted from Reference 53. In yeast, the 32 sister kinetochores form two clusters along the spindle axis, which is ~1 μm in length, similar to the distance between two sister kinetochores in humans. In humans, the sister kinetochores are aligned along the spindle axis. (*b*) Sister kinetochore attachment states. Attachment of sister kinetochores to microtubules from opposite poles is referred to as amphitelic, while attachment of sister kinetochores to microtubules from the same pole is referred to as syntelic. Attachment of a single kinetochore to microtubules from opposite poles is referred to as merotelic. Note that merotely is not possible in budding yeast since each kinetochore has only a single microtubule binding site. (*c*) Types of kinetochore–microtubule attachment. Kinetochores can be captured by the ends of microtubules (end-on) or along the sides of microtubules (side-on or lateral). In the bioriented state, end-on attachments are established. (*d*) In meiosis I, replicated maternal and paternal (homologous) chromosomes are physically connected as a result of crossover recombination, which generates chiasmata, the products of crossover meiotic recombination, together with sister chromatid cohesion distal to the chiasmata. Sister kinetochores are attached to microtubules from the same pole and are said to be cooriented. In anaphase I, cohesin is cleaved only on chromosome arms (pericentromeric cohesin is protected from cleavage by shugoshin-PP2A; reviewed in 156), which resolves chiasma and allows homologous chromosomes to segregate to opposite poles. In meiosis II, sister kinetochores biorient, and the pericentromeric cohesin resists the pulling forces from microtubules. During anaphase II, pericentromeric cohesin is cleaved, and sister kinetochores segregate to opposite poles.

erroneous attachments and promote biorientation (136). These are key during early mitosis when kinetochores are in a mixture of attachment states. For example, in budding yeast, attachments are initially syntelic, meaning that sister kinetochores bind to microtubules from the same pole, and conversion to amphitelic attachment, where sister kinetochores bind to microtubules from opposite poles, relies on error correction (**Figure 1*b***). Once this state of biorientation has been achieved, detachment is rare, explaining why the SAC is nonessential (154). On the other hand, in animal cells, kinetochores are in the unattached state by default, and the SAC is active. Here, kinetochores need to first capture microtubules through either side-on or end-on interactions, giving rise to proper (amphitelic) as well as improper (syntelic or merotelic) attachments that require correction (**Figure 1*c***). Remarkably, these processes are then adapted during meiosis, where sister kinetochores attach to microtubules from the same pole (coorientation, also called monoorientation) during the first division so that sister chromatids cosegregate to allow for a reduction in ploidy (**Figure 1*d***).

Syntelic attachment: attachment of sister kinetochores to microtubules from the same pole

Amphitelic attachment: attachment of sister kinetochores to microtubules from opposite poles

Merotelic attachment: attachment of a single kinetochore by microtubules from opposite poles

Sister kinetochore biorientation: the state in which sister kinetochores are attached to microtubules from opposite poles

To enable all of these functions, kinetochores are built from several copies of multiple proteins and complexes that, although not highly conserved at the sequence level, have recognizable homologs and adopt a similar architecture in most studied eukaryotes, with some variations (165, 238). Notable exceptions are kinetoplastids, a group of unicellular eukaryotes including the *Trypanosoma brucei* parasite, which have divergent kinetochores with distinct protein origins, and some insects, in which kinetochores form a layer across the whole chromosome (106, 239). By contrast, centromeres, the chromatin loci where kinetochores assemble, are highly divergent and rapidly evolving. In their simplest form, as in the budding yeast *Saccharomyces cerevisiae*, centromeres are defined by a specific ~125-bp DNA sequence, which is more or less the same on all 16 chromosomes and referred to as a point centromere (159). However, in most eukaryotes, centromeres are not defined by sequence and consist of highly repetitive DNA sequences, such as tandem repeats and retrotransposons, that are unrelated in different organisms and vary even between chromosomes of the same organism. These complex centromeres are known as regional centromeres and can extend for several megabases (5). In humans, for example, many centromeres

are composed of so-called α-satellite repeats (235). Budding yeast centromeres wrap a single centromeric [centromere protein A (CenpA)-containing] nucleosome, and each kinetochore binds a single microtubule (72, 258). Regional centromeres contain many CenpA nucleosomes and assemble compound kinetochores that bind multiple microtubules (10–15 in human) (44, 120, 183, 208). Each centromere/kinetochore is flanked by a specialized chromosomal domain, called the pericentromere. In most organisms, pericentromeres are large (extending from several kilobases in fission yeast to megabases in humans), repetitive, heterochromatic, and cohesin rich. In budding yeast, pericentromeres are compact (~20 kb) and lack heterochromatin but are nevertheless highly enriched with cohesin (155). Kinetochore structure and function must therefore be considered in the context of a specialized chromatin environment.

Meiosis: a specialized cell cycle where two consecutive chromosome segregation events generate gametes with half the number of chromosomes

Kinetoplastids: a group of unicellular flagellated eukaryotes that lack conventional kinetochores

Centromere: the region of DNA upon which the kinetochore assembles

2. KINETOCHORE ASSEMBLAGE

Conventional kinetochores consist of ~100 proteins (see **Table 1**), many of which are organized into distinct complexes, that self-assemble in a hierarchical manner onto a specialized nucleosome. **Figure 2*a*** sketches out the architecture of the core attachment site (approximate to scale). We use the examples of budding yeast and human kinetochores to introduce these subcomplexes and how they can be assigned to three major subassemblies. Moving from centromeric DNA to microtubules, the subassemblies are specialized CenpA nucleosomes (**Figure 2*b***, subassembly I); the constitutive centromere-associated network (CCAN, also called Ctf19 complex in budding yeast) (**Figure 2*b***, subassembly II); the KMN-S network (incorporating Knl1, Mis12, Ndc80, and Ska complexes or the unrelated Dam1 complex in yeast that couples kinetochore to microtubules), which provides the core microtubule-binding interface and platform for SAC and error correction processes (**Figure 2*b***, subassembly III); and the corona (not in yeast), which incorporates the Rod-Zw10-Zwilch-Spindly (RZZ-S) and CenpF-Nde1-Ndel1-Lis1 (FNNL) complexes and the molecular motors dynein-dynactin (DD), CenpE, and Kif2b (**Figure 2*b***, subassembly IV). The corona facilitates microtubule capture, transport, and SAC activities. Subassemblies III (KMN-S) and IV (corona) are not static but undergo dynamic remodeling throughout the cell cycle (**Figure 2*b***). The corona is a feature of unattached kinetochores, while in animal cells KMN-S loads in early mitosis, undergoes a maturation process as microtubule attachments form, and disassembles in late anaphase (91). Subassembly I is present throughout the cell cycle, although there are hints of changes in organization (6, 21, 160). The stoichiometry, stability, and conformational state of subassemblies are clearly subject to dynamic change in response to mechanical (microtubule attachment and tension) and regulatory inputs, that is, cycles of phosphorylation driven by the major mitotic kinases (Cdk1, Aurora B, Mps1, Bub1, Haspin, and Plk1) and phosphatases (PP1/PP2A) (112, 217). We discuss these subassemblies in turn, highlighting key regulatory and functional features.

2.1. Subassembly I (CenpA Chromatin: a Specialized Nucleosome Specifying Centromere Identity)

Kinetochore assembly must be restricted to a single site to avoid chromosome breakages due to opposing microtubule attachments in mitosis. In most organisms, the site of kinetochore assembly is defined by specialized nucleosomes in which the histone H3 subunit is replaced by the CenpA variant. Understanding how CenpA nucleosomes are specifically deposited at centromeres and specifically recognized by the building blocks of the kinetochore is a key question in understanding centromere identity (for review, see 164).

Budding yeast point centromeres consist of three centromere-determining elements (CDEs). *CDEI* (8 bp) binds the helix-loop-helix transcription factor Cbf1, *CDEII* (80–90-bp AT-rich

Table 1 Parts list of budding yeast and human kinetochores and centromeres

Protein/component		Subcomplex	Complex	Subassembly	Notes
Homo sapiens	*Saccharomyces cerevisiae*				
CenpA	Cse4	NA	Nucleosome	I	Nonspecific DNA binding (wraps AT-rich CDEII in *S. cerevisiae*)
Mis18α	NA	MIS18C	MIS18-HJURP	I	CenpA loading machinery
Mis18β	NA	MIS18C	MIS18-HJURP	I	CenpA loading machinery
Mis18bp	NA	MIS18C	MIS18-HJURP	I	CenpA loading machinery
HJURP	Scm3	NA	MIS18-HJURP	NA	CenpA chaperone (binds CenpA:H4)
Shugoshin-1	Sgo1[a]	NA	NA	NA	Cohesion protection; PP2A receptor
Shugoshin-2	Sgo1[a]	NA	NA	NA	Cohesion protection; PP2A receptor
CenpB	NA	NA	NA	I	DNA binding (CenpB box)
NA	Cbf1	NA	NA	NA	DNA binding (CDEI)
NA	Ndc10	NA	CBF3	I	DNA binding (sequence independent)
NA	Ctf13	CBF3core	CBF3	I	DNA binding (CDEIII)
NA	Cep3	CBF3core	CBF3	I	DNA binding (CCG motif in CDEIII)
NA	Skp1	CBF3core	CBF3	I	F-box protein
CenpC	Mif2	NA	CCAN/CTF19C	II	DNA binding (AT hook); dimer
CenpH	Mcm16	HIKM	CCAN/CTF19C	II	NA
CenpI	Ctf3	HIKM	CCAN/CTF19C	II	NA
CenpK	Mcm22	HIKM	CCAN/CTF19C	II	NA
CenpL	Iml3	NL	CCAN/CTF19C	II	NA
CenpM	Mcm16	HIKM	CCAN/CTF19C	II	NA
CenpN	Chl4	NL	CCAN/CTF19C	II	NA
CenpO	Mcm21	OPQUR/COMA	CCAN/CTF19C	II	NA
CenpP	Ctf19	OPQUR/COMA	CCAN/CTF19C	II	NA
CenpQ	Okp1	OPQUR/COMA	CCAN/CTF19C	II	Ndc80-like MT binding
CenpR	NA	OPQUR	CCAN/CTF19C	II	NA
CenpS	Mhf1[b]	SX	TWSX	II	Histone fold; also DNA repair role
CenpT	Cnn1	TW	TWSX	II	Histone fold
CenpU	Ame1	OPQUR/COMA	CCAN/CTF19C	II	Receptor for Plk1 (*H. sapiens*)
CenpV	NA	NA	NA	NA	GFA domain; *CEN* chromatin structure; meiosis; MT binding
CenpW	Wip1	TW	TWSX	II	Histone fold

(*Continued*)

Table 1 (*Continued*)

Protein/component		Subcomplex	Complex	Subassembly	Notes
Homo sapiens	***Saccharomyces cerevisiae***				
CenpX	Mfh2[b]	SX	TWSX	II	Histone fold; also DNA repair role
NA	Nkp1	Nkp1/Nkp2	CCAN/CTF19C	II	NA
NA	Nkp2	Nkp1/Nkp2	CCAN/CTF19C	II	NA
Spc24	Spc24	NDC80	KMN-S	III	RWD domains
Spc25	Spc25	NDC80	KMN-S	III	RWD domains
Ndc80	Ndc80	NDC80	KMN-S	III	CH domain/MT lattice binding
Nuf1	Nuf2	NDC80	KMN-S	III	CH domain/MT lattice binding
Knl1	Spc105	KNL1	KMN-S	III	MELT array as platform for SAC; PP1 receptor and MT binding (N-terminal)
Zwint	Kre28	KNL1	KMN-S	III	NA
Mis12	Mtw1	MIS12	KMN-S	III	NA
Nnf1 (Pmf1)	Nnf1	MIS12	KMN-S	III	NA
Nsl1	Nsl1	MIS12	KMN-S	III	NA
Dsn1	Dsn1	MIS12	KMN-S	III	Receptor for monopolin
Ska1	NA	SKA	KMN-S	III	Load-bearing device; MT tip tracking; binding curved protofilaments
Ska2	NA	SKA	KMN-S	III	
Ska3	NA	SKA	KMN-S	III	
Cdt1	Tah11[b]	NA	NA	III	Also a replication factor; can bind Ndc80 loop
Ch-TOG	Stu2	NA	NA	III	MT polymerase; docks Ndc80 four-way junction
NA	Dam1	NA	DAM1C	III	MT-encircling coupler
NA	Duo1	NA	DAM1C	III	NA
NA	Dad1	NA	DAM1C	III	NA
NA	Dad2	NA	DAM1C	III	NA
NA	Dad3	NA	DAM1C	III	NA
NA	Dad4	NA	DAM1C	III	NA
NA	Spc34	NA	DAM1C	III	NA
NA	Spc19	NA	DAM1C	III	NA
NA	Ask1	NA	DAM1C	III	NA
NA	Hsk3	NA	DAM1C	III	NA
Astrin	NA	NA	Astrin/Skap	III	NA
Skap	NA	NA	Astrin/Skap	III	NA
MYCBP	NA	NA	Astrin/Skap	III	NA
LC8	NA	NA	Astrin/Skap	III	NA
Bub1	Bub1	BUB1-BUB3	SAC	III	Protein kinase

(*Continued*)

Table 1 (*Continued*)

Protein/component		Subcomplex	Complex	Subassembly	Notes
Homo sapiens	***Saccharomyces cerevisiae***				
Bub3	Bub3	BUB1-BUB3	SAC	III	Phospho-MELT binding
BubR1	Mad3	NA	SAC	III	Mad3 lacking pseudokinase domain found in BubR1
Mad1	Mad1	MAD1-MAD2	SAC	III (& IV)	Forms mitotic checkpoint complex with BubR1 and Cdc20
Mad2	Mad2	MAD1-MAD2	SAC	III (& IV)	
Mps1	Mps1	NA	SAC	III	Protein kinase; spindle assembly checkpoint; biorientation; corona expansion (Knl1's MELTs, Ska, Ndc80, Rod, other substrates)
p31 comet	NA	NA	SAC	NA	SAC inhibitor
CenpE	NA	NA	Corona	IV	Kinesin-7 MT plus-directed molecular motor
CenpF	Slk19	FNNL	Corona	IV	Homology unclear; MT binding and DD regulator in *H. sapiens*
Rod	Sec39 and Sec31[b]	RZZ-S	Corona	IV	Required for vesicle tethering in yeast
Zw10	Dsl1[b] and Tip20	RZZ-S	Corona	IV	Required for vesicle tethering in yeast
Zwilch	NA	RZZ-S	Corona	IV	NA
Spindly	NA	RZZ-S	Corona	IV	Dynein adapter
NA	NA	Cytoplasmic dynein[b]	DD; corona	IV	Minus-directed molecular motor (dynein heavy chain + 5 light/intermediate chains)
NA	NA	Dynactin[b]	DD; corona	IV	Dynein cofactor (11 subunits)
Lis1	Pac1[b]	FNNL	Corona	IV	Dynein cofactor
Nde1	Ndl1[b]	FNNL	Corona	IV	Dynein cofactor
Ndel1	Ndl1[b]	FNNL	Corona	IV	Dynein cofactor
Clasp	Stu1	NA	Corona	IV	MT rescue factor
Nup107	Nup84[b]	NUP107–NUP160 (NPC-Y)	Corona	IV	Also a nuclear pore component
Nup133	Nup133[b]	NUP107–NUP160 (NPC-Y)	Corona	IV	Also a nuclear pore component
Nup96	Nup145C[b]	NUP107–NUP160 (NPC-Y)	Corona	IV	Also a nuclear pore component

(*Continued*)

Table 1 (*Continued*)

Protein/component		Subcomplex	Complex	Subassembly	Notes
Homo sapiens	***Saccharomyces cerevisiae***				
Sec13	Seh1[b]	NUP107–NUP160 (NPC-Y)	Corona	IV	Also a nuclear pore component
Nup160	Nup120[b]	NUP107–NUP160 (NPC-Y)	Corona	IV	Also a nuclear pore component
EB1	Bim1	NA	NA	NA	MT end-tracker
HSET[c]	Kar3	NA	NA	NA	Kinesin-14 MT minus-end directed molecular motor
Kif18a	Kip3	NA	NA	NA	Kinesin-8 MT plus-end directed molecular motor and depolymerase; located on KT proximal k-fiber
NA	Csm1	NA	Monopolin	NA	Also in the nucleolus
NA	Lrs4	NA	Monopolin	NA	Also in the nucleolus
NA	Mam1	NA	Monopolin	NA	Meiosis-specific
CSKN1D[c]	Hrr25	NA	Monopolin	NA	Protein kinase CK1δ
Meikin	Spo13	NA	NA	NA	Mokirs
Aurora B	Ipl1	NA	CPC	NA	Protein kinase
Survivin	Bir1	NA	CPC	NA	CPC localization
Borealin	Nbl1	NA	CPC	NA	CPC localization
INCENP	Sli15	NA	CPC	NA	Kinase activation (IN-box)
MCAK	NA	NA	NA	NA	Kinesin-13 MT catastrophe factor
PP1	Glc7	NA	NA	NA	Protein phosphatase 1
PP2A-B56	PP2A-Rts1	NA	NA	NA	Protein phosphatase 2A
Plk1 (Polo)	Cdc5	NA	NA	NA	Protein kinase
Haspin	Hsk1[b]/Hsk2[b]	NA	NA	NA	Protein kinase (H3T3ph)
Cyclin B	NA	Cyclin B-Cdk1	Corona	IV	Binds Mad1
Bod1	NA	NA	NA	NA	Regulatory subunit for PP2A
SENP family	Ulp2	NA	NA	NA	Docks to Ctf3/CenpI; SUMO protease

