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REVIEW



Cell cycle control of kinetochore assembly

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ABSTRACT

The kinetochore is a large proteinaceous structure assembled on the centromeres of chromosomes. The complex machinery links chromosomes to the mitotic spindle and is essential for accurate chromosome segregation during cell division. The kinetochore is composed of two submodules: the inner and outer kinetochore. The inner kinetochore is assembled on centromeric chromatin and persists with centromeres throughout the cell cycle. The outer kinetochore attaches microtubules to the inner kinetochore, and assembles only during mitosis. The review focuses on recent advances in our understanding of the mechanisms governing the proper assembly of the outer kinetochore during mitosis and highlights open questions for future investigation.

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Introduction

Genetic information must be precisely transmitted to the next generation during cell division. To achieve this end, each chromosome must be duplicated and equally segregated into daughter cells during each cell cycle. Accurate chromosome segregation during mitosis relies on a large protein complex, the kinetochore, a term coined by Lester Whyland Sharp in 1934 [1]. The disc-shaped structure, which contains more than 100 protein subunits in human cells, assembles on a specialized chromatin domain known as the centromere. The kinetochore links chromosomes to microtubule polymers and plays a key role in controlling chromosome movements. It also serves as a hub for the signaling molecules required to control accurate chromosome segregation [2,3]. Under the electron microscope, the kinetochore appears as a two-domain structure at metaphase, consisting of the inner kinetochore and the outer kinetochore. The inner kinetochore is built on the centromeric chromatin and serves as a structural platform for outer kinetochore assembly. The inner kinetochore constitutively associates with centromeres. The outer kinetochore interacts with microtubules and plays an essential role in generating and sensing microtubule attachments [2,4,5]. When cells enter mitosis, the outer kinetochore is quickly

assembled on the platform of the inner kinetochore proteins. At the end of mitosis, the outer kinetochore is rapidly disassembled [6–8]. The kinetochore thus is a dynamic structure and tightly regulated over the course of the cell cycle. However, the molecular mechanisms underlying cell cycle-dependent kinetochore assembly are still not well understood. This review focuses on recent progress on our understanding of how the kinetochore assembly is cell cycle regulated. Due to space limitations, we limit our discussion to kinetochore assembly in mitosis. We refer readers interested in the reorganization of the kinetochore during error correction and meiosis to excellent reviews on these topics [3,9,10].

Centromeres

Centromeres are a specific chromatin structure that is physically linked to the spindle via kinetochores during cell division. It was first defined by Walther Flemming in 1882 as the primary constrictions on chromosomes [11]. The identity of centromeres has not been revealed until recently. Most eukaryotes have large ‘regional centromeres’, which usually consist of AT-rich DNA repeats. The size and sequence of regional centromeres vary significantly across species, spanning from

several kilobases to multiple megabases [12,13]. However, the underlying DNA sequence in centromeres is neither necessary nor sufficient for the centromere formation. Neocentromeres are new centromeres that are ectopically formed at non-centromeric regions when the native centromere is inactivated. Neocentromeres can arise both naturally and by experimental manipulation (for review, see [14,15]), providing strong evidence that centromere formation is an epigenetic event. Recent studies revealed that the regional centromere is epigenetically defined by a conserved centromeric specific histone H3 variant, CENP-A. CENP-A replaces its canonical counterpart and forms specific CENP-A nucleosomes with histone H4, H2A, and H2B, which are interspersed with canonical histone H3-containing nucleosomes in centromeres [16–21]. Mislocalized CENP-A recruits kinetochore proteins to non-centromeric regions, leading to chromosome missegregation defects in a variety of organisms (for review, see [22]). The histone fold domain of CENP-A contains the conserved CENP-A targeting domain (CATD), which is necessary and sufficient for centromeric localization of CENP-A in vertebrates [23,24].

Loading of CENP-A to centromeres is mediated by the conserved histone chaperone HJURP/Scm3 in a cell cycle-dependent manner [25–32]. In metazoans, loading of new CENP-A is restricted in the G1 phase of the cell cycle. The recruitment of HJURP to centromeres is mediated by the Mis18 complex, which is composed of Mis18 α , Mis18 β , and Mis18BP1. The cyclin-dependent kinases 1 and 2 (Cdk1/2) phosphorylate HJURP and Mis18BP1 during S and G2-phase, inhibiting premature CENP-A loading during this time [33–35]. On the other hand, the Polo-like kinase 1 (PLK1) phosphorylates the Mis18 complex during G1 to promote the deposition of new CENP-A to centromeres [36].

Transcription of CENP-A is also cell cycle regulated to ensure that optimal level of CENP-A is generated (for review, see [22]). In fission yeast, the temporal control of CENP-A^{Cnp1} transcription is mediated by the MBF (MluI box-binding factors) complex, which consists of Res1, Res2, Cdc10, Nrm1, and Yox1. The periodic transcription of CENP-A^{Cnp1} is lost in MBF mutants,

resulting in the higher level of CENP-A^{Cnp1} and chromosome segregation defects [37]. A recent study has also shown that the Cdk5 regulatory subunit-associated protein 2 (Cdk5rap2) acts as a positive transcriptional regulator of CENP-A in human cells [38]. Regional centromeres are typically buried in a large pericentric heterochromatin, a condensed and transcriptionally inert chromatin domain lacking CENP-A [39,40]. Heterochromatin, which is epigenetically defined by histone H3 lysine 9 (H3K9) methylation, contributes to the formation of regional centromeres [41–48].

On the other hand, the ‘point’ centromere in the budding yeast *Saccharomyces cerevisiae* is genetically determined by a 125-bp DNA sequence, which contains three centromere-determining elements (CDEI, CDEII, and CDEIII) [49–51]. The budding yeast centromeres also contain a CENP-A homolog, Cse4. CENP-A^{Cse4} is important for kinetochore assembly and chromosome segregation [52–54] and can functionally replace the human CENP-A [55]. The deposition of CENP-A^{Cse4} to centromeres is facilitated by the centromere DNA-binding complex, CBF3 [54,56,57]. In addition, some species in worms, plants, and insects use the whole chromosome as the centromere, which is called the ‘holocentric chromosome’ [58].

The inner kinetochore

The inner kinetochore consists of conserved 16 proteins in vertebrates, collectively known as constitutive centromere-associated network (CCAN). CCAN was originally identified by affinity purification of CENP-A-containing nucleosomes [59]. CCAN constitutively binds to the centromere throughout the cell cycle and can be divided into five subgroups, including CENP-L-N, CENP-H-I-K-M, CENP-O-P-Q-R-U, CENP-T-W-S-X, and CENP-C (Figure 1). CENP-C is a long disordered protein and forms a dimer through the C-terminal Cupin domain [60,61]. It interacts directly with the CENP-A nucleosome through the central domain and the short CENP-C motif [62–65]. CENP-C has multiple contacts with the structure of CCAN, including CENP-L-N and CENP-H-I-K-M, and is required for centromeric localization of other CCAN components [60,65–68], and

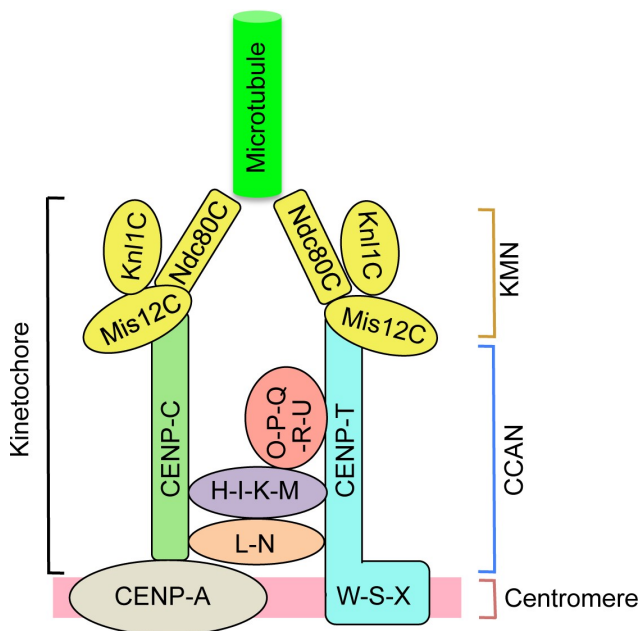


Figure 1. A schematic of a canonical kinetochore. The main structure of the kinetochore consists of constitutive centromere-associated network (CCAN), which includes five sub-groups (CENP-L-N, CENP-H-I-K-M, CENP-O-P-Q-R-U, CENP-T-W-S-X and CENP-C), and the KMN (Knl1, Mis12, and Ndc80 complexes) network. Kinetochore position is specified by CENP-A-containing nucleosomes, upon which CCAN assembles. CCAN recruits KMN, which directly binds microtubules, during mitosis. Two independent pathways, CENP-C and CENP-T, link KMN to CCAN.