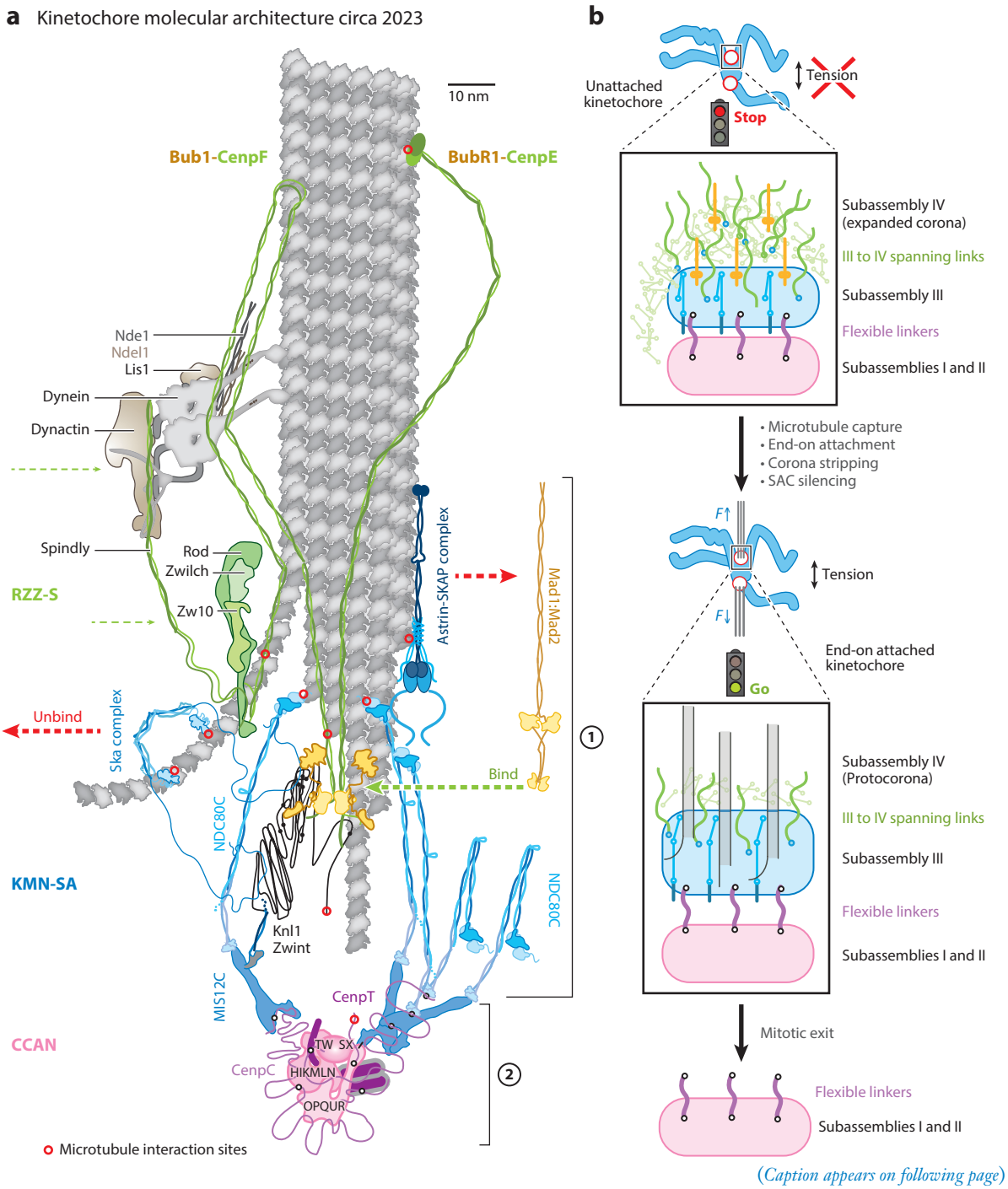
Abbreviations: CCAN, constitutive centromere-associated network; CDE, centromere-determining element; DD, dynein-dynactin; KT, kinetochore; Mokir, meiosis-specific kinase regulator; MT, microtubule; NA, not applicable; SAC, spindle assembly checkpoint.

[a]Budding yeast has a single shugoshin protein that has functions in common with mammalian Sgo1 and Sgo2.

[b]No evidence for kinetochore localization in budding yeast.

[c]No evidence for kinetochore localization in humans.

sequence) wraps a single CenpA nucleosome, and *CDEIII* (~25 bp) binds the four-subunit CBF3 complex (for review, see 19). CBF3 comprises one copy each of Ndc10 and a CBF core (Skp1, Ctf13, and two copies of Cep3). CBF3 binds the essential CCG and TCT motifs of *CDEIII* through the Gal4 domain of one of the Cep3 protomers in a manner resembling transcription



(Caption appears on following page)

Figure 2 (*Figure appears on preceding page*)

Molecular architecture of the kinetochore. (*a*) Architecture of a single microtubule–kinetochore attachment site. For clarity, only one CCAN (*pink*) and the associated molecules are shown (see **Figure 3** for an extension of models to multisubunit kinetochores). All molecules are drawn to scale based on known structural biology, length of coiled-coil sequences, or length of disordered regions. The relative position of molecules is informed by the measured Euclidean distances between the average positions of two labeled proteins in a population of kinetochores (see 211) and/or known binding interfaces. Red circles denote known contact points between a protein and the microtubule. Flexible linkers connect the CCAN to KMN (②), and extended coiled-coil elements span subassembly III to subassembly IV (CenpF, CenpE, Mad1; ①). The detachment of microtubules triggers a switch in composition and architecture: SAC factors (*yellow*) including Mad1-Mad2 load on the Bub1-Bub3 that are bound on the Knl1 phospho-domain (*black dots*), which correlates with rearrangement of NDC80C as they jackknife (fold over) and lose order. Other factors that load or leave are designated by green and red dashed arrows, respectively. Not all factors are shown. (*b*) Dynamic remodeling of kinetochores. At the start of mitosis, kinetochores are not yet binding microtubules end-on (unattached), and the SAC (*yellow*) is actively delaying anaphase onset. In humans, there is expansion of subassembly IV (*green*) into the corona initiated by self-assembly of RZZ (*light green*). As end-on attachments form, the corona (and SAC) is disassembled in part by dynein-driven stripping of corona cargoes. This leaves residual corona molecules spanning to subassembly III. Stretching of linkers separates subassemblies I and II (*pink*) and III (*blue*) when under tension while there are conformational changes within the latter. Abbreviations: CCAN, constitutive centromere-associated network; Cenp, centromere protein; RZZ, Rod-Zw10-Zwilch; SAC, spindle assembly checkpoint.

factor–promoter interactions (86, 140, 262, 269). CBF3 interacts with *CEN* DNA as a head-to-head dimer that includes CDEIII, leaving space for wrapping a Cse4 nucleosome with CDEII DNA (262). Recent data propose an alternative view in which interactions between the CBF3core and the nucleosome facilitate a handover from CBF3 to CenpA nucleosomes at the *CEN* DNA (86). The Ndc10 subunit of CBF3 also recruits Scm3, a specific chaperone for Cse4 (budding yeast CenpA). Recruitment of Scm3 by CBF3 thus defines the deposition and placement of the CenpA nucleosome, though the exact mechanism is unclear (39, 54, 86, 171, 274).

Most human regional centromeres contain repeating units of two alternating 171-bp α-satellite DNA sequences, one of which contains a 17-bp CenpB box to which the CenpB protein binds in a sequence-specific manner. However, unlike in budding yeast, DNA sequence is not sufficient to dictate centromere assembly in humans, and CenpB is not essential, although increasing numbers of these elements bias chromosomes toward faithful segregation (61). Instead, human centromeres, like those of most studied species, are defined epigenetically. At regional centromeres, existing CenpA directs assembly of new CenpA through an epigenetic loop: CenpC, a structural kinetochore component that directly binds the CenpA nucleosome, recruits the Mis18 complex, which in turn binds the HJURP chaperone (equivalent of Scm3 in yeast) to promote CenpA deposition. This process is tightly temporally regulated so that CenpA deposition occurs only at mitotic exit and during G1. During S phase, CenpA nucleosomes distribute onto the two nascent strands, and in this concentration they provide the blueprint for kinetochore assembly and chromosome segregation. Upon CenpA dilution at S phase, canonical H3-containing nucleosomes are thought to act as placeholders that are evicted by transcription at mitotic exit. Centromere specification and CenpA deposition have been discussed in some excellent recent reviews (53, 164, 256).

Three key features of CenpA nucleosomes distinguish them from H3 nucleosomes and are important for defining centromere identity. First, the CenpA centromere-targeting domain (CATD), which is the region with the highest sequence divergence from H3 and sufficient for binding Scm3/HJURP, is critical for CenpA deposition at centromeres (69). Second, partly as a result of increased hydrophobicity of its C-terminal tail, CenpA confers preferential binding of CenpC, which provides the base for kinetochore assembly (118). Third, CenpA nucleosomes differ from H3 nucleosomes in that they wrap less DNA (~100–120 bp rather than 146 bp) and the terminal DNA is less tightly bound, which has important implications for recruitment of the CCAN kinetochore subcomplex (46, 65) (see below).

2.2. Subassembly II [Constitutive Centromere-Associated Network (CCAN)]

Human CCAN is made up of 16 proteins organized into 5 subcomplexes plus CenpC (70, 107, 181). The related Ctf19 complex (CTF19C) similarly has 5 subcomplexes in addition to CenpC/Mif2 made up of 14 proteins, the majority of which are recognizable orthologs of the human CCAN proteins (**Table 1**). Low sequence conservation and disparities in phenotype caused by loss of CCAN/CTF19C subunits led to questions regarding the extent of functional conservation of the yeast and human complexes. However, recent structural analyses of individual subcomplexes and the complete CCAN complex from yeast and human have revealed remarkable structural conservation of the entire complex (95–97, 190, 191, 246, 254, 261, 263, 270). Crucially, reconstitution and cryogenic electron microscopy (cryo-EM) of both the CTF19C and CCAN bound to CenpA nucleosomes indicate highly similar modes of binding (261, 263).

2.2.1. CCAN architecture. CCAN is built upon CenpC/Mif2, which has been termed the blueprint of the kinetochore (126). CenpC binds directly to the CenpA nucleosome and, despite being largely disordered, provides the structural platform upon which the kinetochore is assembled (126, 167, 199). The nucleosome recognition and kinetochore assembly functions are conferred by separate linear binding motifs. The N-terminal region of human CenpC, which contains a Mis12-interacting domain followed by motifs that interact directly with CCAN subcomplexes CenpLN and CenpHIKM, templates kinetochore assembly (126, 188, 191, 224, 263). Two related central and CenpC regions, each composed of a stretch of positively charged residues followed by two aromatic residues, bind to the acidic patch and C-terminal tail, respectively, on the CenpA nucleosome to specify the site of kinetochore assembly (30, 118). Either one of the central or CenpC domains appears to be sufficient for centromere targeting (248). Finally, the C-terminal region of CenpC dimerizes through its structured cupin domain, which, at least in vitro, allows it to bind two nucleosomes, though the significance of this for kinetochore function in vivo is unclear (163, 246).

Although not highly conserved at the sequence level, the overall organization of budding yeast CenpC/Mif2 is similar to that of human CenpC. In addition to connecting to the outer kinetochore through the Mis12 binding motif in its N terminus, budding yeast CenpC also binds CCAN, although this was found to involve the CenpQU (Okp1-Ame1) subunits rather than CenpLN and CenpHIKM as reported for human CenpC (56, 102). Resolving whether these observations underlie structural differences between the budding yeast and human kinetochores or different kinetochore assembly states awaits a complete picture of a fully assembled kinetochore in both systems. In particular, the intrinsic disorder of CenpC has made structural analysis challenging. A further notable difference is that budding yeast CenpC, in common with other nonmammals, lacks the central domain so that CenpA/Cse4 recognition occurs solely through the CenpC domain (45, 259).

Recent cryo-EM structures have shown that human CCAN subcomplexes represent structural modules with CenpOPQUR and CenpHIKM forming two lobes or pillars bound to either side of the arc-shaped CenpLN module. CenpTW forms a base, connecting the two pillars and creating a positively charged CenpLN central channel (191, 263). In cryo-EM structures of the CenpA nucleosome bound to CCAN, this CenpLN channel grips α-satellite linker DNA (263). These protein–DNA contacts appear to represent the major interaction surfaces between the assembled CCAN and CenpA supercomplex, apart from a small protein–protein interaction between CenpL and CenpA, together with the interactions between CenpC and the CenpA nucleosome described above (263). Interestingly, the CenpTWSX subcomplex, which includes four histone fold domains and is structurally related to the H3-H4 tetramer, wraps DNA as it emerges from the CenpLN

channel, introducing curvature into the DNA, which threads through a groove also supported by CenpI (263).

Budding yeast CTF19C has a remarkably similar architecture to human CCAN, although it forms a shallower, wider channel. This is in part due to the absence of CenpM, which is sandwiched between the two pillars of human CCAN, deepening the channel (96, 191, 261, 263). Unwrapped nucleosome DNA, rather than linker DNA, was observed to be gripped by the CTF19C channel (261). Whether DNA is topologically entrapped by CTF19C is also unclear, since CenpTW (Cnn1-Wip1) was not clearly resolved, though modeling indicates that Cnn1-Wip1 have the potential to close the channel (96, 97, 261). The yeast homologs of CenpS and CenpX (Mhf1 and Mhf2) do not appear to be kinetochore proteins (137). Budding yeast also lacks CenpR but substitutes it with Nkp1/Nkp2, forming a cap at the top of pillar 1 (191, 263). There is also evidence of functional divergence in human CCAN: The CenpOPQUR subcomplex is a central receptor for polo-like kinase 1 (Plk1) working alongside Bub1 (15, 116, 175, 226), and it displays Ndc80-like microtubule-binding activity via an N-terminal extension that is absent in budding yeast (190).

Complete CTF19C/CCAN structures are largely consistent with prior studies addressing the arrangement and interaction with the CenpA nucleosome, with one major exception. Isolated vertebrate CenpN binds directly to the L1 loop of nucleosomal CenpA, an interaction that is thought to be important for specifying the site of kinetochore assembly (30, 31, 38, 89, 188). However, in the context of the complete CCAN, CenpN binding to CenpA L1 loop would cause a major steric clash. If the architecture of the complete CCAN-CenpA-nucleosome structure represents that of a fully assembled kinetochore, it is reasonable to assume that CenpN binding to the L1 loop of CenpA is an important assembly intermediate. Similarly, the Ame1-Okp1 (CenpQU) heterodimer binds to the unmodified Cse4 (CenpA) N-terminal tail in budding yeast, and Cse4 and Ame1-Okp1 have been found in proximity by cross-linking mass spectrometry (9, 68). Whether this interaction is indicative of an assembly intermediate or representative of a full kinetochore assembly remains unclear.

2.2.2. Impact of CCAN subunit disruption. In budding yeast, three CCAN subunits (Okp1, Ame1, and Mif2) are encoded on essential genes, while the remainder are indispensable for viability—albeit associated with increased frequency of chromosome missegregation (52, 66, 162, 182, 198). The picture in humans is complicated, since (*a*) results from acute or chronic knockdown and knockout experiments can vary with regard to the penetrance of chromosome alignment phenotypes and (*b*) there is emerging evidence of cell type–specific requirements (70, 161, 175, 190, 200). Differences in essentiality may also underlie the extents to which various organisms rely on functional modules linking the centromeric nucleosome to the microtubule; that is, there are alternative molecular paths that involve distinct interactions between the CenpC and KMN and some organisms rely on some paths more than others (see Section 2.3.2).

2.3. Subassembly III (KMN + SA)

The outer kinetochore is built from the KNL1, MIS12, NDC80, and SKA complexes, which have distinct functions. The KNL1 complex is an assembly hub for regulators that signal the attachment state of the kinetochore. The MIS12 complex (MIS12C) connects the inner and outer kinetochore. The NDC80 and SKA complexes form the main microtubule-binding interface of the kinetochore, with the latter being the major microtubule receptor (257). All are essential genes in yeast with protein inactivation in humans impacting chromosome alignment (to varying extents; see relevant comments above on CCAN in Section 2.2.2).

The MIS12C assembles from four structural paralogs, Dsn1, Mis12, Nsl1, and Pmf1 (Dsn1-Mtw1-Nsl1-Nnf1 in yeast), which bundle in parallel to form an elongated 20-nm-long Y shape (56, 101, 157, 194, 196). The N-terminal regions of Dsn1-Nsl1 and Mis12-Pmf1 form the tips of the Y and connect to the inner kinetochore through a direct interaction of Mis12 with CenpC (224). The stalk of the Y links to both the NDC80 and KNL1 complexes (195).