thus serves as a hub for the assembly of the inner kinetochore. The CENP-L-N complexes also have been shown to directly interact with CENP-A nucleosomes [69,70]. However, a recent structural study using the full human CCAN complex assembled on the CENP-A nucleosome revealed that CENP-L-N interacts with the linker DNA but not the CENP-A nucleosome [68]. It is possible that CENP-L-N has different binding modes depending on cellular states. The CENP-L-N subgroup serves as a structural keystone of CCAN. CENP-H-I-K-M and CENP-O-P-Q-R-U are assembled on either side of the CENP-L-N [68,70].

The CENP-T-W-S-X consists of two heterodimers, CENP-T-W and CENP-S-X. Each subunit in the CENP-T-W-S-X contains the histone fold domain [71,72]. The CENP-T-W in fission yeast and vertebrates is essential for viability, but CENP-S-X is not [66,73–75]. Structural studies revealed that human CENP-T-W-S-X has multiple interactions with neighboring CCAN subunits, including CENP-H-I-K-M, CENP-L-N, and

CENP-O-P-Q-R-U [68]. In addition to the histone fold domain, CENP-T also contains a conserved histone fold extension (HFE) motif at the C-terminus and a long unstructured N-terminus. The histone fold domain of CENP-T forms a dimer with CENP-W, whereas the HFE motif interacts with the CENP-H-K-I-M [71,76]. The CENP-T-W-S-X also directly binds centromeric DNA. The DNA binding activity of the complex depends on the histone fold domain of CENP-T and CENP-W and is essential for kinetochore formation [71,74]. Chicken CENP-T-W-S-X is assembled into a stable heterotetramer in vitro and has been proposed to form a nucleosome-like structure [71]. This was supported by a recent structural study using the full human CCAN with α -satellite DNA [68]. The CENP-T-W-S-X also appears to bind histone H3, not to CENP-A [74,77]. It is believed that deposition of CENP-T is independent of CENP-A, but the association of CENP-T with centromeres is strongly reduced in CENP-A knockout cells [74,78–80]. Whole-proteome genetic analysis of chicken DT40 cells suggested that CENP-T depends on CENP-N for its centromeric localization [81]. A recent study also showed that RbAp48/RbAp46^{Mis16} is required for the deposition of CENP-T to centromeres in fission yeast [82]. Unlike CENP-T-W in vertebrates, CENP-T^{Cnn1} in budding yeast is exactly colocalized with CENP-A^{Cse4} nucleosomes [83,84], suggesting that CENP-T-W-S-X in budding yeast is unlikely to form nucleosome-like structure. The centromeric localization of CENP-T^{Cnn1} depends on CENP-I^{Ctf3} [84].

Although CCAN components localize constitutively at centromeres, the level of individual components, the timing of new protein loading at centromeres, and physical and functional relationships between different sub-groups can change throughout the cell cycle. Thus, the association of CCAN components with centromeres can still quite dynamic (for review, see [8]). The mechanisms underlying the dynamic behavior of CCAN during the cell cycle remain poorly understood.

The outer kinetochore

The outer kinetochore is assembled on the platform of inner kinetochore proteins during mitosis

and directly binds to microtubules. The 10-subunit outer kinetochores are categorized into three complexes: the Ndc80 complex (Ndc80C), the Mis12 complex (Mis12C), and the Knl1 complex (Knl1C). The three complexes form the KMN (Knl1C, Mis12C, and Ndc80C) network [2,85,86] (Figure 1). The Ndc80C contains four subunits: Ndc80, Nuf2, Spc24, and Spc25, and serves as the primary microtubule receptor on the kinetochores. The four subunits form two heterodimers: Ndc80-Nuf2, and Spc24-Spc25 [87–89]. The Ndc80-Nuf2 heterodimer contains a globular N-terminal region and a C-terminal coiled coil, while the Spc24-Spc25 heterodimer contains a globular C-terminal region and an N-terminal coiled coil. Each pair of proteins interacts via the coiled coil regions to form a tetramer, giving an overall dumbbell-like shape with a globular domain at each end. The Ndc80C binds microtubules via the globular N-terminal region of Ndc80-Nuf2, known as the MT-binding module containing calponin-homology (CH) domains [90–92]. The globular C-terminal region of Spc24-Spc25 contains RWD (RING finger and WD-repeats) domains, which interact with the disordered N-terminus of CENP-T [93–96]. The Mis12C consists of Mis12, Nnf1, Nsl1, and Dsn1. The complex is roughly rod-shaped, and binds both Ndc80C and Knl1C [95,97,98]. The Mis12C also interacts with CENP-C and CENP-T and serves as an interaction hub between the inner kinetochore and the outer kinetochore [97,99,100]. The Knl1C is composed of two subunits, Knl1 and ZWINT. Knl1, the largest subunit in the outer kinetochore, contains RWD domains at its C-terminus that directly binds the Mis12C [98,101]. ZWINT is involved in the spindle assembly checkpoint (SAC), a surveillance mechanism that prevents defects in chromosome segregation [102,103]. Interestingly, the KMN network components are conserved and exist in most eukaryotes, whereas the CCAN components are highly divergent across species [104].

Pathways linking the inner kinetochore to the outer kinetochore

Assembly of the outer kinetochore on the inner kinetochore during cell division is a crucial step in establishing microtubule attachments to the

kinetochore. CENP-C and CENP-T are the two major pathways that connect the inner kinetochore with the outer kinetochore [5,7] (Figure 1). Artificial tethering of partial CENP-C and CENP-T into a noncentromeric region indicated that CENP-C and CENP-T form two parallel pathways for the recruitment of the KMN network onto the kinetochore [100,105].

CENP-C exists largely as an elongated protein that may span >100 nm at the kinetochore [106]. The multi-domain protein not only serves as a hub for inner kinetochore assembly but also directly binds the Mis12C via its N-terminal region. CENP-C can recruit one copy of the Ndc80C through its interaction with Mis12 [65,99,100,107,108]. Artificial targeting of the N-terminus of CENP-C to a non-centromeric locus in human and chicken cells results in recruitment of KMN [100,105,107].

CENP-T binds Ndc80C through its extended unstructured N-terminal region. The Ndc80-binding domain at CENP-T forms an alpha-helix that directly interacts with the Spc24-Spc25 heterodimer in Ndc80C [93,94]. The CENP-T N-terminal region also directly interacts with Mis12C, which in turn binds Ndc80C [96]. Artificial tethering of the N-terminus of CENP-T in vertebrate cells is sufficient to recruit KMN to a non-centromeric locus [105]. Human CENP-T has two Ndc80C binding sites, whereas chicken CENP-T only has one site. In addition, CENP-T can indirectly recruit one copy of Ndc80C through its interaction with Mis12C [109,110]. Thus, a total of two copies of the Ndc80C exist on each CENP-T in chicken cells via either direct binding or Mis12C, but three copies for human CENP-T. A recent study in chicken cells demonstrated that both copies of the Ndc80C on chicken CENP-T are required for establishing proper kinetochore-microtubule interaction [109]. Configuration of two copies of Ndc80C has also been suggested for budding yeast CENP-T^{Cnn1} [84]. Artificial tethering assays using various CENP-T mutants in human and chicken cells demonstrated that the direct binding of Ndc80C to the CENP-T N-terminus is required for the CENP-T-Mis12C interaction, suggesting that the direct CENP-T-Ndc80C interaction acts upstream of Mis12C-recruitment to CENP-T [105,107].

The choice of different pathways linking the inner kinetochore and the outer kinetochore is different among different species. In human cancer cell lines, both CENP-C and CENP-T genes are essential for cell growth and proliferation [111], suggesting that both pathways are vital for kinetochore assembly. In chicken cells, although the N-terminus of CENP-C is essential for its interaction with Mis12C, CENP-C-Mis12C interaction is dispensable for cell viability while CENP-T is essential [109,110]. In fission yeast, deletion of CENP-C^{Cnp3} does not result in cell death, but CENP-T^{Cnp20} is indispensable [66], suggesting that the CENP-T pathway plays a more dominant role in kinetochore assembly in these organisms. On the other hand, although CENP-T^{Cnn1} and CENP-C^{Mif2} are conserved in budding yeast, CENP-T^{Cnn1} is not essential for cell viability, whereas CENP-C^{Mif2} is indispensable for chromosome segregation [112–114]. Further analysis revealed that, though CENP-A recognition by CENP-C^{Mif2} is essential for its kinetochore function, its binding to Mis12^{Mtw1} is dispensable [115]. In addition, a third pathway mediated by CENP-U^{Ame1} in budding yeast is used for recruiting KMN to CCAN. CENP-U^{Ame1} has the Mis12^{Mtw1} complex (Mis12^{Mtw1}C)-binding domain containing the first 15 amino acids (aa) in its N-terminus, and deletion of the domain causes cell lethality, suggesting that the CENP-U^{Ame1} pathway is the main pathway for kinetochore assembly in budding yeast [115,116]. CENP-U^{Ame1} also has a homolog in other species, including CENP-U in human and chicken and CENP-U^{Mis17} in fission yeast [94]. Whether CENP-U in these species is involved in KMN recruitment needs further characterization. Interestingly, in some species, such as *Drosophila melanogaster* and *Caenorhabditis elegans*, the CENP-T pathway is lost during evolution, and the CENP-C pathway alone is responsible for the assembly of KMN on the inner kinetochore [108,117–121]. On the other hand, holocentric Lepidoptera (butterflies and moths) lacks both CENP-A and CENP-C homologs [122]. A recent study showed that CENP-T in these insects is sufficient to recruit Mis12C and Ndc80C [123]. The diversity of inner kinetochore architecture may be explained

by the coevolution of inner kinetochore proteins and rapidly changing centromeric DNA sequences [104].

Cell cycle regulation of kinetochore assembly

The inner kinetochore proteins are constitutively localized at centromere in all the stages of the cell cycle, while the outer kinetochore are only assembled at centromeres during mitosis in vertebrates. Nevertheless, in both fission yeast and budding yeast, outer kinetochore proteins, such as Ndc80, appear to associate with centromeres through the different stages of the cell cycle [87,124]. However, a recent work by Jiménez-Martín et al. demonstrated that the outer kinetochore is indeed also reassembled at the onset of mitosis in fission yeast, similar to metazoans [125]. Fission yeast chromatin is characterized by the evolutionarily conserved Rab1 chromosome configuration during interphase, in which centromeres are clustered underneath the nuclear envelope near the spindle pole body (SPB) and telomeres are also attached to the nuclear envelope [126]. The recent study showed that when Rab1 configuration is removed, the outer kinetochore reassembly was then observed during mitosis, indicating that Rab1 configuration masks kinetochore reassembly in fission yeast [125]. Consistent with this, Dong et al. showed that Ndc80 and CENP-T^{Cnp20} strongly interact during mitosis but not interphase [82]. Similarly, in budding yeast, the interaction between Ndc80C and CENP-T^{Cnn1} also occurs predominantly in mitosis, suggesting that the outer kinetochore adopts a structural change during the stage of the cell cycle [94]. These works indicated that outer kinetochore disassembly/assembly program during cell cycle progression is a conserved phenomenon. Recent studies have demonstrated that post-translational modifications, such as phosphorylation, play a crucial role in governing the interaction between CCAN and KMN during mitosis.

Phospho-regulation of outer kinetochore assembly through the cell cycle

CENP-T phosphorylation has been well studied and found to be conserved among different

species. The N-terminus of CENP-T contains the Ndc80C-binding domain, which binds the Spc24-Spc25 heterodimer in Ndc80C. Phosphorylation of the Ndc80C-binding domain in CENP-T by Cdk1 stabilizes the interaction [93,94]. In human cells, Cdk1-mediated phosphorylation at Thr11 and Thr85, both of which reside in the conserved Cdk1 consensus sequence within the Ndc80C-binding domain, promotes the recruitment of two copies of Ndc80C to CENP-T [96,107]. Chicken CENP-T has one Cdk1 consensus sequence in the Ndc80C-binding domain, and phosphorylation of Thr72 within the sequence is important for recruitment of Ndc80C to CENP-T [93]. Budding yeast CENP-T^{Cnn1} also contains a conserved Ndc80C binding motif at its N-terminus [94]. The N-terminus of CENP-T^{Cnn1} can be phosphorylated by both Cdk1 and mitotic kinase Mps1. However, Cdk1 is not required for the interaction of CENP-T^{Cnn1} and Ndc80C. Instead, Mps1-mediated Ser74 phosphorylation contributes to the interaction [95,127]. The interaction between Ndc80C and CENP-T^{Cnn1} in budding yeast is not essential, while the Ndc80C-Mis12^{Mtw1}C interaction is. It has been proposed that Mps1 promotes the formation of essential Ndc80C-Mis12^{Mtw1}C during S phase and early mitosis by inhibiting the Ndc80C and CENP-T^{Cnn1} interaction [95].

Cdk1-mediated phosphorylation also plays an important role in CENP-T-Mis12C interaction. The aa 201–230 region in human CENP-T is critical for Mis12C recruitment. Thr195 and Ser201 in this region have been shown to be critical for recruitment of Mis12C to CENP-T [96,107]. However, in chicken cells, a phospho-null mutant of Thr184 (corresponding to human CENP-T S201) is viable [110]. In fact, the phospho-null mutant of all the Ser/Thr from aa 161 to 216 in the Mis12C binding region does not have any defect [109]. These data suggest that an additional regulatory mechanism controls the CENP-T-Mis12C interaction in chicken cells.

In addition, the CENP-C-Mis12C interaction is also mediated by mitotic phosphorylation. Dsn1 in the Mis12C can auto-inhibit the CENP-C-Mis12C interaction via its conserved basic domain by masking the CENP-C-binding interface in Mis12C [128,129]. Aurora B kinase, which is

known to be involved in error correction and SAC response, phosphorylates the basic domain of Dsn1 during mitosis. The phosphorylation results in an increased binding affinity between CENP-C and Mis12C in vitro and centromeric localization of Mis12C in mitotic cells [128,130,131].

Hara et al. also showed that Cdk1-mediated phosphorylation contributes to the Mis12C-Ndc80C interaction [110]. Dsn1 in Mis12C binds Ndc80C through its C-terminus, which contains a sequence similar to the Ndc80C-binding region in the CENP-T N-terminus. The Ndc80C-binding region in Dsn1 contains one Cdk1 consensus sequence [110,128]. Interestingly, while phosphorylation of CENP-T by Cdk1 stabilizes its interaction with Ndc80C, Cdk1-mediated phosphorylation in the Ndc80-binding region of Dsn1 decreases its binding affinity with Spc24-Spc25, leading to unstable interaction between Mis12C and Ndc80C. Thus, Cdk1 phosphorylation is a negative regulator of Mis12C-Ndc80C interaction, which may explain why a majority of Ndc80C localized to the CENP-T pathway, but less to Mis12C on the CENP-C pathway during mitosis in chicken DT40 cells [110].