The NDC80 complex (NDC80C) is a 62-nm dumbbell-shaped heterotetramer formed from the Ndc80/Hec1-Nuf2 and Spc24-Spc25 dimers. Each dimer forms an N-terminal globular domain and a coiled-coil stalk. The coiled-coil C termini of the two dimers intercalate in a tetrameric junction to assemble the NDC80C. A break in the Nuf2-Ndc80 coiled coil forms a loop that is reported to interact with other various kinetochore/microtubule proteins, depending on the organism (234), and provide rotational freedom. In the absence of microtubules, NDC80C jackknifes into an autoinhibited state (218). In cells, this jackknifed state correlates with SAC activation (Mad1-Mad2 binding) and may function as a microtubule occupancy sensor (10, 211, 247) (**Figure 2*a***, step 1). At the centromere-facing end, Spc24-Spc25 form RWD domains that bind MIS12C or CenpT, as part of two distinct connections between the inner kinetochore and microtubules (see Section 2.3.2). At the other end, Nuf2 and Ndc80 form calponin homology (CH) domains that comprise the main microtubule-binding interface of the kinetochore (35, 40, 41, 104, 240, 251–253). Part of the Ndc80 CH domain, known as the toe, interacts directly with the microtubule lattice, binding both at the interface between α- and β-tubulin monomers and at the interface between α- and β-tubulin dimers (7, 8). The disordered, positively charged, N-terminal tail of Ndc80, which has been extensively studied, also contributes to microtubule attachment, which is negatively regulated by phosphorylation (see Section 2.3.1 on error correction; reviewed in 257). NDC80Cs bind microtubule lattices with low affinity and prefer straight versus curved protofilaments (35). In vitro experiments show that clusters of two or more NDC80Cs can track with depolymerizing microtubules and can stall and rescue microtubule depolymerization in a force-dependent manner (245).

The KNL1 complex is a heterodimer of Knl1 and Zwint (known as Spc105 and Kre28 in budding yeast). A region toward the C terminus of Knl1, which is predicted to form a coiled coil, binds Zwint and is followed by tandem RWD domains that bind the stalk of Mis12 and a C-terminal motif in Nsl1 (195). The remainder of Knl1 is a large, disordered element (predicted ~400 nm in human) (79) containing a series of motifs that bind key kinetochore regulators to provide an assembly platform for signaling the attachment state of the kinetochore. These motifs include a binding site for the PP1 phosphatase close to the N terminus and multiple MELT motifs that, upon phosphorylation by the Mps1 kinase, dock a complex of the Bub1-Bub3 SAC proteins, which facilitates recruitment of Bub3-BubR1-PP2A (79, 130). The Bub1-Bub3 complex also recruits Mad1-Mad2 complexes that catalyze the generation of a wait anaphase signal (reviewed in 138 and references therein). A series of feedback and feedforward loops between Mps1/Aurora B kinases and PP1/PP2A regulates the stability of attachments and promotes checkpoint silencing (217).

Metazoans contain an additional outer subassembly component: the SKA complex (SKAC). Ska1, Ska2, and Ska3 form a trimer that dimerizes to form a W-shaped complex with a long axis of ~18 nm (1, 110, 222). At the tip of the W is the Ska1 microtubule-binding domain, which contains a winged helix–like domain (1), and an unstructured extension from Ska3, which mediates phospho-dependent interactions with the coiled coils of NDC80Cs and enhances microtubule binding (2, 32, 92, 268). SKACs are able to autonomously track with the ends of depolymerizing microtubules and interact with both straight and curved protofilaments (1, 92, 105, 108, 149, 172, 222). Unlike KMN, the SKAC is largely missing from unattached kinetochores and progressively loads as microtubules bind the kinetochore (13, 34). Experiments in vivo and in vitro with purified

proteins show that the SKAC operates as a load-bearing device within the kinetochore (13, 92). This feature of the SKAC is reinforced by NDC80C and reduces the detachment rate from depolymerizing microtubules (92).

The budding yeast Dam1 complex (DAM1C) is a heterodecamer of 10 polypeptides (**Table 1**) and is unrelated to the SKAC but performs an analogous and essential function. A single DAM1C heterodecamer forms a rod-shaped complex with a near-perpendicular Spc19-Spc34 protrusion in the middle of the rod (109). Rings are assembled from 16 DAM1C heterodecamers that make head-to-tail contacts and thereby encircle microtubules (109, 169, 204, 255). Each kinetochore appears to have two DAM1C rings (123). Like the SKAC, DAM1C association with kinetochores requires microtubules (144). The plus end–tracking protein, Bim1 (yeast EB1), binds to the DAM1C protrusion in a phospho-dependent manner, promoting its oligomerization and potentially handing over the assembly to Ndc80 (60). The DAM1C also has similar biochemical properties to the SKAC, acting as a force coupler through interactions with both microtubules and the NDC80C (133, 134, 236).

In mammals, the formation of a mature microtubule–kinetochore interface further involves recruitment of the microtubule-binding Astrin-Skap-MYCBP-LC8 complex, which is positioned close to the NDC80C (62, 71, 119, 153, 223). Unlike KMN-S, the Astrin-Skap complex is proposed to reduce friction in the kinetochore–microtubule interface (210).

In summary, KMN-S-Astrin-Skap (KMN-SA) is a key feature of mammalian kinetochores that enables coupling of chromosomes to dynamic microtubules, thus harnessing energy for powering chromosome movement. The molecular mechanisms are a combination of biased diffusion on the microtubule lattice (by NDC80C); binding to curved protofilaments (by Skap), which is a feature of growing and shrinking MT tips (87); or an encircling coupler (by DAM1C). Motorized tethering by kinesin and dynein motors also contributes (for a review and more discussion of the biophysics, see 12, 50, 147) (see Section 3.4).

2.3.1. KMN-SA is the major target for error correction processes. The resolution of improper kinetochore–microtubule attachments involves a number of tension-dependent and -independent mechanisms (for a detailed discussion, see 136). Briefly, the latter basic mechanism depends on geometric constraints (i.e., sisters are back to back) and the natural turnover rate of kinetochore–microtubule interfaces. The tension-dependent mechanism is linked to the Aurora B–dependent phosphorylation of key kinetochore substrates (including Ndc80, Knl1, and Ska1/Dam1). These modifications reduce the affinity of the kinetochore for microtubules, promoting either release or depolymerization (reviewed in 58, 257). We note that the SAC kinase Mps1 is also implicated in promoting biorientation, in part through phosphorylation of the Ska3 hinge region and Ndc80 tail (149, 215). A key challenge is to understand how different attachment states, for example, amphitelic versus syntelic versus merotelic, are coupled to changes in the phosphorylation state of the outer kinetochore. Spatial separation of kinases and substrates between the centromere and kinetochore or between intrakinetochore positions has been proposed (135, 136). Aurora B is localized to centromeres and kinetochores through multiple receptors, suggesting that both types of model may be relevant (28, 94). For example, preventing survivin-based Aurora B (Ipl1)-targeting in yeast (29) is compatible with tension sensing because the C-terminal region of Ctf19 is an Ipl1-binding site (68, 75). Nevertheless, once a kinetochore forms an end-on attachment, further attachment stabilization takes place due to the aforementioned maturation of the outer kinetochore, BubR1-PP2A activity, and recruitment of PP1 to Knl1 (217). Recruitment of PP2A and PP1 phosphatases likely overwhelms kinase activity and explains why metaphase kinetochores do not detach under natural fluctuations in tension.

2.3.2. Connectivity between subassemblies II and III. To act as a force coupler that allows chromosome movement, the kinetochore must maintain connectivity between the inner and outer kinetochore. Several distinct paths of connectivity have been described, and the relative importance of these differs between organisms. Details of phosphorylation and other posttranslational modifications that modulate subcomplex interactions are also beginning to emerge. However, despite remarkable insights into the organization of individual subcomplexes, the overall architecture of a complete kinetochore and the regulatory events that permit this superassembly have yet to be revealed. Two pathways of connectivity between the inner and outer kinetochore exist in both yeast and humans and involve disordered extensions of CenpC and CenpT that have the potential to project several tens of nanometers outwards from CCAN (**Figure 2*a***, step 2):

1. CenpC is bound directly by MIS12C, which, in turn, binds one NDC80C and a single KNL1C. The CenpC interaction with MIS12C is facilitated by phosphorylation of two conserved serine residues on Dsn1 by Aurora B (3, 25, 90, 124, 202, 273). This displaces an autoinhibitory fragment, exposing a binding site on Mis12/Mtw1 for CenpC/Mif2 (56, 194). In yeast, Mis12/Mtw1 also binds Ame1 (102), but the reciprocal third CenpU-Mis12 linkage has not yet been shown in humans. An autoinhibitory mechanism similarly prevents CenpC that is not bound to the centromeric nucleosome from binding to MIS12C (121). In yeast, Aurora B may further stabilize the kinetochore through phosphorylation of CenpA (23).
2. CCAN subunit CenpT can bind directly to two NDC80Cs (56, 77, 152, 157, 177, 187, 194, 202, 221, 224). Both MIS12C and CenpT bind the NDC80C through the same interaction surface in the RWD motifs of Spc24-Spc25 (56, 100, 152, 177, 221). CenpT can also recruit one MIS12C, which, in turn, brings an additional NDC80C (104).

In sum, each CCAN has the potential to recruit five NDC80Cs, three MIS12Cs, and two KNL1s, with CenpC and CenpT providing independent links to the outer kinetochore (113, 126, 161, 232). Whether these different subpopulations of NDC80 have differential functions or mechanical properties remains unknown. These connectors are flexible (211) and likely operate as a compliant linkage between CCAN and KMN-SA that can withstand hundreds of piconewtons of force when microtubules are driving chromosome movement (232, 264) (**Figure 2*a***).

2.4. Subassembly IV (Corona: a Metazoan Specialization)

The corona is the outermost layer of the kinetochore and was originally identified in electron micrographs as a diffuse fibrous network that appears when microtubules are not engaged with the kinetochore (114). The corona is highly plastic, able to undergo a time-dependent expansion to form crescents, and ultimately a structure that can encircle the entire pair of sister chromatids at the primary constriction (for review, see 128). Several proteins are known to be part of this expansion, including RZZ-S, DD, CenpE, CenpF, Clasp, Clip170, Mad1-Mad2, Cyclin B, and Nup107–Nup160 (**Table 1**).

The core of the fibrous corona is the RZZ complex, which dimerizes to form a head-to-tail overlapping 42-nm-long dimer that recruits Spindly through an interaction with Rod's beta-propeller (173, 189, 203). This interaction requires the farnesylation of the C-terminal CAAX box, which releases Spindly from an autoinhibited state (212). Spindly, in turn, recruits the DD motor complex, which is important for future compaction (see below). RZZ-S then drives the process of kinetochore expansion, and this requires Mps1 phosphorylation of Rod (209, 212) and Zwilch (189). Early experiments showed how purified Rod-Zw10 dimers can self-assemble into filament-like structures (189) although self-assembly of full RZZ complexes requires farnesylated

Spindly with Mps1 acting as a catalyst (203, 212). The similarity of Rod to membrane-coating proteins (i.e., Clathrin and COP I/COP II) that can form high-order assemblies points to common mechanistic principles (43, 173).

Cells deficient of RZZ do not form a fibrous corona when observed by electron microscopy (209). However, other corona proteins remain kinetochore bound, albeit without undergoing expansion [i.e., CenpF/CenpE/Mad1 (42, 209)]. Corona proteins must therefore dock through RZZ-independent mechanisms, presumably to the outer kinetochore. CenpF is an ~3,000-amino-acid microtubule-binding protein that contains extensive coiled coils, enabling it to physically bridge the corona and outer kinetochore, where it docks onto the kinase domain of Bub1 (18, 42, 200). Similarly, CenpE, which is a member of the Kinesin-7 family, docks through its C terminus to the kinase domain of BubR1, while the N-terminal motor domain is projected via the extended coiled-coil region into the corona. Both BubR1-CenpE and Bub1-CenpF are assembled onto Knl1 (which does not show expansion behavior). Their common features have raised the possibility they are distantly related paralogs (42).

How the RZZ-S assembles onto subassembly III (on the outer kinetochore) is less well understood. Depletion of Zwint or Knl1 reduces—but does not abolish—the binding of RZZ to kinetochores (129, 225, 242). Consistently, loss of the Knl1-dependent BubR1-CenpE or Bub1-CenpF linkage does not affect RZZ binding (14, 51, 209). Thus, there must be linkages beyond Knl1 axes, with one possibility being the reported interaction between Rod and the NDC80C (33). This could be consistent with nanoscale mapping experiments that locate RZZ to the outside of, but close to, the Ndc80 N terminus on unexpanded kinetochores (211).

It is well established that Mad1 is recruited to kinetochores through a direct interaction with Bub1, which is located in the outer kinetochore and does not itself undergo expansion [Bub1 is the only kinetochore receptor for Mad1 in yeast (146)]. The C terminus of Mad1 (close to the Mad2 binding site) is located proximal to outer kinetochore Bub1, while the N terminus is ~50 nm outside. This is consistent with Mad1 bridging the outer kinetochore and the corona (211). As kinetochores expand, Mad1-Mad2 is enriched in the corona, suggesting a second population and receptor. Several lines of evidence support this idea: (*a*) Mad1-Mad2 binds detached coronas that do not contain Knl1-Bub1 (189), (*b*) Rod-deficient cells (which lack the corona) still recruit Mad1 to outer kinetochores, and (*c*) the N terminus of Mad1 binds directly to corona-associated Cyclin B (4).

Overall, current data suggest that kinetochores project several highly flexible molecules beyond the outer kinetochore to form, with RZZ-S, a protocorona that can operate as a nucleating center for expansion through Mps1-triggered self-assembly of corona proteins (**Figure 2*b***). These new self-assemblies would not necessarily connect to the outer kinetochore, thus explaining how the corona can be disassociated as a single unit from the kinetochore (189).

As end-on attachments form, the corona disassembles because the minus end–directed motor activity of DD strips the corona from the kinetochore. Hence the corona sets up its own destruction through recruitment of DD and its activator Spindly (78, 103, 212). This could be a passive process that initiates the moment a microtubule forms an end-on attachment. Consistent with this idea is the finding that loss of Mps1 activation is neither necessary nor sufficient to trigger corona disassembly (212). Stripping may also provide a feedback to further promote end-on attachment by relieving inhibition of NDC80C by RZZ (33)—perhaps gating the straightening of NDC80Cs and associated loss of Mad1-Mad2 (211). However, regulation is clearly important because stripping does not fully eliminate all corona proteins from kinetochores (Mad1-Mad2 is an exception). This likely reflects the observations that some factors are needed to trigger expansion (see above), while others, for example, CenpF and CenpE, are directly implicated in coupling kinetochores to dynamic end-on attached microtubules (88, 115, 244). CenpF (via Nde1/Ndel1/Lis1)

also functions as a dynein brake to limit stripping of corona cargoes (14). This is noteworthy because slowing or accelerating the stripping process leads to mitotic defects (14, 78). Determining whether each corona cargo is stripped at different times and kinetics and how this is coordinated with cycles of microtubule attachment and detachment will be important.