The kinetochore-microtubule attachment is stabilized by the Ska complex, which contains three subunits, Ska1, Ska2, and Ska3 [132–134]. During mitosis, Ska3 is also phosphorylated by Cdk1. Phosphorylated Ska3 binds to Ndc80C which in turn recruits the Ska complex to kinetochores. Ska3 mutants lacking Cdk1 phosphorylation are defective in kinetochore localization [135,136].

Mutually exclusive binding modes establish distinct configurations of the outer kinetochore

A recent study in fission yeast revealed that phosphorylation-mediated competitive exclusion of different proteins at the N-terminus of CENP-T also regulates the recruitment of KMN. Fission yeast contains regional centromeres, epigenetically defined by CENP-A^{Cnp1} [137,138]. Dong et al. previously identified that Ccp1, a conserved nucleosome assembly protein (NAP) domain-containing protein, antagonizes the loading of CENP-A in both centromere and non-centromeric regions in

fission yeast [139]. Interestingly, Ccp1 associates with centromeres during interphase but dissociates from centromeres during mitosis [139,140]. The biological significance of this cell cycle-regulated association of Ccp1 with centromeres is unknown. Dong et al. recently revealed that Ccp1 binds to CENP-T^{Cnp20} and further showed that CENP-T^{Cnp20} is required for Ccp1 centromeric localization [82].

CENP-T^{Cnp20} in fission yeast is essential for viability and contains a Ndc80-binding region (aa 70–87) at its N-terminus. The Ndc80 binding region contains a conserved Cdk1 consensus sequence [94]. Ccp1 binds to the first 1–55 amino acids of CENP-T^{Cnp20}, which is immediately adjacent to the Ndc80-binding region. The Ccp1-binding motif in CENP-T^{Cnp20} contains four conserved Cdk1 consensus sequences and can be phosphorylated by Cdk1 in vitro [82]. Phosphorylation of the Ccp1-binding motif in CENP-T^{Cnp20} decreases its interaction with Ccp1. Consistent with this, in the phosphomimetic mutant of the Ccp1 binding motif, Ccp1 dissociates from centromeres through all stages of the cell cycle. In contrast, Ccp1

associates with centromeres during both mitosis and interphase in the phospho-null mutant of the motif. Furthermore, the phospho-null mutant of Ccp1-binding motif disrupts the positioning of Ndc80C during mitosis and displays severe chromosome missegregation defects, suggesting that occupancy of Ccp1 at the Ccp1-binding domain in CENP-T prevents the interaction of the adjacent Ndc80-binding domain with Ndc80C [82]. Together, this study suggests the following model: at the onset of mitosis, Cdk1 mediated phosphorylation of CENP-T^{Cnp20} at the Ccp1-binding motif ejected the Ccp1 and makes the room for the binding of Ndc80C to CENP-T^{Cnp20}; at the end of mitosis, the Ccp1-binding motif is dephosphorylated, leading to the recruitment of Ccp1 that blocks the binding of Ndc80C to CENP-T (Figure 2). Phosphorylation-mediated competitive exclusion between Ccp1 and Ndc80C represents a new mechanism governing the cell cycle-dependent kinetochore assembly. The principle explains the observation that Ccp1 dissociates from centromeres during mitosis.

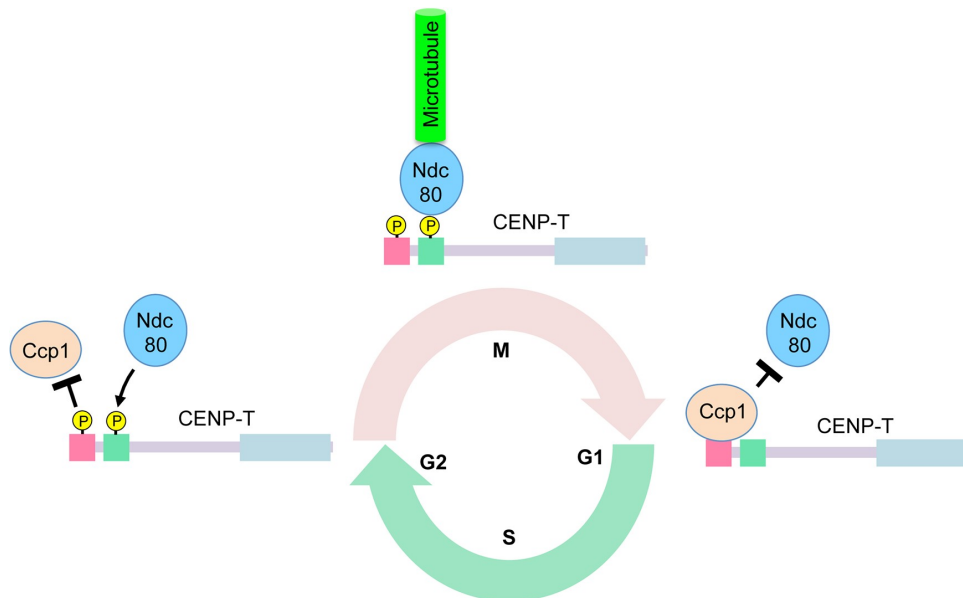


Figure 2. Phosphorylation-mediated competitive exclusion between Ccp1 and Ndc80 at the N-terminus of CENP-T regulates the recruitment of KMN. The Ccp1-binding domain of CENP-T is localized adjacent to the Ndc80-binding domain at the N-terminal region of CENP-T. When cells enter mitosis, the Ccp1-binding domain of CENP-T is phosphorylated by the Cdk1 kinase. Phosphorylation of the Ccp1-binding domain dissociates Ccp1 from CENP-T, allowing Ndc80C to bind to the Ndc80-binding domain. Ndc80C then directly interacts with microtubules to facilitate chromosome segregation. When cells exit from mitosis, the Ccp1-binding domain is dephosphorylated, which recruits Ccp1. Reassociation of Ccp1 with the Ccp1-binding domain blocks the binding of Ndc80C to CENP-T during interphase. P: phosphorylation.

Concluding remarks

The kinetochore plays a fundamental role in accurate chromosome segregation during mitosis. The large protein machine must be precisely assembled on centromeric chromatin during mitosis to generate a microtubule-binding interface that links chromosomes to the mitotic spindle. Kinetochore dysfunctions can lead to chromosomal instability (CIN) and aneuploidy, both of which are common characteristics of cancer cells [141–143]. However, in spite of recent achievements in understanding the components and functions of the kinetochore, there remains much to be learned about how kinetochore assembly is cell cycle regulated. Phosphorylation has been heavily studied as regulators of the events leading to kinetochore assembly. The role of other posttranslational modifications in kinetochore assembly during mitosis is less well understood. Future studies are needed to address this issue. A variety of different strategies were adopted by different species to recruit KMN to the inner kinetochores. A detailed understanding of how CENP-C and CENP-T pathways are used and coordinated to recruit KMN in these organisms will shed important light on the kinetochore's role in chromosome segregation. Phosphorylation-mediated competitive exclusion between Ccp1-Ndc80 provides a new insight into the cell cycle-dependent kinetochore assembly. Similar to Ccp1, multiple other centromeric proteins in fission yeast, including HJURP/Scm3, Mis16, Mis18, Eic1/Mis19/Kis1, and Eic2/Mis20, also display the same distribution pattern through the cell cycle [26,28,29,144–147]. In addition, Mis18 α in chicken DT40 cells is localized at centromeres during interphase, but is lost during mitosis [148]. It will be interesting to know whether these centromeric proteins use a similar mechanism to regulate kinetochore assembly. While much of the focus has been on understanding the assembly of kinetochores at the onset of mitosis, how kinetochores are disassembled at the end of mitosis have received less attention. The PP2A (protein phosphatase 2A), which is able to

dephosphorylate CDK1 substrates during anaphase, has been implicated in kinetochore disassembly [6]. The molecular basis of PP2A-mediated kinetochore disassembly will need to be addressed in the future.