Chiasmata: product of crossover recombination between homologous chromosomes during meiosis; chiasmata and distal arm cohesin hold homologous chromosomes together during meiosis I

3. STRUCTURAL ORGANIZATION OF CENTROMERIC AND PERICENTROMERIC CHROMATIN

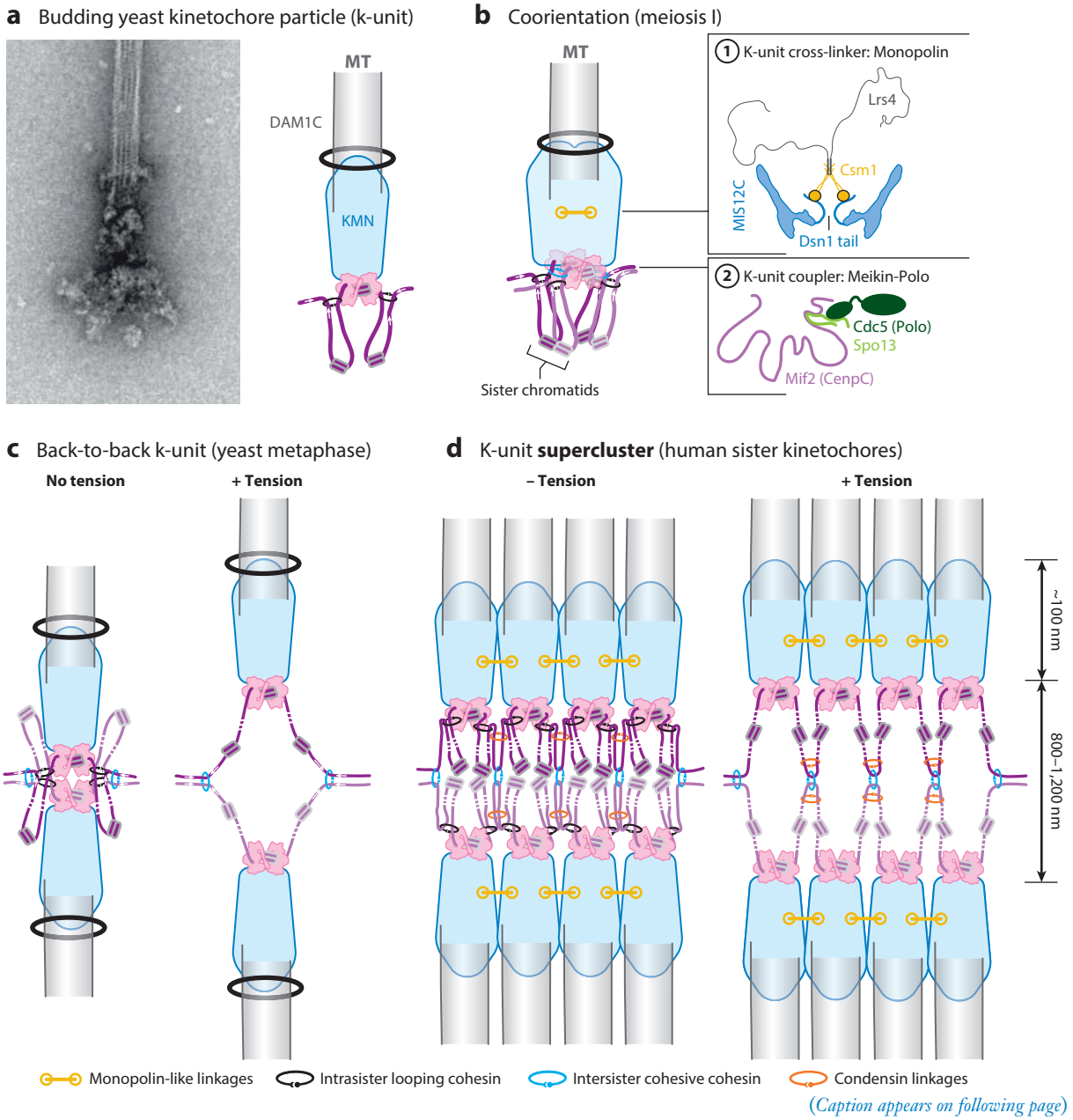
Regional centromeres contain blocks of CenpA nucleosomes linearly interspersed with lysine 4 dimethylated H3-containing nucleosomes in which CenpA nucleosomes are at a density of just ~1:25 CenpA to H3 (20, 22, 207, 229, 241). CenpA nucleosomes are gathered at one face of the chromatin to form a kinetochore assembly platform, with H3K4 dimethylated nucleosomes residing underneath (229). Evidence from chicken neocentromeres suggests that centromeric nucleosomes are densely packed (176). Intriguing recent data showed that the kinetochore protein CenpN is capable of stacking CenpA nucleosomes, suggesting that it may contribute to the higher-order structure of centromeric chromatin (272). This core domain of centromeric chromatin is flanked by H3 lysine 9 trimethylated (H3K9Me3) and HP1-bound pericentromeric heterochromatin, which is highly enriched with the chromosome-organizing complex, cohesin (76). In fission yeast, HP1 is required for pericentromeric cohesin enrichment, while in humans HP1 may have an indirect role (17, 127, 178, 260). The repetitive nature of centromeric chromatin has precluded a detailed picture of its architecture, but super-resolution and chromatin-unfolding experiments in chicken cells have suggested a modular structure (207, 241); proposals include a solenoid or a boustrophedon, meaning a structure where the chromatin folds back on itself in alternative lines from left to right and right to left (207, 229).

By contrast, the absence of heterochromatin and repetitive sequences has allowed the application of next-generation sequencing–based approaches to probe the structure of budding yeast pericentromeres (**Figure 3*a***). Cohesin is enriched over ~20 kb surrounding the ~125-bp yeast centromere and plays a central role in pericentromere folding (64, 66, 67, 80, 184, 250). Cohesin is specifically targeted to centromeres through a direct interaction between a conserved patch on the Scc4 subunit of the Scc2-Scc4 cohesin loader and the N terminus of the kinetochore component Ctf19 (a subunit of CCAN) upon its phosphorylation by the Dbf4-dependent kinase (DDK) (98, 99). Although initial studies had suggested that the budding yeast pericentromere forms a cruciform structure (266), later high-resolution chromosome capture (Hi-C) analyses found that centromeres strongly suppressed interactions between flanking chromatin on each side (49, 139, 184, 219), discounting this model. Instead, Ctf19-anchored cohesin extrudes an intrachromosomal loop on each side of the centromere until it is stalled by convergent gene pairs that form boundaries at the pericentromere borders, which is also where cohesin links the sister chromatids (184). Upon sister kinetochore biorientation in mitosis, the loop-extruding cohesin is released from chromosomes, and pericentromeres adopt a V-shaped structure with borders at their apices (184) (**Figure 3*c***).

4. MEIOTIC KINETOCHORES

Kinetochore adaptations during meiosis support the segregation of homologous chromosomes in meiosis I followed by sister chromatids in meiosis II (27, 63) (**Figure 1*d***). Following DNA replication and the establishment of sister chromatid cohesion in S phase, homologous chromosomes pair and undergo meiotic recombination. This generates crossovers, which are sites of exchange of genetic material between the homologs and the precursors to chiasmata. Together with sister chromatid cohesion, chiasmata hold homologs together, allowing for their biorientation on the

meiosis I spindle. During meiosis I, sister kinetochores are monooriented so that they attach to microtubules from the same pole. Homolog segregation during meiosis I is triggered by loss of arm cohesion, while pericentromeric cohesion is protected to keep sister chromatids together. During meiosis II, sister kinetochores biorient, pericentromeric cohesin is deprotected, and sister chromatids segregate to opposite poles. Kinetochores play central roles in multiple aspects of meiosis, including (*a*) preventing crossover recombination in pericentromeres; (*b*) homolog



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Supercluster for centromere–kinetochore multimerization. (*a*, *left*) Cryo-electron microscopy image of isolated budding yeast kinetochore particles bound to microtubules. In the image, globular domains contact the microtubule, which is encircled by a ring-like structure, likely DAM1C. There is also a central hub that does not contact the microtubule directly. Panel *a* adapted with permission from Reference 82. (*a*, *right*) Model for the architecture of a single k-unit where one kinetochore superassembly contacts one microtubule, as in budding yeast. Each CCAN anchors cohesin, which forms intramolecular loops on each side of the kinetochore. (*b*) Model for sister kinetochore coorientation during meiosis I in budding yeast. Two k-units—the sister kinetochores—are clamped together in a side-by-side orientation due to two kinds of linkages: Monopolin binds to the Dsn1 subunit of the MIS12C and fuses the sister kinetochores together, and Spo13-Polo associates with CenpC/Mif2 and promotes coorientation, possibly by facilitating cohesin-dependent linkages of sister centromeres. Note that a fused pair of sister kinetochores binds a single microtubule in meiosis I (259). (*c*) Schematic showing the architecture of the budding yeast kinetochore in mitosis in the presence and absence of spindle tension. (*Left*) CCAN-anchored cohesin extrudes a loop on either side of the centromere until blocked by convergent genes at pericentromere borders. Borders also retain intersister, cohesive cohesin. This state, as shown, is short-lived because the attachment of sister kinetochores to microtubules from opposite poles results in the generation of tension. (*Right*) Under tension, the chromatin loops extend into a V-shaped structure. Intramolecular, loop-extruding cohesin slides off, but intermolecular, cohesive cohesin is trapped at the borders and holds the sister chromatids together. (*d*) Speculative model for the architecture of the mammalian kinetochore, inspired by the structure of the budding yeast kinetochore and pericentromeric chromatin in mitosis and meiosis. Ordered arrays of k-units are clustered together. This clustering is facilitated by cohesin anchored on CCAN and stabilized by cross-linkers between KMN, analogous to monopolin. Chromatin-organizing complexes such as condensin may further serve to stabilize interactions between adjacent k-units. Abbreviations: CCAN, constitutive centromere-associated network; Cenp, centromere protein; MT, microtubule.

pairing, which is the process by which homologs find each other as a prerequisite for their recombination; (*c*) nucleation of the synaptonemal complex, a zipper-like structure that assembles between the homologous chromosomes to support meiotic recombination; (*d*) the establishment of pericentromeric cohesin protection; and (*e*) sister kinetochore monoorientation (see reviews in 27, 63, 131, 156, 179). Here, we focus on our current understanding of how meiotic kinetochore architecture is adapted to bring about these functions.

4.1. Architecture of Meiotic Kinetochores

Budding yeast meiotic kinetochores have a similar composition to mitotic kinetochores, with the addition of meiosis-specific factors (26, 214) (see also Section 4.2). However, the pathways that govern the assembly and maintenance of kinetochores may differ in meiosis compared to mitosis since kinetochore integrity and viability in budding yeast meiosis rely on CCAN subunits that are dispensable for mitotic growth (26). In the budding yeast mitotic cell cycle, kinetochores remain fully assembled except for a brief period during S phase (125). By contrast, kinetochore subassembly III disassembles during meiotic prophase as a result of both reduced synthesis and increased degradation of the Ndc80 protein (11, 36, 37, 166, 168). This is reminiscent of kinetochores in mammalian somatic cells where subcomplex III assembles only at mitotic entry to make kinetochores competent to bind microtubules (91). Ndc80 degradation in meiotic prophase is promoted by Aurora B kinase (Ipl1), which also severs kinetochore–microtubule attachments, reminiscent of its role during error correction in mitosis (36, 166) (see Section 2.3.1). The loss of the outer kinetochore may facilitate the remodeling of the kinetochore for meiosis. Indeed, components of the synaptonemal initiation complex and monopolin are recruited by the inner kinetochore in meiotic prophase (26). Kinetochore disassembly in meiotic prophase may also prevent centromere–microtubule attachments at a time when telomeres are attached to microtubules to bring about the coordinated chromosome movements known as the meiotic bouquet, which is thought to facilitate homology search (220). However, preventing Ndc80 degradation in meiotic prophase does not have any obvious adverse effects on unchallenged meiosis, so the role of outer kinetochore disassembly remains unclear (36).

Sister kinetochore monoorientation: the state in which sister kinetochores are attached to microtubules from the same pole (also called coorientation)

Mammalian meiotic kinetochores have not been intensively studied, but components of the major subassemblies appear to be present (186, 276). Furthermore, kinetochores in human oocytes are prone to fragmentation (275), raising the interesting possibility that the links between individual kinetochore assemblies (which we refer to as k-units below) become weakened over time.

4.2. Sister Kinetochore Monoorientation

The segregation of homologs, rather than sister chromatids, in meiosis I underlies Mendel's law of segregation and requires that sister kinetochores be monooriented. Sister kinetochore monoorientation was shown to be a property of the kinetochore, rather than microtubules, by pioneering transplantation experiments in grasshopper spermatocytes (185) and more recently in mouse oocytes (180). Electron microscopy in male *Drosophila* revealed that sister kinetochores orient in a side-by-side fashion and converge into a single structure in meiosis I, while light microscopy in maize indicated that sister kinetochores may be fused by a Mis12 bridge (81, 143). Further evidence for sister kinetochore fusion in meiosis I came from biophysical experiments, which showed that kinetochore particles isolated from budding yeast meiosis I cells are larger and can withstand higher forces than those from mitotic or meiosis II cells (214).

The molecular basis of monoorientation is poorly understood. In budding yeast, a four-subunit complex called monopolin is required for monoorientation in vivo and is sufficient to alter the biophysical properties of kinetochores in vitro (193, 201, 214, 237). Monopolin comprises two nucleolar proteins, Lrs4 and Csm1; CK1δ kinase Hrr25; and a meiosis-specific protein, Mam1. The Polo kinase Cdc5 promotes the release of Lrs4 and Csm1 from the nucleolus to form the four-protein monopolin complex at kinetochores (193, 201). Monopolin is a V-shaped complex in which Csm1 homodimers are linked at the end of their coiled-coil N termini by two Lrs4 subunits (47, 48) (**Figure 3*b***). The Csm1 globular heads at the apices of the V bind to a region in the N terminus of Dsn1 that is also required for sister kinetochore monoorientation (48, 197, 216). A flexible linker separates Mam1's C-terminal domain, which wraps around a Csm1 head, and its N-terminal domain, which binds CK1δ to tether it to the complex (47, 265). Monopolin is thought to fuse sister kinetochores by bridging Dsn1 molecules in sister kinetochores (47, 48, 197). A key question in this model is how does monopolin avoid cross-linking Dsn1 molecules in the same kinetochore or homologous kinetochores? It is likely that phosphorylation controls monoorientation specificity. Indeed, two residues (S109 and S110) within the monopolin-binding site on Dsn1 are phosphorylated in vivo, and phosphomimetic mutations increase Csm1–Dsn1 binding in vitro, though whether CK1δ or some other kinase is responsible remains unknown (197). Mam1 has not been identified outside budding yeast, CK1δ is widely conserved, and although homologs of Csm1-Lrs4 exist in some species, they are dispensable for sister kinetochore monoorientation (83, 197). Therefore, monopolin-directed monoorientation may be a point centromere adaptation.

A group of meiosis-specific kinase regulators (Mokirs), which include Spo13 in budding yeast, Moa1 in fission yeast, and Meikin in mouse, appear to have a more widespread role in sister kinetochore monoorientation (74). Mokirs are not conserved at the sequence level, except at a small motif that binds Polo kinase through its Polo-binding domain (PBD) and recruits it to kinetochores. In the case of Moa1 and Meikin, kinetochore association occurs through a direct interaction with a region near the C terminus of CenpC (24, 73, 122, 151, 158, 233) (**Figure 3*b***). The critical role of Spo13 and Moa1 in monoorientation appears to be recruitment of Polo kinase to kinetochores (73, 148, 170). Forced kinetochore association of budding yeast Polo kinase Cdc5 to kinetochores induces monoorientation independently of monopolin, suggesting a mechanism in common with organisms that lack monopolin (73). However, retention of monopolin at kinetochores in meiosis I requires Spo13, indicating that it elicits monoorientation through both

monopolin-dependent and -independent mechanisms (117, 142). Mokir-Polo substrates responsible for kinetochore monoorientation have not been identified; however, budding yeast monopolin subunit Lrs4 is one likely target (158). Another attractive candidate is cohesin at core centromeres, which is required for monoorientation in budding yeast, fission yeast, and mouse and requires fission yeast Moa1 for its establishment (16, 174, 180, 213, 249). In further support of this idea, merged meiosis I kinetochores in mouse oocytes are individualized in anaphase I, dependent on separase activity, though whether cohesin is the relevant substrate has not been demonstrated (85, 180). Meikin is also cleaved by separase, generating a fragment that retains the ability to bind Polo and kinetochores, but with a distinct function in promoting chromosome alignment in meiosis II (151). Understanding exactly how Mokirs and cohesin direct monoorientation and the relationship between them is an important priority for the future.

In human oocytes, our understanding lags far behind. Sister kinetochores appear unfused in meiosis I, and the distance between them increases with maternal age (186, 276). This is a potential cause of the high levels of aneuploidy characteristic of human oocytes, and although the underlying molecular reasons are unknown, age-related cohesin loss could be a contributing factor (84).

5. WORKING MODEL FOR THE KINETOCHORE–CENTROMERE SUPERCLUSTER

The modular hierarchical architecture of the kinetochore is largely conserved, strongly suggesting that regional kinetochores may resemble repeated arrays of the budding yeast kinetochore, though the exact nature of this could take many forms. Expanding on the original idea from Brinkley and colleagues (277) as extended by Musacchio and colleagues (192) to include the underlying chromatin, we propose molecular ideas for how regional kinetochores are built from multiple kinetochore units (herein termed k-units), where one k-unit is equivalent to the one kinetochore–one microtubule yeast kinetochore (**Figure 3*a***). The yeast k-unit is built around a single octameric Cse4 nucleosome (111) that wraps *CEN* DNA and associates with two CTF19Cs when reconstituted (254, 261). These k-units have been isolated from cells and can be visualized by electron microscopy (82) (**Figure 3*a***). The copy number of other factors has been estimated in vivo and points to the presence of 2 CTF19Cs, ~6 MIS12Cs, and ~8 NDC80Cs. This agrees well with the stoichiometry of CCAN versus KMN assemblies (see Section 3.3.1):

$$2^{*}\mathrm{CCAN}^{*} \rightarrow 2^{*}\mathrm{CenpC}\,(2\,\mathrm{NDC80C}) + 2^{*}\mathrm{CenpT}\,(6\,\mathrm{NDC80C}) = 8\,\mathrm{NDC80C}.$$

Similar counting experiments in human cells estimated ~244 NDC80Cs per kinetochore (231). Recent electron tomography now indicates there may be only ~10 microtubules per human kinetochore (120, 183, 271). This gives ~24 NDC80Cs per microtubule (MT) in humans, hinting that either there are two k-units per microtubule or the number of NDC80Cs is overestimated. The reverse calculation starting from the estimated number of nucleosomes per kinetochore (22) gives

$$44\,\mathrm{CenpA-nucleosomes} \rightarrow 88^{*}\mathrm{CCAN} \rightarrow 352^{*}\mathrm{NDC80} = 35^{*}\mathrm{NDC80C/MT}.$$

This equation assumes that every CenpA nucleosome brings 2*CCAN. Given the degree of structural conservation, we suggest that this is not the case and that the number of NDC80Cs per k-unit is ~10 with a fraction of CenpA nucleosomes in position to bind CCAN. Future work using knock-in human cell lines rather than transgenes should settle this. It will then be crucial to measure molecule numbers for all other components, defining stoichiometries and building molecular-scale models of the full ensemble.

How these complexes and proteins are arranged within the k-unit is also of importance. Eyeballing the electron micrographs of yeast kinetochore particles suggests an ordered structure with

what are presumably NDC80Cs projecting outwards along the microtubule axis from an inner core (**Figure 3*a***). High-resolution fluorescence microscopy experiments provide evidence that NDC80Cs (as well as Mad1 and RZZ) have a high nematic order (a measure of the degree of alignment of molecules) in human kinetochores (211). This ensemble-level view further suggests that k-units must be well aligned. Consistently, fluorescence resonance energy transfer (FRET) experiments show that Ndc80-Nuf2 clusters and aligns in both yeast and humans, while only in the latter do Mis12-Spc25 components cluster (132). This hints at differences that likely reflect analyses of single k-units versus k-unit arrays.

Alignment and clustering of k-units into a single unidirectional microtubule-binding surface would require that individual k-units cluster together in a side-by-side manner to form a compound kinetochore (**Figure 3*d***). We suggest that two types of linkages couple adjacent k-units: (*a*) protein–protein bridges juxtaposing KMN assemblies in adjacent k-units and (*b*) topological chromatin organizers that link loops anchored at each k-unit. We speculate about the molecular nature of both types of linkages, taking inspiration from yeast pericentromeres and meiotic kinetochores, respectively.

5.1. Inter-K-Unit Protein Cross-Linkers

An innovation that is required at regional centromeres but not point centromeres is the ability of k-units on each chromosome to act in unison and form a single, complex kinetochore. We invoke a requirement for k-unit to k-unit cross-linkers in the formation of a compound kinetochore. Such cross-linkers are already known to function in budding yeast meiosis, where the monopolin complex bridges two microtubule-binding sites representing the single k-unit sister kinetochores, through a direct interaction with the MIS12C subunit, Dsn1 (47, 48, 197, 214). Similarly, the Pcs1-Mds4 complex, which is the fission yeast equivalent of the Csm1-Lrs4 monopolin sub-complex of budding yeast, clamps microtubule-binding sites together in mitosis to prevent merotely—the attachment of a single kinetochore to microtubules from opposite poles (83). Therefore, the idea that monopolin also links k-units in the compound mammalian kinetochore by bridging their KMN assemblies is attractive. However, monopolin orthologs have not been identified in metazoans, though they are found in some plants (197), and factors that link k-units have not been described. This suggests that as-yet-unidentified proteins might perform this function to stabilize the compound kinetochore.

5.2. Inter-K-Unit Chromatin Linkages

The ensemble emerging from multiple k-units and hundreds of proteins is not static and should not be thought of as ribosome-like: The shape of the kinetochore is heterogeneous and can be deformed along its microtubule axis with the outer kinetochore capable of swiveling and tilting relative to the inner—all aspects responding to changes in microtubule binding and/or force (150, 205, 211, 227, 247). This likely reflects a degree of spacing and flexibility between k-units. At the same time, there must be sufficient stiffness to withstand force, and k-units should presumably be cooriented.

Inspired by the structure of the yeast pericentromere where cohesin organizes separate chromatin loops on each side of the centromere, we suggest that each k-unit may adopt a bilateral loop structure, which would facilitate the coalescence of k-units into a complex kinetochore. Interestingly, CenpU harbors a cohesin-binding motif (145), raising the possibility that human CCAN anchors loop-extruding cohesin similar to yeast Ctf19C, albeit through a different CCAN subunit (98, 184) (**Table 1**). Reminiscent of the convergent genes at pericentromere borders in yeast (184), human k-units may be flanked by transcriptional units that act as boundaries to halt

loop extrusion. There is abundant evidence for noncoding transcription in centromeres supporting this possibility (141). Compound kinetochores may also require stabilization via intramolecular linkages between chromatin loops or between CenpA chromatin blocks. The condensin complex may contribute to this function since it is required for centromere rigidity and kinetochore geometry in both yeast and humans (206, 228, 243).

5.3. Other Models

Our supercluster k-unit model for the mammalian kinetochore is broadly compatible with previous proposals. Hill's (93) sleeve model asserts that individual microtubules insert into channels on the outer surface of the kinetochore to form multiple low-affinity binding sites. In our model, each k-unit would be equivalent to a single channel. Our repeating k-unit model is also consistent with chromatin unfolding data (241) and recent work demonstrating the importance of cohesin and condensin in defining microtubule-binding domains of kinetochores (212a). The fibrous network model advocates that a flexible meshwork of NDC80Cs on the surface of the outer kinetochore embeds microtubule ends (57). Zaytsev and colleagues (267) argue that NDC80Cs have low cooperativity and make multiple low-affinity and independent interactions with microtubules rather than acting as part of an oligomeric assembly. The clustered k-units we propose could result in overlapping NDC80C extensions to form such a meshwork and be compatible with independent NDC80C binding, consistent with all of these models.

6. CONCLUSIONS

The past few years have seen major advances in our understanding of kinetochore biology. The molecular structure of the majority of individual subcomplexes has emerged, and we now have a working model for how they connect together into a microtubule-binding supercluster. Questions of how force is generated at kinetochores and coupled to chromosome movement have begun to be addressed, owing to developments in technology that have allowed physical properties to be measured, although making such measurements in living cells remains a major challenge, with exciting recent progress in this direction (230). Kinetochores have also been revealed to influence and organize the surrounding chromatid, establishing them as much more than machines that couple chromosomes to microtubules. Indeed, in situ cryo-electron tomography now shows how the human kinetochore is sitting within a centromeric chromatin pocket with expected filament-like linkages to microtubules visible (271).

In this review, we have focused on the best-understood kinetochores—those of budding yeast and humans, emphasizing their similarities and differences (see **Figure 1*a***, which highlights the dramatic difference in scale from the spindle-level viewpoint; **Table 1**). Nevertheless, the structural organization of yeast and human kinetochore subcomplexes is remarkably conserved, leading us to propose a modular k-unit repeat structure for the human kinetochore, based on the simpler yeast kinetochore. The deviations in complexity presumably reflect the need to upscale the kinetochore in humans. Such upscaling allows the attachment of multiple microtubules to a single chromosome, which together form a k-fiber, providing resilience for the movement of larger chromosomes over longer distances. The multimicrotubule compound kinetochore also signifies a change in force couplers from a ring around the microtubule (DAM1C; yeast) to a system involving coupling Ndc80 to the lateral surface of microtubules (Ska; humans). A further innovation in human, but not yeast, kinetochores is the corona, which can undergo considerable expansion. In human cells, but not yeast, the nuclear membrane breaks down at mitotic entry, spilling chromosomes into the cytoplasm and posing a significant challenge for kinetochore capture by microtubules at metaphase. The corona may have evolved to meet this challenge by providing a larger surface

area both for kinetochore capture and to generate a robust wait anaphase signal in response to unattached kinetochores.

Building on this foundational work, the next frontiers in kinetochore research are to reveal the ultrastructure and dynamics of the kinetochore in vivo. Atomic resolution cell biology will uncover how kinetochores bind microtubules and how different microtubule-binding sites are coordinated within a single kinetochore. To dissect mechanisms underlying canonical and noncanonical functions of kinetochores, structure-guided designer mutations are needed to disrupt key interfaces, preclude posttranslational modifications, and prevent enzyme docking. Much is also to be learned from studying the diversity of kinetochores. This includes organisms that use a variation on the theme of yeast and human kinetochores discussed here, such as CCAN-lacking fruit flies and holocentric worms, and those with a completely distinct blueprint for kinetochores, such as noncanonical trypanosomes and dinoflagellates where the kinetochore remains embedded in the nuclear envelope (59). Kinetochore proteins can even play roles away from the chromosome, having been shown to interact with cytoplasmic microtubules to direct neuronal development (reviewed in 55). Only through analysis of kinetochores and their constituent parts in these distinct contexts will we gain a holistic view of kinetochore assembly and function.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Lina Germanova for discussion and help developing the approximate scale model of kinetochore architecture and Alex Zwetsloot for DD schematics. We apologize to our colleagues whose work we could not cite due to space. A.D.M. and A.L.M. are supported by a Wellcome collaborator award (215625), Investigator awards (106151 to A.D.M. and 220780 to A.L.M.), a Wellcome Centre award (203149), and the Biotechnology and Biological Sciences Research Council (BB/R009503/1 to A.D.M.).

LITERATURE CITED

1. Abad MA, Medina B, Santamaria A, Zou J, Plasberg-Hill C, et al. 2014. Structural basis for microtubule recognition by the human kinetochore Ska complex. *Nat. Commun.* 5:2964
2. Abad MA, Zou J, Medina-Pritchard B, Nigg EA, Rappsilber J, et al. 2016. Ska3 ensures timely mitotic progression by interacting directly with microtubules and Ska1 microtubule binding domain. *Sci. Rep.* 6(1):34042
3. Akiyoshi B, Nelson CR, Biggins S. 2013. The Aurora B kinase promotes inner and outer kinetochore interactions in budding yeast. *Genetics* 194(3):785–89
4. Allan LA, Camacho Reis M, Ciossani G, Huis in 't Veld PJ, Wohlgemuth S, et al. 2020. Cyclin B1 scaffolds MAD1 at the kinetochore corona to activate the mitotic checkpoint. *EMBO J.* 39(12):e103180
5. Allshire RC, Karpen GH. 2008. Epigenetic regulation of centromeric chromatin: old dogs, new tricks? *Nat. Rev. Genet.* 9(12):923–37
6. Allu PK, Dawicki-McKenna JM, Van Eeuwen T, Slavin M, Braitbard M, et al. 2019. Structure of the human core centromeric nucleosome complex. *Curr. Biol.* 29(16):2625–39.e5
7. Alushin GM, Musinipally V, Matson D, Tooley J, Stukenberg PT, Nogales E. 2012. Multimodal microtubule binding by the Ndc80 kinetochore complex. *Nat. Struct. Mol. Biol.* 19(11):1161–67
8. Alushin GM, Ramey VH, Pasqualato S, Ball DA, Grigorieff N, et al. 2010. The Ndc80 kinetochore complex forms oligomeric arrays along microtubules. *Nature* 467(7317):805–10

9. Anedchenko EA, Samel-Pommerencke A, Tran Nguyen TM, Shahnejat-Bushehri S, Pöpsel J, et al. 2019. The kinetochore module Okp1^{CENP-Q}/Ame1^{CENP-U} is a reader for N-terminal modifications on the centromeric histone Cse4^{CENP-A}. *EMBO J.* 38(1):e98991
10. Aravamudhan P, Goldfarb AA, Joglekar AP. 2015. The kinetochore encodes a mechanical switch to disrupt spindle assembly checkpoint signalling. *Nat. Cell Biol.* 17(7):868–79
11. Asakawa H, Hayashi A, Haraguchi T, Hiraoka Y. 2005. Dissociation of the Nuf2-Ndc80 complex releases centromeres from the spindle-pole body during meiotic prophase in fission yeast. *Mol. Biol. Cell* 16(5):2325–38
12. Asbury CL, Tien JF, Davis TN. 2011. Kinetochores' gripping feat: conformational wave or biased diffusion? *Trends Cell Biol.* 21(1):38–46
13. Auckland P, Clarke NI, Royle SJ, McAinsh AD. 2017. Congressing kinetochores progressively load Ska complexes to prevent force-dependent detachment. *J. Cell Biol.* 216(6):1623–39
14. Auckland P, Roscioli E, Coker HLE, McAinsh AD. 2020. CENP-F stabilizes kinetochore-microtubule attachments and limits dynein stripping of corona cargoes. *J. Cell Biol.* 219(5):e201905018
15. Bancroft J, Auckland P, Samora CP, McAinsh AD. 2015. Chromosome congression is promoted by CENP-Q- and CENP-E-dependent pathways. *J. Cell Sci.* 128(1):171–84
16. Barton RE, Massari LF, Robertson D, Marston AL. 2022. Eco1-dependent cohesin acetylation anchors chromatin loops and cohesion to define functional meiotic chromosome domains. *eLife* 11:e74447
17. Bernard P, Schmidt CK, Vaur S, Dheur S, Drogat J, et al. 2008. Cell-cycle regulation of cohesin stability along fission yeast chromosomes. *EMBO J.* 27(1):111–21
18. Berto A, Yu J, Morchoisne-Bolhy S, Bertipaglia C, Vallee R, et al. 2018. Disentangling the molecular determinants for Cenp-F localization to nuclear pores and kinetochores. *EMBO Rep.* 19(5):19:e44742
19. Biggins S. 2013. The composition, functions, and regulation of the budding yeast kinetochore. *Genetics* 194(4):817–46
20. Blower MD, Sullivan BA, Karpen GH. 2002. Conserved organization of centromeric chromatin in flies and humans. *Dev. Cell* 2(3):319–30
21. Bock LJ, Pagliuca C, Kobayashi N, Grove RA, Oku Y, et al. 2012. Cnn1 inhibits the interactions between the KMN complexes of the yeast kinetochore. *Nat. Cell Biol.* 14(6):614–24
22. Bodor DL, Mata JF, Sergeev M, David AF, Salimian KJ, et al. 2014. The quantitative architecture of centromeric chromatin. *eLife* 3(3):e02137
23. Boeckmann L, Takahashi Y, Au W-C, Mishra PK, Choy JS, et al. 2013. Phosphorylation of centromeric histone H3 variant regulates chromosome segregation in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 24(12):2034–44
24. Bonner AM, Hughes SE, Hawley RS. 2020. Regulation of Polo kinase by matrimony is required for cohesin maintenance during *Drosophila melanogaster* female meiosis. *Curr. Biol.* 30(4):715–22.e3
25. Bonner MK, Haase J, Swinderman J, Halas H, Miller Jenkins LM, Kelly AE. 2019. Enrichment of Aurora B kinase at the inner kinetochore controls outer kinetochore assembly. *J. Cell Biol.* 218(10):3237–57
26. Borek WE, Vincenten N, Duro E, Makrantoni V, Spanos C, et al. 2021. The proteomic landscape of centromeric chromatin reveals an essential role for the Ctf19^{CCAN} complex in meiotic kinetochore assembly. *Curr. Biol.* 31(2):283–96.e7
27. Brar GA, Amon A. 2008. Emerging roles for centromeres in meiosis I chromosome segregation. *Nat. Rev. Genet.* 9(12):899–910
28. Broad AJ, DeLuca KF, DeLuca JG. 2020. Aurora B kinase is recruited to multiple discrete kinetochore and centromere regions in human cells. *J. Cell Biol.* 219(3):e201905144
29. Campbell CS, Desai A. 2013. Tension sensing by Aurora B kinase is independent of survivin-based centromere localization. *Nature* 497(7447):118–21
30. Carroll CW, Milks KJ, Straight AF. 2010. Dual recognition of CENP-A nucleosomes is required for centromere assembly. *J. Cell Biol.* 189(7):1143–55
31. Carroll CW, Silva MCC, Godek KM, Jansen LET, Straight AF. 2009. Centromere assembly requires the direct recognition of CENP-A nucleosomes by CENP-N. *Nat. Cell Biol.* 11(7):896–902
32. Chan YW, Jeyaprakash AA, Nigg EA, Santamaria A. 2012. Aurora B controls kinetochore–microtubule attachments by inhibiting Ska complex–KMN network interaction. *J. Cell Biol.* 196(5):563–71