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References

- [1] Sharp LW. Introduction to cytology. New York: McGraw-Hill Book Company, inc; 1934.
- [2] Cheeseman IM, Desai A. Molecular architecture of the kinetochore-microtubule interface. *Nat Rev Mol Cell Biol.* 2008;9(1):33–46.
- [3] Santaguida S, Musacchio A. The life and miracles of kinetochores. *EMBO J.* 2009;28(17):2511–2531.
- [4] Cleveland DW, Mao Y, Sullivan KF. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. *Cell.* 2003;112(4):407–421.
- [5] Musacchio A, Desai A. A molecular view of kinetochore assembly and function. *Biology (Basel).* 2017;6(1):5.
- [6] Gascoigne KE, Cheeseman IM. CDK-dependent phosphorylation and nuclear exclusion coordinately control kinetochore assembly state. *J Cell Biol.* 2013;201(1):23–32.
- [7] Nagpal H, Fukagawa T. Kinetochore assembly and function through the cell cycle. *Chromosoma.* 2016;125(4):645–659.
- [8] Navarro AP, Cheeseman IM. Kinetochore assembly throughout the cell cycle. *Semin Cell Dev Biol.* 2021;117:62–74.
- [9] Lara-Gonzalez P, Pines J, Desai A. Spindle assembly checkpoint activation and silencing at kinetochores. *Semin Cell Dev Biol.* 2021;117:86–98.
- [10] Watanabe Y. Geometry and force behind kinetochore orientation: lessons from meiosis. *Nat Rev Mol Cell Biol.* 2012;13(6):370–382.
- [11] Westhorpe FG, Straight AF. The centromere: epigenetic control of chromosome segregation during

- mitosis. *Cold Spring Harb Perspect Biol.* **2014**;7:a015818.
- [12] McKinley KL, Cheeseman IM. The molecular basis for centromere identity and function. *Nat Rev Mol Cell Biol.* **2016**;17(1):16–29.
- [13] Kalitsis P, Choo KH. The evolutionary life cycle of the resilient centromere. *Chromosoma.* **2012**;121(4):327–340.
- [14] DeBose-Scarlett EM, Sullivan BA. Genomic and Epigenetic Foundations of Neocentromere Formation. *Annu Rev Genet.* **2021**;55(1):331–348.
- [15] Burrack LS, Berman J. Neocentromeres and epigenetically inherited features of centromeres. *Chromosome Res.* **2012**;20(5):607–619.
- [16] Tachiwana H, Kagawa W, Shiga T, et al. Crystal structure of the human centromeric nucleosome containing CENP-A. *Nature.* **2011**;476(7359):232–235. DOI:10.1038/nature10258.
- [17] Sullivan BA, Karpen GH. Centromeric chromatin exhibits a histone modification pattern that is distinct from both euchromatin and heterochromatin. *Nat Struct Mol Biol.* **2004**;11(11):1076–1083.
- [18] Bodor DL, Mata JF, Sergeev M, et al. The quantitative architecture of centromeric chromatin. *Elife.* **2014**;3:e02137.
- [19] Kixmoeller K, Allu PK, Black BE. The centromere comes into focus: from CENP-A nucleosomes to kinetochore connections with the spindle. *Open Biol.* **2020**;10(6):200051.
- [20] Mendiburo MJ, Padeken J, Fulop S, et al. *Drosophila* CENH3 is sufficient for centromere formation. *Science.* **2011**;334(6056):686–690.
- [21] Stirpe A, Heun P. The ins and outs of CENP-A: chromatin dynamics of the centromere-specific histone. *Semin Cell Dev Biol.* **2022**;S1084-9521(22):00128-8.
- [22] Dong Q, Yang J, Gao J, et al. Recent insights into mechanisms preventing ectopic centromere formation. *Open Biol.* **2021**;11(9):210189.
- [23] Black BE, Brock MA, Bedard S, et al. An epigenetic mark generated by the incorporation of CENP-A into centromeric nucleosomes. *Proc Natl Acad Sci U S A.* **2007**;104(12):5008–5013.
- [24] Black BE, Jansen LE, Maddox PS, et al. Centromere identity maintained by nucleosomes assembled with histone H3 containing the CENP-A targeting domain. *Mol Cell.* **2007**;25(2):309–322.
- [25] Dunleavy EM, Roche D, Tagami H, et al. HJURP is a cell-cycle-dependent maintenance and deposition factor of CENP-A at centromeres. *Cell.* **2009**;137(3):485–497.
- [26] Foltz DR, Jansen LE, Bailey AO, et al. Centromere-specific assembly of CENP-a nucleosomes is mediated by HJURP. *Cell.* **2009**;137(3):472–484.
- [27] Mizuguchi G, Xiao H, Wisniewski J, et al. Nonhistone Scm3 and histones CenH3-H4 assemble the core of centromere-specific nucleosomes. *Cell.* **2007**;129(6):1153–1164.
- [28] Pidoux AL, Choi ES, Abbott JK, et al. Fission yeast Scm3: a CENP-A receptor required for integrity of subkinetochore chromatin. *Mol Cell.* **2009**;33(3):299–311. DOI:10.1016/j.molcel.2009.01.019.
- [29] Williams JS, Hayashi T, Yanagida M, et al. Fission yeast Scm3 mediates stable assembly of Cnp1/CENP-A into centromeric chromatin. *Mol Cell.* **2009**;33(3):287–298.
- [30] Sanchez-Pulido L, Pidoux AL, Ponting CP, et al. Common ancestry of the CENP-A chaperones Scm3 and HJURP. *Cell.* **2009**;137(7):1173–1174.
- [31] Dunleavy EM, Beier NL, Gorgescu W, et al. The cell cycle timing of centromeric chromatin assembly in *Drosophila* meiosis is distinct from mitosis yet requires CAL1 and CENP-C. *PLoS Biol.* **2012**;10(12):e1001460.
- [32] Mellone BG, Grive KJ, Shteyn V, et al. Assembly of *Drosophila* centromeric chromatin proteins during mitosis. *PLoS Genet.* **2011**;7(5):e1002068.
- [33] Silva MC, Bodor DL, Stellfox ME, et al. Cdk activity couples epigenetic centromere inheritance to cell cycle progression. *Dev Cell.* **2012**;22(1):52–63.
- [34] Spiller F, Medina-Pritchard B, Abad MA, et al. Molecular basis for Cdk1-regulated timing of Mis18 complex assembly and CENP-A deposition. *EMBO Rep.* **2017**;18(6):894–905.
- [35] Stankovic A, Guo LY, Mata JF, et al. A dual inhibitory mechanism sufficient to maintain cell-cycle-restricted CENP-A assembly. *Mol Cell.* **2017**;65(2):231–246. DOI:10.1016/j.molcel.2016.11.021.
- [36] McKinley KL, Cheeseman IM. Polo-like kinase 1 licenses CENP-A deposition at centromeres. *Cell.* **2014**;158(2):397–411.
- [37] Aristizabal-Corrales D, Yang J, Li F. Cell cycle-regulated transcription of CENP-A by the MBF complex ensures optimal level of CENP-A for centromere formation. *Genetics.* **2019**;211(3):861–875.
- [38] Wang X, Rosales JL, Gao X, et al. Centromeric chromatin integrity is compromised by loss of Cdk5rap2, a transcriptional activator of CENP-A. *Biomed Pharmacother.* **2021**;138:111463.
- [39] Yang J, Li F. Are all repeats created equal? Understanding DNA repeats at an individual level. *Curr Genet.* **2017**;63(1):57–63.
- [40] Steiner FA, Henikoff S. Diversity in the organization of centromeric chromatin. *Curr Opin Genet Dev.* **2015**;31:28–35.
- [41] Ishii K, Ogiyama Y, Chikashige Y, et al. Heterochromatin integrity affects chromosome reorganization after centromere dysfunction. *Science.* **2008**;321(5892):1088–1091.
- [42] Folco HD, Pidoux AL, Urano T, et al. Heterochromatin and RNAi are required to establish CENP-A chromatin at centromeres. *Science.* **2008**;319(5859):94–97.
- [43] He H, Zhang S, Wang D, et al. Condensin promotes position effects within tandem DNA repeats via the RITS complex. *Cell Rep.* **2016**;14(5):1018–1024.