33. Cheerambathur DK, Gassmann R, Cook B, Oegema K, Desai A. 2013. Crosstalk between microtubule attachment complexes ensures accurate chromosome segregation. *Science* 342(6163):1239–42
34. Cheerambathur DK, Prevo B, Hattersley N, Lewellyn L, Corbett KD, et al. 2017. Dephosphorylation of the Ndc80 tail stabilizes kinetochore-microtubule attachments via the Ska complex. *Dev. Cell* 41(4):424–37.e4
35. Cheeseman IM, Chappie JS, Wilson-Kubalek EM, Desai A. 2006. The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell* 127(5):983–97
36. Chen J, Liao A, Powers EN, Liao H, Kohlstaedt LA, et al. 2020. Aurora B-dependent Ndc80 degradation regulates kinetochore composition in meiosis. *Genes Dev.* 34(3–4):209–25
37. Chen J, Tresenrider A, Chia M, McSwiggen DT, Spedale G, et al. 2017. Kinetochore inactivation by expression of a repressive mRNA. *eLife* 6:e27417
38. Chittori S, Hong J, Saunders H, Feng H, Ghirlando R, et al. 2018. Structural mechanisms of centromeric nucleosome recognition by the kinetochore protein CENP-N. *Science* 359(6373):339–43
39. Cho US, Harrison SC. 2011. Ndc10 is a platform for inner kinetochore assembly in budding yeast. *Nat. Struct. Mol. Biol.* 19(1):48–56
40. Ciferri C, De Luca J, Monzani S, Ferrari KJ, Ristic D, et al. 2005. Architecture of the human Ndc80-Hec1 complex, a critical constituent of the outer kinetochore. *J. Biol. Chem.* 280(32):29088–95
41. Ciferri C, Pasqualato S, Screpanti E, Varetti G, Santaguida S, et al. 2008. Implications for kinetochore-microtubule attachment from the structure of an engineered Ndc80 complex. *Cell* 133(3):427–39
42. Ciossani G, Overlack K, Petrovic A, Huis in 't Veld PJ, Koerner C, et al. 2018. The kinetochore proteins CENP-E and CENP-F directly and specifically interact with distinct BUB mitotic checkpoint Ser/Thr kinases. *J. Biol. Chem.* 293(26):10084–101
43. Çivril F, Wehenkel A, Giorgi FM, Santaguida S, Di Fonzo A, et al. 2010. Structural analysis of the RZZ complex reveals common ancestry with multisubunit vesicle tethering machinery. *Structure* 18(5):616–26
44. Cleveland DW, Mao Y, Sullivan KF. 2003. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. *Cell* 112(4):407–21
45. Cohen RL, Espelin CW, De Wulf P, Sorger PK, Harrison SC, Simons KT. 2008. Structural and functional dissection of Mif2p, a conserved DNA-binding kinetochore protein. *Mol. Biol. Cell* 19(10):4480–91
46. Cole HA, Howard BH, Clark DJ. 2011. The centromeric nucleosome of budding yeast is perfectly positioned and covers the entire centromere. *PNAS* 108(31):12687–92
47. Corbett KD, Harrison SC. 2016. Molecular architecture of the yeast monopolin complex. *Cell Rep.* 17(3):583–89
48. Corbett KD, Yip CK, Ee L-S, Walz T, Amon A, Harrison SC. 2010. The monopolin complex crosslinks kinetochore components to regulate chromosome-microtubule attachments. *Cell* 142(4):556–67
49. Costantino L, Hsieh T-HS, Lamothe R, Darzacq X, Koshland D. 2020. Cohesin residency determines chromatin loop patterns. *eLife* 9:e59889
50. Cross RA, McAinsh A. 2014. Prime movers: the mechanochemistry of mitotic kinesins. *Nat. Rev. Mol. Cell Biol.* 15(4):257–71
51. Currie CE, Mora-Santos M, Smith CA, McAinsh AD, Millar JBA. 2018. Bub1 is not essential for the checkpoint response to unattached kinetochores in diploid human cells. *Curr. Biol.* 28(17):R929–30
52. De Wulf P, McAinsh AD, Sorger PK. 2003. Hierarchical assembly of the budding yeast kinetochore from multiple subcomplexes. *Genes Dev.* 17(23):2902–21
53. Debose-Scarlett EM, Sullivan BA. 2021. Genomic and epigenetic foundations of neocentromere formation. *Annu. Rev. Genet.* 55:331–48
54. Dechassa ML, Wyns K, Luger K. 2014. Scm3 deposits a $(Cse4–H4)_2$ tetramer onto DNA through a Cse4–H4 dimer intermediate. *Nucleic Acids Res.* 42(9):5532–42
55. del Castillo U, Norkett R, Gelfand VI. 2019. Unconventional roles of cytoskeletal mitotic machinery in neurodevelopment. *Trends Cell Biol.* 29(11):901–11
56. Dimitrova YN, Jenni S, Valverde R, Khin Y, Harrison SC. 2016. Structure of the MIND complex defines a regulatory focus for yeast kinetochore assembly. *Cell* 167(4):1014–27.e12
57. Dong Y, Vanden Beldt KJ, Meng X, Khodjakov A, McEwen BF. 2007. The outer plate in vertebrate kinetochores is a flexible network with multiple microtubule interactions. *Nat. Cell Biol.* 9(5):516–22

58. Doodhi H, Tanaka TU. 2022. Swap and stop—Kinetochores play error correction with microtubules: mechanisms of kinetochore–microtubule error correction. *Bioessays* 44:e2100246
59. Drechsler H, McAinsh AD. 2012. Exotic mitotic mechanisms. *Open Biol*. 2(12):120140
60. Dudziak A, Engelhard L, Bourque C, Klink BU, Rombaut P, et al. 2021. Phospho-regulated Bim1/EB1 interactions trigger Dam1c ring assembly at the budding yeast outer kinetochore. *EMBO J*. 40(18):e108004
61. Dumont M, Gamba R, Gestraud P, Klaasen S, Worrall JT, et al. 2020. Human chromosome-specific aneuploidy is influenced by DNA-dependent centromeric features. *EMBO J*. 39(2):e102924
62. Dunsch AK, Linnane E, Barr FA, Gruneberg U. 2011. The astrin–kinastrin/SKAP complex localizes to microtubule plus ends and facilitates chromosome alignment. *J. Cell Biol.* 192(6):959–68
63. Duro E, Marston AL. 2015. From equator to pole: splitting chromosomes in mitosis and meiosis. *Genes Dev*. 29(2):109–22
64. Eckert CA, Gravdahl DJ, Megee PC. 2007. The enhancement of pericentric cohesin association by conserved kinetochore components promotes high-fidelity chromosome segregation and is sensitive to microtubule-based tension. *Genes Dev*. 21(3):278–91
65. Falk SJ, Guo LY, Sekulic N, Smoak EM, Mani T, et al. 2015. CENP-C reshapes and stabilizes CENP-A nucleosomes at the centromere. *Science* 348(6235):699–703
66. Fernius J, Marston AL. 2009. Establishment of cohesion at the pericentromere by the Ctf19 kinetochore subcomplex and the replication fork-associated factor, Csm3. *PLOS Genet*. 5(9):e1000629
67. Fernius J, Nerusheva OO, Galander S, de Lima Alves F, Rappsilber J, Marston AL. 2013. Cohesin-dependent association of Scc2/4 with the centromere initiates pericentromeric cohesion establishment. *Curr. Biol.* 23(7):599–606
68. Fischböck-Halwachs J, Singh S, Potocnjak M, Hagemann G, Solis-Mezarino V, et al. 2019. The COMA complex interacts with Cse4 and positions Sli15/Ipl1 at the budding yeast inner kinetochore. *eLife* 8:e42879
69. Foltz DR, Jansen LET, Bailey AO, Yates JR, Bassett EA, et al. 2009. Centromere-specific assembly of CENP-A nucleosomes is mediated by HJURP. *Cell* 137(3):472–84
70. Foltz DR, Jansen LET, Black BE, Bailey AO, Yates JR, Cleveland DW. 2006. The human CENP-A centromeric nucleosome-associated complex. *Nat. Cell Biol.* 8(5):458–69
71. Friese A, Faesen AC, Huis in 't Veld PJ, Fischböck J, Prumbaum D, et al. 2016. Molecular requirements for the inter-subunit interaction and kinetochore recruitment of SKAP and Astrin. *Nat. Commun.* 7:11407
72. Furuyama S, Biggins S. 2007. Centromere identity is specified by a single centromeric nucleosome in budding yeast. *PNAS* 104(37):14706–11
73. Galander S, Barton RE, Borek WE, Spanos C, Kelly DA, et al. 2019. Reductional meiosis I chromosome segregation is established by coordination of key meiotic kinases. *Dev. Cell* 49(4):526–41.e5
74. Galander S, Marston AL. 2020. Meiosis I kinase regulators: conserved orchestrators of reductional chromosome segregation. *Bioessays* 42(10):2000018
75. García-Rodríguez LJ, Kasciukovic T, Denninger V, Tanaka TU. 2019. Aurora B-INCENP localization at centromeres/inner kinetochores is required for chromosome bi-orientation in budding yeast. *Curr. Biol.* 29(9):1536–44.e4
76. Gartenberg M. 2009. Heterochromatin and the cohesion of sister chromatids. *Chromosom. Res.* 17(2):229–38
77. Gascoigne KE, Takeuchi K, Suzuki A, Hori T, Fukagawa T, Cheeseman IM. 2011. Induced ectopic kinetochore assembly bypasses the requirement for CENP-A nucleosomes. *Cell* 145(3):410–22
78. Gassmann R, Holland AJ, Varma D, Wan X, Çivril F, et al. 2010. Removal of Spindly from microtubule-attached kinetochores controls spindle checkpoint silencing in human cells. *Genes Dev*. 24(9):957–71
79. Ghongane P, Kapanidou M, Asghar A, Elowe S, Bolanos-Garcia VM. 2014. The dynamic protein Knl1—a kinetochore rendezvous. *J. Cell Sci.* 127(Part 16):3415–23
80. Glynn EF, Megee PC, Yu H-G, Mistrot C, Unal E, et al. 2004. Genome-wide mapping of the cohesin complex in the yeast *Saccharomyces cerevisiae*. *PLOS Biol*. 2(9):e259
81. Goldstein LS. 1981. Kinetochore structure and its role in chromosome orientation during the first meiotic division in male *D. melanogaster*. *Cell* 25(3):591–602

82. Gonen S, Akiyoshi B, Iadanza MG, Shi D, Duggan N, et al. 2012. The structure of purified kinetochores reveals multiple microtubule-attachment sites. *Nat. Struct. Mol. Biol.* 19(9):925–29
83. Gregan J, Riedel CG, Pidoux AL, Katou Y, Rumpf C, et al. 2007. The kinetochore proteins Pcs1 and Mde4 and heterochromatin are required to prevent merotelic orientation. *Curr. Biol.* 17(14):1190–1200
84. Gruhn JR, Zielinska AP, Shukla V, Blanshard R, Capalbo A, et al. 2019. Chromosome errors in human eggs shape natural fertility over reproductive life span. *Science* 365(6460):1466–69
85. Gryaznova Y, Keating L, Touati SA, Cladière D, El Yakoubi W, et al. 2021. Kinetochore individualization in meiosis I is required for centromeric cohesin removal in meiosis II. *EMBO J.* 40(7):e106797
86. Guan R, Lian T, Zhou B-R, He E, Wu C, et al. 2021. Structural and dynamic mechanisms of CBF3-guided centromeric nucleosome formation. *Nat. Commun.* 12(1):1763
87. Gudimchuk NB, McIntosh JR. 2021. Regulation of microtubule dynamics, mechanics and function through the growing tip. *Nat. Rev. Mol. Cell Biol.* 22(12):777–95
88. Gudimchuk NB, Vitre B, Kim Y, Kiyatkin A, Cleveland DW, et al. 2013. Kinetochore kinesin CENP-E is a processive bi-directional tracker of dynamic microtubule tips. *Nat. Cell Biol.* 15(9):1079–88
89. Guo LY, Allu PK, Zandarashvili L, McKinley KL, Sekulic N, et al. 2017. Centromeres are maintained by fastening CENP-A to DNA and directing an arginine anchor-dependent nucleosome transition. *Nat. Commun.* 8:15775
90. Hara M, Ariyoshi M, Okumura E-I, Hori T, Fukagawa T. 2018. Multiple phosphorylations control recruitment of the KMN network onto kinetochores. *Nat. Cell Biol.* 20(12):1378–88
91. Hara M, Fukagawa T. 2020. Dynamics of kinetochore structure and its regulations during mitotic progression. *Cell. Mol. Life Sci.* 77:2981–95
92. Helgeson LA, Zelter A, Riffle M, MacCoss MJ, Asbury CL, Davis TN. 2018. Human Ska complex and Ndc80 complex interact to form a load-bearing assembly that strengthens kinetochore–microtubule attachments. *PNAS* 115(11):2740–45
93. Hill TL. 1985. Theoretical problems related to the attachment of microtubules to kinetochores. *PNAS* 82(13):4404–8
94. Hindriksen S, Lens SMA, Hadders MA. 2017. The ins and outs of Aurora B inner centromere localization. *Front. Cell Dev. Biol.* 5:112
95. Hinshaw SM, Dates AN, Harrison SC. 2019. The structure of the yeast Ctf3 complex. *eLife* 8:e48215
96. Hinshaw SM, Harrison SC. 2019. The structure of the Ctf19c/CCAN from budding yeast. *eLife* 8:e44239
97. Hinshaw SM, Harrison SC. 2020. The structural basis for kinetochore stabilization by Cnn1/CENP-T. *Curr. Biol.* 30(17):3425–31.e3
98. Hinshaw SM, Makrantoni V, Harrison SC, Marston AL. 2017. The kinetochore receptor for the cohesin loading complex. *Cell* 171(1):72–84.e13
99. Hinshaw SM, Makrantoni V, Kerr A, Marston AL, Harrison SC. 2015. Structural evidence for Scc4-dependent localization of cohesin loading. *eLife* 4:e06057
100. Hori T, Amano M, Suzuki A, Backer CB, Welburn JP, et al. 2008. CCAN makes multiple contacts with centromeric DNA to provide distinct pathways to the outer kinetochore. *Cell* 135(6):1039–52
101. Hornung P, Maier M, Alushin GM, Lander GC, Nogales E, Westermann S. 2011. Molecular architecture and connectivity of the budding yeast Mtw1 kinetochore complex. *J. Mol. Biol.* 405(2):548–59
102. Hornung P, Troc P, Malvezzi F, Maier M, Demianova Z, et al. 2014. A cooperative mechanism drives budding yeast kinetochore assembly downstream of CENP-A. *J. Cell Biol.* 206(4):509–24
103. Howell BJ, McEwen BF, Canman JC, Hoffman DB, Farrar EM, et al. 2001. Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. *J. Cell Biol.* 155(7):1159–72
104. Huis in 't Veld PJ, Jeganathan S, Petrovic A, Singh P, John J, et al. 2016. Molecular basis of outer kinetochore assembly on CENP-T. *eLife* 5:e21007
105. Huis in 't Veld PJ, Volkov VA, Stender ID, Musacchio A, Dogterom M. 2019. Molecular determinants of the Ska-Ndc80 interaction and their influence on microtubule tracking and force-coupling. *eLife* 8:e49539
106. Ishii M, Akiyoshi B. 2022. Plasticity in centromere organization and kinetochore composition: lessons from diversity. *Curr. Opin. Cell Biol.* 74:47–54

107. Izuta H, Ikeno M, Suzuki N, Tomonaga T, Nozaki N, et al. 2006. Comprehensive analysis of the ICEN (Interphase Centromere Complex) components enriched in the CENP-A chromatin of human cells. *Genes Cells* 11(6):673–84
108. Janczyk P, Skorupka KA, Tooley JG, Matson DR, Kestner CA, et al. 2017. Mechanism of Ska recruitment by Ndc80 complexes to kinetochores. *Dev. Cell* 41(4):438–49.e4
109. Jenni S, Harrison SC. 2018. Structure of the DASH/Dam1 complex shows its role at the yeast kinetochore-microtubule interface. *Science* 360(6388):552–58
110. Jeyaprakash AA, Santamaria A, Jayachandran U, Chan YW, Benda C, et al. 2012. Structural and functional organization of the Ska complex, a key component of the kinetochore-microtubule interface. *Mol. Cell* 46(3):274–86
111. Joglekar AP, Bloom K, Salmon ED. 2009. In vivo protein architecture of the eukaryotic kinetochore with nanometer scale accuracy. *Curr. Biol.* 19(8):694–99
112. Joglekar AP, Kukreja AA. 2017. How kinetochore architecture shapes the mechanisms of its function. *Curr. Biol.* 27(16):R816–24
113. Johnston K, Joglekar A, Hori T, Suzuki A, Fukagawa T, Salmon ED. 2010. Vertebrate kinetochore protein architecture: protein copy number. *J. Cell Biol.* 189(6):937–43
114. Jokelainen PT. 1967. The ultrastructure and spatial organization of the metaphase kinetochore in mitotic rat cells. *J. Ultrastruct. Res.* 19(1):19–44
115. Kanfer G, Peterka M, Arzhanik VK, Drobyshev AL, Ataullakhanov FI, et al. 2017. CENP-F couples cargo to growing and shortening microtubule ends. *Mol. Biol. Cell* 28(18):2400–9
116. Kang YH, Park CH, Kim T-S, Soung N-K, Bang JK, et al. 2011. Mammalian polo-like kinase 1-dependent regulation of the PBIP1-CENP-Q complex at kinetochores. *J. Biol. Chem.* 286(22):19744–57
117. Katis VL, Matos J, Mori S, Shirahige K, Zachariae W, Nasmyth K. 2004. Spo13 facilitates monopolin recruitment to kinetochores and regulates maintenance of centromeric cohesion during yeast meiosis. *Curr. Biol.* 14(24):2183–96
118. Kato H, Jiang J, Zhou BR, Rozendaal M, Feng H, et al. 2013. A conserved mechanism for centromeric nucleosome recognition by centromere protein CENP-C. *Science* 340(6136):1110–13
119. Kern DM, Monda JK, Su KC, Wilson-Kubalek EM, Cheeseman IM. 2017. Astrin-SKAP complex reconstitution reveals its kinetochore interaction with microtubule-bound Ndc80. *eLife* 6:e26866
120. Kiewisz R, Fabig G, Conway W, Baum D, Needleman DJ, Müller-Reichert T. 2022. Three-dimensional structure of kinetochore-fibers in human mitotic spindles. *eLife* 11:e75459
121. Killinger K, Böhm M, Steinbach P, Hagemann G, Blüggel M, et al. 2020. Auto-inhibition of Mif2/CENP-C ensures centromere-dependent kinetochore assembly in budding yeast. *EMBO J.* 39:e102938
122. Kim J, Ishiguro K-I, Nambu A, Akiyoshi B, Yokobayashi S, et al. 2015. Meikin is a conserved regulator of meiosis-I-specific kinetochore function. *Nature* 517(7535):466–71
123. Kim JO, Zelter A, Umbreit NT, Bollozos A, Riffle M, et al. 2017. The Ndc80 complex bridges two Dam1 complex rings. *eLife* 6:e21069
124. Kim S, Yu H. 2015. Multiple assembly mechanisms anchor the KMN spindle checkpoint platform at human mitotic kinetochores. *J. Cell Biol.* 208(2):181–96
125. Kitamura E, Tanaka K, Kitamura Y, Tanaka TU. 2007. Kinetochore–microtubule interaction during S phase in *Saccharomyces cerevisiae*. *Genes Dev*. 21(24):3319–30
126. Klare K, Weir JR, Basilico F, Zimniak T, Massimiliano L, et al. 2015. CENP-C is a blueprint for constitutive centromere-associated network assembly within human kinetochores. *J. Cell Biol.* 210(1):11–22
127. Koch B, Kueng S, Ruckenbauer C, Wendt KS, Peters JM. 2008. The Suv39h–HP1 histone methylation pathway is dispensable for enrichment and protection of cohesin at centromeres in mammalian cells. *Chromosoma* 117(2):199–210
128. Kops GJPL, Gassmann R. 2020. Crowning the kinetochore: the fibrous corona in chromosome segregation. *Trends Cell Biol*. 30(8):653–67
129. Kops GJPL, Kim Y, Weaver BAA, Mao Y, McLeod I, et al. 2005. ZW10 links mitotic checkpoint signaling to the structural kinetochore. *J. Cell Biol.* 169(1):49–60

130. Krenn V, Wehenkel A, Li X, Santaguida S, Musacchio A. 2012. Structural analysis reveals features of the spindle checkpoint kinase Bub1–kinetochore subunit Knl1 interaction. *J. Cell Biol.* 196(4):451–67
131. Kuhl LM, Vader G. 2019. Kinetochores, cohesin, and DNA breaks: controlling meiotic recombination within pericentromeres. *Yeast* 36(3):121–27
132. Kukreja AA, Kavuri S, Joglekar AP. 2020. Microtubule attachment and centromeric tension shape the protein architecture of the human kinetochore. *Curr. Biol.* 30(24):4869–81.e5
133. Lampert F, Hornung P, Westermann S. 2010. The Dam1 complex confers microtubule plus end-tracking activity to the Ndc80 kinetochore complex. *J. Cell Biol.* 189(4):641–49
134. Lampert F, Mieck C, Alushin GM, Nogales E, Westermann S. 2013. Molecular requirements for the formation of a kinetochore–microtubule interface by Dam1 and Ndc80 complexes. *J. Cell Biol.* 200(1):21–30
135. Lampson MA, Cheeseman IM. 2011. Sensing centromere tension: Aurora B and the regulation of kinetochore function. *Trends Cell Biol.* 21(3):133–40
136. Lampson MA, Grishchuk EL. 2017. Mechanisms to avoid and correct erroneous kinetochore-microtubule attachments. *Biology* 6(1):1
137. Lang J, Barber A, Biggins S. 2018. An assay for de novo kinetochore assembly reveals a key role for the CENP-T pathway in budding yeast. *eLife* 7:e37819
138. Lara-Gonzalez P, Pines J, Desai A. 2021. Spindle assembly checkpoint activation and silencing at kinetochores. *Semin. Cell Dev. Biol.* 117:86–98
139. Lazar-Stefanita L, Scolari VF, Mercy G, Muller HH, Guérin TM, et al. 2017. Cohesins and condensins orchestrate the 4D dynamics of yeast chromosomes during the cell cycle. *EMBO J.* 36(18):2684–97
140. Leber V, Nans A, Singleton MR. 2018. Structural basis for assembly of the CBF3 kinetochore complex. *EMBO J.* 37(2):269–81
141. Leclerc S, Kitagawa K. 2021. The role of human centromeric RNA in chromosome stability. *Front. Mol. Biosci.* 8:170
142. Lee BH, Kiburz BM, Amon A. 2004. Spo13 maintains centromeric cohesion and kinetochore coorientation during meiosis I. *Curr. Biol.* 14(24):2168–82
143. Li X, Dawe RK. 2009. Fused sister kinetochores initiate the reductional division in meiosis I. *Nat. Cell Biol.* 11(9):1103–8
144. Li Y, Bachant J, Alcasabas AA, Wang Y, Qin J, Elledge SJ. 2002. The mitotic spindle is required for loading of the DASH complex onto the kinetochore. *Genes Dev.* 16(2):183–97
145. Li Y, Haarhuis JHI, Sedeño Cacciatore Á, Oldenkamp R, van Ruiten MS, et al. 2020. The structural basis for cohesin–CTCF-anchored loops. *Nature* 578(7795):472–76
146. London N, Biggins S. 2014. Mad1 kinetochore recruitment by Mps1-mediated phosphorylation of Bub1 signals the spindle checkpoint. *Genes Dev.* 28(2):140–52
147. Long AF, Kuhn J, Dumont S. 2019. The mammalian kinetochore-microtubule interface: robust mechanics and computation with many microtubules. *Curr. Opin. Cell Biol.* 60:60–67
148. Ma W, Zhou J, Chen J, Carr AM, Watanabe Y. 2021. Meikin synergizes with shugoshin to protect cohesin Rec8 during meiosis I. *Genes Dev.* 35(9–10):692–97
149. Maciejowski J, Drechsler H, Grundner-Culemann K, Ballister ER, Rodriguez-Rodriguez JA, et al. 2017. Mps1 regulates kinetochore-microtubule attachment stability via the Ska complex to ensure error-free chromosome segregation. *Dev. Cell* 41(2):143–56.e6
150. Magidson V, He J, Ault JG, O'Connell CB, Yang N, et al. 2016. Unattached kinetochores rather than intrakinetochore tension arrest mitosis in taxol-treated cells. *J. Cell Biol.* 212(3):307–19
151. Maier NK, Ma J, Lampson MA, Cheeseman IM. 2021. Separase cleaves the kinetochore protein Meikin at the meiosis I/II transition. *Dev. Cell* 56(15):2192–206.e8
152. Malvezzi F, Litos G, Schleiffer A, Heuck A, Mechtler K, et al. 2013. A structural basis for kinetochore recruitment of the Ndc80 complex via two distinct centromere receptors. *EMBO J.* 32(3):409–23
153. Manning AL, Bakhoum SF, Maffini S, Correia-Melo C, Maiato H, Compton DA. 2010. CLASP1, astrin and Kif2b form a molecular switch that regulates kinetochore-microtubule dynamics to promote mitotic progression and fidelity. *EMBO J.* 29(20):3531–43
154. Marco E, Dorn JF, Hsu PH, Jaqaman K, Sorger PK, Danuser G. 2013. *S. cerevisiae* chromosomes biorient via gradual resolution of syntely between S phase and anaphase. *Cell* 154(5):1127–39

155. Marston AL. 2014. Chromosome segregation in budding yeast: sister chromatid cohesion and related mechanisms. *Genetics* 196(1):31–63
156. Marston AL. 2015. Shugoshins: tension-sensitive pericentromeric adaptors safeguarding chromosome segregation. *Mol. Cell Biol.* 35(4):634–48
157. Maskell DP, Hu XW, Singleton MR. 2010. Molecular architecture and assembly of the yeast kinetochore MIND complex. *J. Cell Biol.* 190(5):823–34
158. Matos J, Lipp JJ, Bogdanova A, Guillot S, Okaz E, et al. 2008. Dbf4-dependent Cdc7 kinase links DNA replication to the segregation of homologous chromosomes in meiosis I. *Cell* 135(4):662–78
159. McAinsh AD, Tytell JD, Sorger PK. 2003. Structure, function, and regulation of budding yeast kinetochores. *Annu. Rev. Cell Dev. Biol.* 19:519–39
160. McClelland SE, Borusu S, Amaro AC, Winter JR, Belwal M, et al. 2007. The CENP-A NAC/CAD kinetochore complex controls chromosome congression and spindle bipolarity. *EMBO J.* 26(24):5033–47
161. McKinley KL, Sekulic N, Guo LY, Tsinman T, Black BE, Cheeseman IM. 2015. The CENP-L-N complex forms a critical node in an integrated meshwork of interactions at the centromere-kinetochore interface. *Mol. Cell* 60(6):886–98
162. Measday V, Hailey DW, Pot I, Givan SA, Hyland KM, et al. 2002. Ctf3p, the Mis6 budding yeast homolog, interacts with Mcm22p and Mcm16p at the yeast outer kinetochore. *Genes Dev.* 16(1):101–13
163. Medina-Pritchard B, Lazou V, Zou J, Byron O, Abad MA, et al. 2020. Structural basis for centromere maintenance by *Drosophila* CENP-A chaperone CAL1. *EMBO J.* 39(7):e103234
164. Mellone BG, Fachinetti D. 2021. Diverse mechanisms of centromere specification. *Curr. Biol.* 31(22):R1491–504
165. Meraldi P, McAinsh AD, Rheinbay E, Sorger PK. 2006. Phylogenetic and structural analysis of centromeric DNA and kinetochore proteins. *Genome Biol.* 7(3):R23
166. Meyer RE, Chuong HH, Hild M, Hansen CL, Kinter M, Dawson DS. 2015. Ipl1/Aurora-B is necessary for kinetochore restructuring in meiosis I in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 26(17):2986–3000
167. Milks KJ, Moree B, Straight AF. 2009. Dissection of CENP-C-directed centromere and kinetochore assembly. *Mol. Biol. Cell* 20(19):4246–55
168. Miller MP, Ünal E, Brar GA, Amon A. 2012. Meiosis I chromosome segregation is established through regulation of microtubule-kinetochore interactions. *eLife* 2012(1):e00117
169. Miranda JJL, De Wulf P, Sorger PK, Harrison SC. 2005. The yeast DASH complex forms closed rings on microtubules. *Nat. Struct. Mol. Biol.* 12(2):138–43
170. Miyazaki S, Kim J, Yamagishi Y, Ishiguro T, Okada Y, et al. 2017. Meikin-associated polo-like kinase specifies Bub1 distribution in meiosis I. *Genes Cells* 22(6):552–67
171. Mizuguchi G, Xiao H, Wisniewski J, Smith MM, Wu C. 2007. Nonhistone Scm3 and histones CenH3-H4 assemble the core of centromere-specific nucleosomes. *Cell* 129(6):1153–64
172. Monda JK, Whitney IP, Tarasovetc EV, Wilson-Kubalek E, Milligan RA, et al. 2017. Microtubule tip tracking by the spindle and kinetochore protein Ska1 requires diverse tubulin-interacting surfaces. *Curr. Biol.* 27(23):3666–75.e6
173. Mosalaganti S, Keller J, Altenfeld A, Winzker M, Rombaut P, et al. 2017. Structure of the RZZ complex and molecular basis of its interaction with Spindly. *J. Cell Biol.* 216(4):961–81
174. Nambu M, Kishikawa A, Yamada T, Ichikawa K, Kira Y, et al. 2022. Direct evaluation of cohesin-mediated sister kinetochore associations at meiosis I in fission yeast. *J. Cell Sci.* 135(1):jcs259102
175. Nguyen AL, Fadel MD, Cheeseman IM. 2021. Differential requirements for the CENP-O complex reveal parallel PLK1 kinetochore recruitment pathways. *Mol. Biol. Cell* 32(8):712–21
176. Nishimura K, Komiya M, Hori T, Itoh T, Fukagawa T. 2019. 3D genomic architecture reveals that neocentromeres associate with heterochromatin regions. *J. Cell Biol.* 218(1):134–49
177. Nishino T, Rago F, Hori T, Tomii K, Cheeseman IM, Fukagawa T. 2013. CENP-T provides a structural platform for outer kinetochore assembly. *EMBO J.* 32(3):424–36
178. Nonaka N, Kitajima T, Yokobayashi S, Xiao G, Yamamoto M, et al. 2002. Recruitment of cohesin to heterochromatic regions by Swi6/HP1 in fission yeast. *Nat. Cell Biol.* 4(1):89–93
179. Obeso D, Pezza RJ, Dawson D. 2014. Couples, pairs, and clusters: mechanisms and implications of centromere associations in meiosis. *Chromosoma* 123(1–2):43–55

180. Ogushi S, Rattani A, Godwin J, Metson J, Schermelleh L, Nasmyth K. 2021. Loss of sister kinetochore co-orientation and peri-centromeric cohesin protection after meiosis I depends on cleavage of centromeric REC8. *Dev. Cell* 56(22):3100–14.e4
181. Okada M, Cheeseman IM, Hori T, Okawa K, McLeod IX, et al. 2006. The CENP-H-I complex is required for the efficient incorporation of newly synthesized CENP-A into centromeres. *Nat. Cell Biol.* 8(5):446–57
182. Ortiz J, Stemmann O, Rank S, Lechner J. 1999. A putative protein complex consisting of Ctf19, Mcm21, and Okp1 represents a missing link in the budding yeast kinetochore. *Genes Dev.* 13(9):1140–55
183. O'Toole E, Morphew M, McIntosh JR. 2020. Electron tomography reveals aspects of spindle structure important for mechanical stability at metaphase. *Mol. Biol. Cell* 31(3):184–95
184. Paldi F, Alver B, Robertson D, Schalbetter SA, Kerr A, et al. 2020. Convergent genes shape budding yeast pericentromeres. *Nature* 582(7810):119–23
185. Paliulis LV, Nicklas RB. 2000. The reduction of chromosome number in meiosis is determined by properties built into the chromosomes. *J. Cell Biol.* 150(6):1223–31
186. Patel J, Tan SL, Hartshorne GM, McAinsh AD. 2015. Unique geometry of sister kinetochores in human oocytes during meiosis I may explain maternal age-associated increases in chromosomal abnormalities. *Biol. Open* 5(2):178–84
187. Pekgöz Altunkaya G, Malvezzi F, Demianova Z, Zimniak T, Litos G, et al. 2016. CCAN assembly configures composite binding interfaces to promote cross-linking of Ndc80 complexes at the kinetochore. *Curr. Biol.* 26(17):2370–78
188. Pentakota S, Zhou K, Smith C, Maffini S, Petrovic A, et al. 2017. Decoding the centromeric nucleosome through CENP-N. *eLife* 6:e33442
189. Pereira C, Reis RM, Gama JB, Celestino R, Cheerambathur DK, et al. 2018. Self-assembly of the RZZ complex into filaments drives kinetochore expansion in the absence of microtubule attachment. *Curr. Biol.* 28(21):3408–21.e8
190. Pesenti ME, Prumbaum D, Auckland P, Smith CM, Faesen AC, et al. 2018. Reconstitution of a 26-subunit human kinetochore reveals cooperative microtubule binding by CENP-OPQUR and NDC80. *Mol. Cell* 71(6):923–39.e10
191. Pesenti ME, Raisch T, Conti D, Hoffmann I, Vogt D, et al. 2022. Structure of the human inner kinetochore CCAN complex and its significance for human centromere organization. *Mol. Cell* 82:2113–31.e8
192. Pesenti ME, Weir JR, Musacchio A. 2016. Progress in the structural and functional characterization of kinetochores. *Curr. Opin. Struct. Biol.* 37:152–63
193. Petronczki M, Matos J, Mori S, Gregan J, Bogdanova A, et al. 2006. Monopolar attachment of sister kinetochores at meiosis I requires casein kinase 1. *Cell* 126(6):1049–64
194. Petrovic A, Keller J, Liu Y, Overlack K, John J, et al. 2016. Structure of the MIS12 complex and molecular basis of its interaction with CENP-C at human kinetochores. *Cell* 167(4):1028–40.e15
195. Petrovic A, Mosalaganti S, Keller J, Mattiuzzo M, Overlack K, et al. 2014. Modular assembly of RWD domains on the Mis12 complex underlies outer kinetochore organization. *Mol. Cell* 53(4):591–605
196. Petrovic A, Pasqualato S, Dube P, Krenn V, Santaguida S, et al. 2010. The MIS12 complex is a protein interaction hub for outer kinetochore assembly. *J. Cell Biol.* 190(5):835–52
197. Plowman R, Singh N, Tromer EC, Payan A, Duro E, et al. 2019. The molecular basis of monopolin recruitment to the kinetochore. *Chromosoma* 128(3):331–54
198. Pot I, Measday V, Snydsman B, Cagney G, Fields S, et al. 2003. Chl4p and Iml3p are two new members of the budding yeast outer kinetochore. *Mol. Biol. Cell* 14(2):460–76
199. Przewloka MR, Venkei Z, Bolanos-Garcia VM, Debski J, Dadlez M, Glover DM. 2011. CENP-C is a structural platform for kinetochore assembly. *Curr. Biol.* 21(5):399–405
200. Raaijmakers JA, van Heesbeen RGHP, Blomen VA, Janssen LME, van Diemen F, et al. 2018. BUB1 is essential for the viability of human cells in which the spindle assembly checkpoint is compromised. *Cell Rep.* 22(6):1424–38
201. Rabitsch KP, Petronczki M, Javerzat J-P, Genier S, Chwalla B, et al. 2003. Kinetochore recruitment of two nucleolar proteins is required for homolog segregation in meiosis I. *Dev. Cell* 4(4):535–48
202. Rago F, Gascoigne KE, Cheeseman IM. 2015. Distinct organization and regulation of the outer kinetochore KMN network downstream of CENP-C and CENP-T. *Curr. Biol.* 25(5):671–77