- [44] Allshire RC, Madhani HD. Ten principles of heterochromatin formation and function. *Nat Rev Mol Cell Biol.* **2018**;19(4):229–244.
- [45] Olszak AM, van Essen D, Pereira AJ, et al. Heterochromatin boundaries are hotspots for de novo kinetochore formation. *Nat Cell Biol.* **2011**;13(7):799–808.
- [46] Kagansky A, Folco HD, Almeida R, et al. Synthetic heterochromatin bypasses RNAi and centromeric repeats to establish functional centromeres. *Science.* **2009**;324(5935):1716–1719.
- [47] Smith KM, Phatale PA, Sullivan CM, et al. Heterochromatin is required for normal distribution of *Neurospora crassa* CenH3. *Mol Cell Biol.* **2011**;31(12):2528–2542.
- [48] Yang J, Sun S, Zhang S, et al. Heterochromatin and RNAi regulate centromeres by protecting CENP-A from ubiquitin-mediated degradation. *PLoS Genet.* **2018**;14(8):e1007572.
- [49] Malik HS, Henikoff S. Major evolutionary transitions in centromere complexity. *Cell.* **2009**;138(6):1067–1082.
- [50] Clarke L, Carbon J. Isolation of a yeast centromere and construction of functional small circular chromosomes. *Nature.* **1980**;287(5782):504–509.
- [51] Cottarel G, Shero JH, Hieter P, et al. A 125-base-pair CEN6 DNA fragment is sufficient for complete meiotic and mitotic centromere functions in *Saccharomyces cerevisiae*. *Mol Cell Biol.* **1989**;9(8):3342–3349.
- [52] Stoler S, Keith KC, Curnick KE, et al. A mutation in CSE4, an essential gene encoding a novel chromatin-associated protein in yeast, causes chromosome nondisjunction and cell cycle arrest at mitosis. *Genes Dev.* **1995**;9(5):573–586.
- [53] Meluh PB, Yang P, Glowczewski L, et al. Cse4p is a component of the core centromere of *Saccharomyces cerevisiae*. *Cell.* **1998**;94(5):607–613.
- [54] Furuyama S, Biggins S. Centromere identity is specified by a single centromeric nucleosome in budding yeast. *Proc Natl Acad Sci U S A.* **2007**;104(37):14706–14711.
- [55] Wieland G, Orthaus S, Ohndorf S, et al. Functional Complementation of Human Centromere Protein A (CENP-A) by Cse4p from *Saccharomyces cerevisiae*. *Mol Cell Biol.* **2004**;24(15):6620–6630.
- [56] Cole HA, Howard BH, Clark DJ. The centromeric nucleosome of budding yeast is perfectly positioned and covers the entire centromere. *Proc Natl Acad Sci U S A.* **2011**;108(31):12687–12692.
- [57] Krassovsky K, Henikoff JG, Henikoff S. Tripartite organization of centromeric chromatin in budding yeast. *Proc Natl Acad Sci U S A.* **2012**;109(1):243–248.
- [58] Melters DP, Paliulis LV, Korf IF, et al. Holocentric chromosomes: convergent evolution, meiotic adaptations, and genomic analysis. *Chromosome Res.* **2012**;20(5):579–593.
- [59] Foltz DR, Jansen LE, Black BE, et al. The human CENP-A centromeric nucleosome-associated complex. *Nat Cell Biol.* **2006**;8(5):458–469.
- [60] Klare K, Weir JR, Basilico F, et al. CENP-C is a blueprint for constitutive centromere-associated network assembly within human kinetochores. *J Cell Biol.* **2015**;210(1):11–22.
- [61] Cohen RL, Espelin CW, De Wulf P, et al. Structural and functional dissection of Mif2p, a conserved DNA-binding kinetochore protein. *Mol Biol Cell.* **2008**;19(10):4480–4491.
- [62] Kato H, Jiang J, Zhou BR, et al. A conserved mechanism for centromeric nucleosome recognition by centromere protein CENP-C. *Science.* **2013**;340(6136):1110–1113.
- [63] Carroll CW, Milks KJ, Straight AF. Dual recognition of CENP-A nucleosomes is required for centromere assembly. *J Cell Biol.* **2010**;189(7):1143–1155.
- [64] Chik JK, Moiseeva V, Goel PK, et al. Structures of CENP-C cupin domains at regional centromeres reveal unique patterns of dimerization and recruitment functions for the inner pocket. *J Biol Chem.* **2019**;294(38):14119–14134.
- [65] Walstein K, Petrovic A, Pan D, et al. Assembly principles and stoichiometry of a complete human kinetochore module. *Sci Adv.* **2021**;7(27). DOI:10.1126/sciadv.abg1037
- [66] Tanaka K, Chang HL, Kagami A, et al. CENP-C functions as a scaffold for effectors with essential kinetochore functions in mitosis and meiosis. *Dev Cell.* **2009**;17(3):334–343.
- [67] Yan K, Yang J, Zhang Z, et al. Structure of the inner kinetochore CCAN complex assembled onto a centromeric nucleosome. *Nature.* **2019**;574(7777):278–282.
- [68] Yatskevich S, Muir KW, Bellini D, et al. Structure of the human inner kinetochore bound to a centromeric CENP-A nucleosome. *Science.* **2022**;376(6595):844–852.
- [69] Tian T, Li X, Liu Y, et al. Molecular basis for CENP-N recognition of CENP-A nucleosome on the human kinetochore. *Cell Res.* **2018**;28(3):374–378.
- [70] McKinley KL, Sekulic N, Guo LY, et al. The CENP-L-N complex forms a critical node in an integrated meshwork of interactions at the centromere-kinetochore interface. *Mol Cell.* **2015**;60(6):886–898.
- [71] Nishino T, Takeuchi K, Gascoigne KE, et al. CENP-T-W-S-X forms a unique centromeric chromatin structure with a histone-like fold. *Cell.* **2012**;148(3):487–501.
- [72] Takeuchi K, Nishino T, Mayanagi K, et al. The centromeric nucleosome-like CENP-T-W-S-X complex induces positive supercoils into DNA. *Nucleic Acids Res.* **2014**;42(3):1644–1655.
- [73] Zafar F, Okita AK, Onaka AT, et al. Regulation of mitotic recombination between DNA repeats in