203. Raisch T, Ciossani G, d'Amico E, Cmentowski V, Carmignani S, et al. 2022. Structure of the RZZ complex and molecular basis of Spindly-driven corona assembly at human kinetochores. *EMBO J.* 41:e110411
204. Ramey VH, Wang H-W, Nakajima Y, Wong A, Liu J, et al. 2011. The Dam1 ring binds to the E-hook of tubulin and diffuses along the microtubule. *Mol. Biol. Cell* 22(4):457–66
205. Renda F, Khodjakov A. 2021. Role of spatial patterns and kinetochore architecture in spindle morphogenesis. *Semin. Cell Dev. Biol.* 117:75–85
206. Ribeiro SA, Gatlin JC, Dong Y, Joglekar A, Cameron L, et al. 2009. Condensin regulates the stiffness of vertebrate centromeres. *Mol. Biol. Cell* 20(9):2371–80
207. Ribeiro SA, Vagnarelli P, Dong Y, Hori T, McEwen BF, et al. 2010. A super-resolution map of the vertebrate kinetochore. *PNAS* 107(23):10484–89
208. Rieder CL. 1982. The formation, structure, and composition of the mammalian kinetochore and kinetochore fiber. *Int. Rev. Cytol.* 79:1–58
209. Rodriguez-Rodriguez JA, Lewis C, McKinley KL, Sikirzhytski V, Corona J, et al. 2018. Distinct roles of RZZ and Bub1-KNL1 in mitotic checkpoint signaling and kinetochore expansion. *Curr. Biol.* 28(21):3422–29.e5
210. Rosas-Salvans M, Sutanto R, Suresh P, Dumont S. 2022. The Astrin-SKAP complex reduces friction at the kinetochore-microtubule interface. *Curr. Biol.* 32:2621–31
211. Roscioli E, Germanova TE, Smith CA, Embacher PA, Erent M, et al. 2020. Ensemble-level organization of human kinetochores and evidence for distinct tension and attachment sensors. *Cell Rep*. 31(4):107535
212. Sacristan C, Ahmad MUD, Keller J, Fermie J, Groenewold V, et al. 2018. Dynamic kinetochore size regulation promotes microtubule capture and chromosome biorientation in mitosis. *Nat. Cell Biol.* 20(7):800–10
212a. Sacristan C, Samejima K, Ruiz LA, Lambers MLA, Buckle A, et al. 2022. Condensin reorganizes centromeric chromatin during mitotic entry into a bipartite structure stabilized by cohesin. bioRxiv 2022.08.01.502248. **https://doi.org/10.1101/2022.08.01.502248**
213. Sakuno T, Tada K, Watanabe Y. 2009. Kinetochore geometry defined by cohesion within the centromere. *Nature* 458(7240):852–58
214. Sarangapani KK, Duro E, Deng Y, De Lima Alves F, Ye Q, et al. 2014. Sister kinetochores are mechanically fused during meiosis I in yeast. *Science* 346(6206):248–51
215. Sarangapani KK, Koch LB, Nelson CR, Asbury CL, Biggins S. 2021. Kinetochore-bound Mps1 regulates kinetochore–microtubule attachments via Ndc80 phosphorylation. *J. Cell Biol.* 220(12):e202106130
216. Sarkar S, Shenoy RT, Dalgaard JZ, Newnham L, Hoffmann E, et al. 2013. Monopolin subunit Csm1 associates with MIND complex to establish monopolar attachment of sister kinetochores at meiosis I. *PLOS Genet*. 9(7):e1003610
217. Saurin AT. 2018. Kinase and phosphatase cross-talk at the kinetochore. *Front. Cell Dev. Biol.* 6:62
218. Scarborough EA, Davis TN, Asbury CL. 2019. Tight bending of the Ndc80 complex provides intrinsic regulation of its binding to microtubules. *eLife* 8:e44489
219. Schalbetter SA, Goloborodko A, Fudenberg G, Belton J-M, Miles C, et al. 2017. SMC complexes differentially compact mitotic chromosomes according to genomic context. *Nat. Cell Biol.* 19(9):1071–80
220. Scherthan H. 2001. A bouquet makes ends meet. *Nat. Rev. Mol. Cell Biol.* 2:621–27
221. Schleiffer A, Maier M, Litos G, Lampert F, Hornung P, et al. 2012. CENP-T proteins are conserved centromere receptors of the Ndc80 complex. *Nat. Cell Biol.* 14(6):604–13
222. Schmidt JC, Arthanari H, Boeszoermenyi A, Dashkevich NM, Wilson-Kubalek EM, et al. 2012. The kinetochore-bound Ska1 complex tracks depolymerizing microtubules and binds to curved protofilaments. *Dev. Cell* 23(5):968–80
223. Schmidt JC, Kiyomitsu T, Hori T, Backer CB, Fukagawa T, Cheeseman IM. 2010. Aurora B kinase controls the targeting of the Astrin-SKAP complex to bioriented kinetochores. *J. Cell Biol.* 191(2):269–80
224. Screpanti E, De Antoni A, Alushin GM, Petrovic A, Melis T, et al. 2011. Direct binding of Cenp-C to the Mis12 complex joins the inner and outer kinetochore. *Curr. Biol.* 21(5):391–98

225. Silió V, McAinsh AD, Millar JB. 2015. KNL1-Bubs and RZZ provide two separable pathways for checkpoint activation at human kinetochores. *Dev. Cell* 35(5):600–13
226. Singh P, Pesenti ME, Maffini S, Carmignani S, Hedtfeld M, et al. 2021. BUB1 and CENP-U, primed by CDK1, are the main PLK1 kinetochore receptors in mitosis. *Mol. Cell* 81(1):67–87.e9
227. Smith CA, McAinsh AD, Burroughs NJ. 2016. Human kinetochores are swivel joints that mediate microtubule attachments. *eLife* 5:e16159
228. Stephens AD, Haase J, Vicci L, Taylor RM, Bloom K. 2011. Cohesin, condensin, and the intramolecular centromere loop together generate the mitotic chromatin spring. *J. Cell Biol.* 193(7):1167–80
229. Sullivan BA, Karpen GH. 2004. Centromeric chromatin exhibits a histone modification pattern that is distinct from both euchromatin and heterochromatin. *Nat. Struct. Mol. Biol.* 11(11):1076–83
230. Suresh P, Long AF, Dumont S. 2020. Microneedle manipulation of the mammalian spindle reveals specialized, short-lived reinforcement near chromosomes. *eLife* 9:e53807
231. Suzuki A, Badger BL, Salmon ED. 2015. A quantitative description of Ndc80 complex linkage to human kinetochores. *Nat. Commun.* 6:8161
232. Suzuki A, Badger BL, Wan X, DeLuca JG, Salmon ED. 2014. The architecture of CCAN proteins creates a structural integrity to resist spindle forces and achieve proper intrakinetochore stretch. *Dev. Cell* 30(6):717–30
233. Tanaka K, Chang HL, Kagami A, Watanabe Y. 2009. CENP-C functions as a scaffold for effectors with essential kinetochore functions in mitosis and meiosis. *Dev. Cell* 17(3):334–43
234. Tang NH, Toda T. 2013. Ndc80 Loop as a protein-protein interaction motif. *Cell Div.* 8(1):2
235. Thakur J, Packiaraj J, Henikoff S. 2021. Sequence, chromatin and evolution of satellite DNA. *Int. J. Mol. Sci.* 22(9):4309
236. Tien JF, Umbreit NT, Gestaut DR, Franck AD, Cooper J, et al. 2010. Cooperation of the Dam1 and Ndc80 kinetochore complexes enhances microtubule coupling and is regulated by aurora B. *J. Cell Biol.* 189(4):713–23
237. Tóth A, Rabitsch KP, Gálová M, Schleiffer A, Buonomo SB, Nasmyth K. 2000. Functional genomics identifies monopolin: a kinetochore protein required for segregation of homologs during meiosis I. *Cell* 103(7):1155–68
238. Tromer EC, van Hooff JJE, Kops GJPL, Snel B. 2019. Mosaic origin of the eukaryotic kinetochore. *PNAS* 116(26):12873–82
239. Tromer EC, Wemyss TA, Ludzia P, Waller RF, Akiyoshi B. 2021. Repurposing of synaptonemal complex proteins for kinetochores in Kinetoplastida. *Open Biol.* 11(5):210049
240. Valverde R, Ingram J, Harrison SC. 2016. Conserved tetramer junction in the kinetochore Ndc80 complex. *Cell Rep.* 17(8):1915–22
241. Vargiu G, Makarov AA, Allan J, Fukagawa T, Booth DG, Earnshaw WC. 2017. Stepwise unfolding supports a subunit model for vertebrate kinetochores. *PNAS* 114(12):3133–38
242. Varma D, Wan X, Cheerambathur D, Gassmann R, Suzuki A, et al. 2013. Spindle assembly checkpoint proteins are positioned close to core microtubule attachment sites at kinetochores. *J. Cell Biol.* 202(5):735–46
243. Verzijlbergen KF, Nerusheva OO, Kelly D, Kerr A, Clift D, et al. 2014. Shugoshin biases chromosomes for biorientation through condensin recruitment to the pericentromere. *eLife* 3:e01374
244. Volkov VA, Grissom PM, Arzhanik VK, Zaytsev AV, Renganathan K, et al. 2015. Centromere protein F includes two sites that couple efficiently to depolymerizing microtubules. *J. Cell Biol.* 209(6):813–28
245. Volkov VA, Huis in 't Veld PJ, Dogterom M, Musacchio A. 2018. Multivalency of NDC80 in the outer kinetochore is essential to track shortening microtubules and generate forces. *eLife* 7:e36764
246. Walstein K, Petrovic A, Pan D, Hagemeier B, Vogt D, et al. 2021. Assembly principles and stoichiometry of a complete human kinetochore module. *Sci. Adv.* 7(27):eabg1037
247. Wan X, O'Quinn RP, Pierce HL, Joglekar AP, Gall WE, et al. 2009. Protein architecture of the human kinetochore microtubule attachment site. *Cell* 137(4):672–84

248. Watanabe R, Hara M, Okumura EI, Hervé S, Fachinetti D, et al. 2019. CDK1-mediated CENP-C phosphorylation modulates CENP-A binding and mitotic kinetochore localization. *J. Cell Biol.* 218(12):4042–62
249. Watanabe Y, Nurse P. 1999. Cohesin Rec8 is required for reductional chromosome segregation at meiosis. *Nature* 400(6743):461–64
250. Weber SA, Gerton JL, Polancic JE, DeRisi JL, Koshland D, et al. 2004. The kinetochore is an enhancer of pericentric cohesin binding. *PLOS Biol.* 2(9):e260
251. Wei RR, Al-Bassam J, Harrison SC. 2007. The Ndc80/HEC1 complex is a contact point for kinetochore-microtubule attachment. *Nat. Struct. Mol. Biol.* 14(1):54–59
252. Wei RR, Schnell JR, Larsen NA, Sorger PK, Chou JJ, Harrison SC. 2006. Structure of a central component of the yeast kinetochore: the Spc24p/Spc25p globular domain. *Structure* 14(6):1003–9
253. Wei RR, Sorger PK, Harrison SC. 2005. Molecular organization of the Ndc80 complex, an essential kinetochore component. *PNAS* 102(15):5363–67
254. Weir JR, Faesen AC, Klare K, Petrovic A, Basilico F, et al. 2016. Insights from biochemical reconstitution into the architecture of human kinetochores. *Nature* 537(7619):249–53
255. Westermann S, Avila-Sakar A, Wang HW, Niederstrasser H, Wong J, et al. 2005. Formation of a dynamic kinetochore-microtubule interface through assembly of the Dam1 ring complex. *Mol. Cell* 17(2):277–90
256. Westhorpe FG, Straight AF. 2014. The centromere: epigenetic control of chromosome segregation during mitosis. *Cold Spring Harb. Perspect. Biol.* 7(1):a015818
257. Wimbish RT, DeLuca JG. 2020. Hec1/Ndc80 tail domain function at the kinetochore-microtubule interface. *Front. Cell Dev. Biol.* 8:43
258. Winey M, Mamay CL, O'Toole ET, Mastronarde DN, Giddings THJ, et al. 1995. Three-dimensional ultrastructural analysis of the *Saccharomyces cerevisiae* mitotic spindle. *J. Cell Biol.* 129(6):1601–15
259. Xiao H, Wang F, Wisniewski J, Shaytan AK, Ghirlando R, et al. 2017. Molecular basis of CENP-C association with the CENP-A nucleosome at yeast centromeres. *Genes Dev.* 31(19):1958–72
260. Yamagishi Y, Sakuno T, Shimura M, Watanabe Y. 2008. Heterochromatin links to centromeric protection by recruiting shugoshin. *Nature* 455(7210):251–55
261. Yan K, Yang J, Zhang Z, McLaughlin SH, Chang L, et al. 2019. Structure of the inner kinetochore CCAN complex assembled onto a centromeric nucleosome. *Nature* 574:278–82
262. Yan K, Zhang Z, Yang J, McLaughlin SH, Barford D. 2018. Architecture of the CBF3-centromere complex of the budding yeast kinetochore. *Nat. Struct. Mol. Biol.* 25(12):1103–10
263. Yatskevich S, Muir KW, Bellini D, Zhang Z, Yang J, et al. 2022. Structure of the human inner kinetochore bound to a centromeric CENP-A nucleosome. *Science* 376:844–52
264. Ye AA, Cane S, Maresca TJ. 2016. Chromosome biorientation produces hundreds of piconewtons at a metazoan kinetochore. *Nat. Commun.* 7:13221
265. Ye Q, Ur SN, Su TY, Corbett KD. 2016. Structure of the *Saccharomyces cerevisiae* Hrr25:Mam1 monopolin subcomplex reveals a novel kinase regulator. *EMBO J.* 35(19):2139–51
266. Yeh E, Haase J, Paliulis LV, Joglekar A, Bond L, et al. 2008. Pericentric chromatin is organized into an intramolecular loop in mitosis. *Curr. Biol.* 18(2):81–90
267. Zaytsev AV, Mick JE, Maslennikov E, Nikashin B, De Luca JG, Grishchuk EL. 2015. Multisite phosphorylation of the NDC80 complex gradually tunes its microtubule-binding affinity. *Mol. Biol. Cell* 26(10):1829–44
268. Zhang Q, Sivakumar S, Chen Y, Gao H, Yang L, et al. 2017. Ska3 phosphorylated by Cdk1 binds Ndc80 and recruits Ska to kinetochores to promote mitotic progression. *Curr. Biol.* 27(10):1477–84.e4
269. Zhang W, Lukoynova N, Miah S, Lucas J, Vaughan CK. 2018. Insights into centromere DNA bending revealed by the cryo-EM structure of the core centromere binding factor 3 with Ndc10. *Cell Rep.* 24(3):744–54
270. Zhang Z, Bellini D, Barford D. 2020. Crystal structure of the Cenp-HIKHead-TW sub-module of the inner kinetochore CCAN complex. *Nucleic Acids Res.* 48(19):11172–84
271. Zhao W, Jensen GJ. 2022. *In situ* architecture of human kinetochore-microtubule interface visualized by cryo-electron tomography. bioRxiv 2022.02.17.480955. **https://doi.org/10.1101/2022.02.17.480955**

272. Zhou K, Gebala M, Woods D, Sundararajan K, Edwards G, et al. 2022. CENP-N promotes the compaction of centromeric chromatin. *Nat. Struct. Mol. Biol.* 29:403–13
273. Zhou X, Zheng F, Wang C, Wu M, Zhang XX, et al. 2017. Phosphorylation of CENP-C by Aurora B facilitates kinetochore attachment error correction in mitosis. *PNAS* 114(50):E10667–76
274. Zhou Z, Feng H, Zhou BR, Ghirlando R, Hu K, et al. 2011. Structural basis for recognition of centromere histone variant CenH3 by the chaperone Scm3. *Nature* 472(7342):234–38
275. Zielinska AP, Bellou E, Sharma N, Frombach AS, Seres KB, et al. 2019. Meiotic kinetochores fragment into multiple lobes upon cohesin loss in aging eggs. *Curr. Biol.* 29(22):3749–65.e7
276. Zielinska AP, Holubcová Z, Blayney M, Elder K, Schuh M. 2015. Sister kinetochore splitting and precocious disintegration of bivalents could explain the maternal age effect. *eLife* 4:e11389
277. Zinkowski RP, Meyne J, Brinkley BR. 1991. The centromere-kinetochore complex: a repeat subunit model. *J. Cell Biol.* 113(5):1091–110