- centromeres. *Nucleic Acids Res.* **2017**;45(19):11222–11235.
- [74] Hori T, Amano M, Suzuki A, et al. CCAN makes multiple contacts with centromeric DNA to provide distinct pathways to the outer kinetochore. *Cell.* **2008**;135(6):1039–1052. DOI:[10.1016/j.cell.2008.10.019](https://doi.org/10.1016/j.cell.2008.10.019).
- [75] Amano M, Suzuki A, Hori T, et al. The CENP-S complex is essential for the stable assembly of outer kinetochore structure. *J Cell Biol.* **2009**;186(2):173–182.
- [76] Zhang Z, Bellini D, Barford D. Crystal structure of the Cenp-HIKHead-TW sub-module of the inner kinetochore CCAN complex. *Nucleic Acids Res.* **2020**;48(19):11172–11184.
- [77] Tschernyschkow S, Herda S, Gruenert G, et al. Rule-based modeling and simulations of the inner kinetochore structure. *Prog Biophys Mol Biol.* **2013**;113(1):33–45.
- [78] Fachinetti D, Folco HD, Nechemia-Arbely Y, et al. A two-step mechanism for epigenetic specification of centromere identity and function. *Nat Cell Biol.* **2013**;15(9):1056–1066. DOI:[10.1038/ncb2805](https://doi.org/10.1038/ncb2805).
- [79] Sathyan KM, Fachinetti D, Foltz DR. alpha-amino trimethylation of CENP-A by NRMT is required for full recruitment of the centromere. *Nat Commun.* **2017**;8(1):14678.
- [80] Hoffmann S, Fachinetti D. A time out for CENP-A. *Mol Cell Oncol.* **2017**;4(3):e1293596.
- [81] Samejima I, Spanos C, Alves Fde L, et al. Whole-proteome genetic analysis of dependencies in assembly of a vertebrate kinetochore. *J Cell Biol.* **2015**;211(6):1141–1156.
- [82] Dong Q, Liu XL, Wang XH, et al. Ccp1-Ndc80 switch at the N terminus of CENP-T regulates kinetochore assembly. *Proc Natl Acad Sci U S A.* **2021**;118(48). DOI:[10.1073/pnas.2104459118](https://doi.org/10.1073/pnas.2104459118)
- [83] Foltz DR, Stukenberg PT. A new histone at the centromere? *Cell.* **2012**;148(3):394–396.
- [84] Pekgoz Altunkaya G, Malvezzi F, Demianova Z, et al. CCAN Assembly Configures Composite Binding Interfaces to Promote Cross-Linking of Ndc80 Complexes at the Kinetochore. *Curr Biol.* **2016**;26(17):2370–2378.
- [85] Cheeseman IM, Niessen S, Anderson S, et al. A conserved protein network controls assembly of the outer kinetochore and its ability to sustain tension. *Genes Dev.* **2004**;18(18):2255–2268.
- [86] Cheeseman IM, Chappie JS, Wilson-Kubalek EM, et al. The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell.* **2006**;127(5):983–997.
- [87] Wigge PA, Kilmartin JV. The Ndc80p complex from *Saccharomyces cerevisiae* contains conserved centromere components and has a function in chromosome segregation. *J Cell Biol.* **2001**;152(2):349–360.
- [88] McClelland ML, Gardner RD, Kallio MJ, et al. The highly conserved Ndc80 complex is required for kinetochore assembly, chromosome congression, and spindle checkpoint activity. *Genes Dev.* **2003**;17(1):101–114.
- [89] Bharadwaj R, Qi W, Yu H. Identification of two novel components of the human NDC80 kinetochore complex. *J Biol Chem.* **2004**;279(13):13076–13085.
- [90] Ciferri C, De Luca J, Monzani S, et al. Architecture of the human ndc80-hec1 complex, a critical constituent of the outer kinetochore. *J Biol Chem.* **2005**;280(32):29088–29095.
- [91] Wei RR, Schnell JR, Larsen NA, et al. Structure of a central component of the yeast kinetochore: the Spc24p/Spc25p globular domain. *Structure.* **2006**;14(6):1003–1009.
- [92] Ciferri C, Pasqualato S, Screpanti E, et al. Implications for kinetochore-microtubule attachment from the structure of an engineered Ndc80 complex. *Cell.* **2008**;133(3):427–439. DOI:[10.1016/j.cell.2008.03.020](https://doi.org/10.1016/j.cell.2008.03.020).
- [93] Nishino T, Rago F, Hori T, et al. CENP-T provides a structural platform for outer kinetochore assembly. *EMBO J.* **2013**;32(3):424–436.
- [94] Schleiffer A, Maier M, Litos G, et al. CENP-T proteins are conserved centromere receptors of the Ndc80 complex. *Nat Cell Biol.* **2012**;14(6):604–613.
- [95] Malvezzi F, Litos G, Schleiffer A, et al. A structural basis for kinetochore recruitment of the Ndc80 complex via two distinct centromere receptors. *EMBO J.* **2013**;32(3):409–423.
- [96] Huis In 't Veld PJ, Jeganathan S, Petrovic A. Molecular basis of outer kinetochore assembly on CENP-T. *Elife.* **2016**;5:e21007.
- [97] Petrovic A, Pasqualato S, Dube P, et al. The MIS12 complex is a protein interaction hub for outer kinetochore assembly. *J Cell Biol.* **2010**;190(5):835–852. DOI:[10.1083/jcb.201002070](https://doi.org/10.1083/jcb.201002070).
- [98] Petrovic A, Mosalaganti S, Keller J, et al. Modular assembly of RWD domains on the Mis12 complex underlies outer kinetochore organization. *Mol Cell.* **2014**;53(4):591–605. DOI:[10.1016/j.molcel.2014.01.019](https://doi.org/10.1016/j.molcel.2014.01.019).
- [99] Screpanti E, De Antoni A, Alushin GM, et al. Direct binding of Cenp-C to the Mis12 complex joins the inner and outer kinetochore. *Curr Biol.* **2011**;21(5):391–398.
- [100] Gascoigne KE, Takeuchi K, Suzuki A, et al. Induced ectopic kinetochore assembly bypasses the requirement for CENP-A nucleosomes. *Cell.* **2011**;145(3):410–422.
- [101] Schmitzberger F, Harrison SC. RWD domain: a recurring module in kinetochore architecture shown by a Ctf19-Mcm21 complex structure. *EMBO Rep.* **2012**;13(3):216–222.
- [102] Wang H, Hu X, Ding X, et al. Human Zwint-1 specifies localization of Zeste White 10 to kinetochores and is essential for mitotic checkpoint signaling. *J Biol Chem.* **2004**;279(52):54590–54598. DOI:[10.1074/jbc.M407588200](https://doi.org/10.1074/jbc.M407588200).
- [103] Varma D, Wan X, Cheerambathur D, et al. Spindle assembly checkpoint proteins are positioned close to

- core microtubule attachment sites at kinetochores. *J Cell Biol.* **2013**;202(5):735–746.
- [104] Hamilton GE, Davis TN. Biochemical evidence for diverse strategies in the inner kinetochore. *Open Biol.* **2020**;10(11):200284.
- [105] Hori T, Shang W-H, Takeuchi K, et al. The CCAN recruits CENP-A to the centromere and forms the structural core for kinetochore assembly. *J Cell Biol.* **2013**;200(1):45–60.
- [106] Guo LY, Allu PK, Zandarashvili L, et al. Centromeres are maintained by fastening CENP-A to DNA and directing an arginine anchor-dependent nucleosome transition. *Nat Commun.* **2017**;8(1):15775. DOI:10.1038/ncomms15775.
- [107] Rago F, Gascoigne KE, Cheeseman IM. Distinct organization and regulation of the outer kinetochore KMN network downstream of CENP-C and CENP-T. *Curr Biol.* **2015**;25(5):671–677.
- [108] Przewlaka MR, Venkei Z, Bolanos-Garcia VM, et al. CENP-C is a structural platform for kinetochore assembly. *Curr Biol.* **2011**;21(5):399–405.
- [109] Takenoshita Y, Hara M, Fukagawa T. Recruitment of two Ndc80 complexes via the CENP-T pathway is sufficient for kinetochore functions. *Nat Commun.* **2022**;13(1):851.
- [110] Hara M, Ariyoshi M, Okumura EI, et al. Multiple phosphorylations control recruitment of the KMN network onto kinetochores. *Nat Cell Biol.* **2018**;20(12):1378–1388.
- [111] Wang T, Birsoy K, Hughes NW, et al. Identification and characterization of essential genes in the human genome. *Science.* **2015**;350(6264):1096–1101.
- [112] Lang J, Barber A, Biggins S. An assay for de novo kinetochore assembly reveals a key role for the CENP-T pathway in budding yeast. *eLife.* **2018**;7:e37819.
- [113] Bock LJ, Pagliuca C, Kobayashi N, et al. Cnn1 inhibits the interactions between the KMN complexes of the yeast kinetochore. *Nat Cell Biol.* **2012**;14(6):614–624. DOI:10.1038/ncb2495.
- [114] Meluh PB, Koshland D. Evidence that the MIF2 gene of *Saccharomyces cerevisiae* encodes a centromere protein with homology to the mammalian centromere protein CENP-C. *Mol Biol Cell.* **1995**;6(7):793–807.
- [115] Hornung P, Troc P, Malvezzi F, et al. A cooperative mechanism drives budding yeast kinetochore assembly downstream of CENP-A. *J Cell Biol.* **2014**;206(4):509–524. DOI:10.1083/jcb.201403081.
- [116] Hamilton GE, Helgeson LA, Noland CL, et al. Reconstitution reveals two paths of force transmission through the kinetochore. *Elife.* **2020**;9:e56582.
- [117] van Hooff JJ, Tromer E, van Wijk LM, et al. Evolutionary dynamics of the kinetochore network in eukaryotes as revealed by comparative genomics. *EMBO Rep.* **2017**;18(9):1559–1571.
- [118] Barth TK, Schade GO, Schmidt A, et al. Identification of novel *Drosophila* centromere-associated proteins. *Proteomics.* **2014**;14(19):2167–2178.
- [119] Przewlaka MR, Zhang W, Costa P, et al. Molecular analysis of core kinetochore composition and assembly in *Drosophila melanogaster*. *PLoS One.* **2007**;2(5):e478.
- [120] Desai A, Rybina S, Muller-Reichert T, et al. KNL-1 directs assembly of the microtubule-binding interface of the kinetochore in *C. elegans*. *Genes Dev.* **2003**;17(19):2421–2435.
- [121] Oegema K, Desai A, Rybina S, et al. Functional analysis of kinetochore assembly in *Caenorhabditis elegans*. *J Cell Biol.* **2001**;153(6):1209–1226.
- [122] Drinnenberg IA, deYoung D, Henikoff S, et al. Recurrent loss of CenH3 is associated with independent transitions to holocentricity in insects. *Elife.* **2014**;3:e03676.
- [123] Cortes-Silva N, Ulmer J, Kiuchi T, et al. CenH3-Independent kinetochore assembly in *Lepidoptera* requires CCAN, including CENP-T. *Curr Biol.* **2020**;30(4):561–572 e510.
- [124] Sanchez-Perez I, Renwick SJ, Crawley K, et al. The DASH complex and Klp5/Klp6 kinesin coordinate bipolar chromosome attachment in fission yeast. *EMBO J.* **2005**;24(16):2931–2943.
- [125] Jimenez-Martin A, Pineda-Santaella A, Pinto-Cruz J, et al. The Rabl chromosome configuration masks a kinetochore reassembly mechanism in yeast mitosis. *Mol Biol Cell.* **2022**;33(5):br8.
- [126] Matsuda A, Asakawa H, Haraguchi T, et al. Spatial organization of the *Schizosaccharomyces pombe* genome within the nucleus. *Yeast.* **2017**;34(2):55–66.
- [127] Thapa KS, Oldani A, Pagliuca C, et al. The Mps1 kinase modulates the recruitment and activity of Cnn1/CENP-T at *Saccharomyces cerevisiae* kinetochores. *Genetics.* **2015**;200(1):79–90.
- [128] Petrovic A, Keller J, Liu Y, et al. Structure of the MIS12 complex and molecular basis of its interaction with CENP-C at human kinetochores. *Cell.* **2016**;167(4):1028–1040 e1015. DOI:10.1016/j.cell.2016.10.005.
- [129] Dimitrova YN, Jenni S, Valverde R, et al. Structure of the MIND complex defines a regulatory focus for yeast kinetochore assembly. *Cell.* **2016**;167(4):1014–1027 e1012.
- [130] Welburn JP, Vleugel M, Liu D, et al. Aurora B phosphorylates spatially distinct targets to differentially regulate the kinetochore-microtubule interface. *Mol Cell.* **2010**;38(3):383–392.
- [131] Kim S, Yu H. Multiple assembly mechanisms anchor the KMN spindle checkpoint platform at human mitotic kinetochores. *J Cell Biol.* **2015**;208(2):181–196.
- [132] Gaitanos TN, Santamaria A, Jeyaprakash AA, et al. Stable kinetochore-microtubule interactions depend on the Ska complex and its new component Ska3/C13Orf3. *EMBO J.* **2009**;28(10):1442–1452.
- [133] Raaijmakers JA, Tanenbaum ME, Maia AF, et al. RAMA1 is a novel kinetochore protein involved in

- kinetochore-microtubule attachment. *J Cell Sci.* **2009**;122(14):2436–2445.
- [134] Welburn JP, Grishchuk EL, Backer CB, et al. The human kinetochore Ska1 complex facilitates microtubule depolymerization-coupled motility. *Dev Cell.* **2009**;16(3):374–385.
- [135] Zhang Q, Sivakumar S, Chen Y, et al. Ska3 phosphorylated by Cdk1 binds Ndc80 and recruits Ska to kinetochores to promote mitotic progression. *Curr Biol.* **2017**;27(10):1477–1484 e1474.
- [136] Huis In 't Veld PJ, Volkov VA, Stender ID, et al.; Huis In. Molecular determinants of the Ska-Ndc80 interaction and their influence on microtubule tracking and force-coupling. *Elife.* **2019**;8:e49539.
- [137] He H, Gonzalez M, Zhang F, et al. DNA replication components as regulators of epigenetic inheritance—lesson from fission yeast centromere. *Protein Cell.* **2014**;5(6):411–419.
- [138] Gonzalez M, He H, Dong Q, et al. Ectopic centromere nucleation by CENP-A in fission yeast. *Genetics.* **2014**;198(4):1433–1446.
- [139] Dong Q, Yin FX, Gao F, et al. Ccp1 Homodimer Mediates Chromatin Integrity by Antagonizing CENP-A Loading. *Mol Cell.* **2016**;64(1):79–91. DOI:10.1016/j.molcel.2016.08.022.
- [140] Lu M, He X. Ccp1 modulates epigenetic stability at centromeres and affects heterochromatin distribution in *Schizosaccharomyces pombe*. *J Biol Chem.* **2018**;293(31):12068–12080.
- [141] Bakhoum SF, Compton DA. Kinetochore and disease: keeping microtubule dynamics in check! *Curr Opin Cell Biol.* **2012**;24(1):64–70.
- [142] Yuen KW, Montpetit B, Hieter P. The kinetochore and cancer: what's the connection? *Curr Opin Cell Biol.* **2005**;17(6):576–582.
- [143] Pfau SJ, Amon A. Chromosomal instability and aneuploidy in cancer: from yeast to man. *EMBO Rep.* **2012**;13(6):515–527.
- [144] Hayashi T, Fujita Y, Iwasaki O, et al. Mis16 and Mis18 are required for CENP-A loading and histone deacetylation at centromeres. *Cell.* **2004**;118(6):715–729.
- [145] Subramanian L, Toda NR, Rappsilber J, et al. Eic1 links Mis18 with the CCAN/Mis6/Ctf19 complex to promote CENP-A assembly. *Open Biol.* **2014**;4(4):140043.
- [146] Hirai H, Arai K, Kariyazono R, et al. The kinetochore protein Kis1/Eic1/Mis19 ensures the integrity of mitotic spindles through maintenance of kinetochore factors Mis6/CENP-I and CENP-A. *PLoS One.* **2014**;9(11):e111905.
- [147] Hayashi T, Ebe M, Nagao K, et al. *Schizosaccharomyces pombe* centromere protein Mis19 links Mis16 and Mis18 to recruit CENP-A through interacting with NMD factors and the SWI/SNF complex. *Genes Cells.* **2014**;19(7):541–554.
- [148] Hori T, Shang WH, Hara M, et al. Association of M18BP1/KNL2 with CENP-A nucleosome is essential for centromere formation in non-mammalian vertebrates. *Dev Cell.* **2017**;42(2):181–189 e183.