The smooth and stable operation of centromeres

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The centromere functions as a unique chromosomal attachment site for microtubules. Appropriate microtubule attachment is fundamental for organized chromosome behavior during mitosis and meiosis. Hence, centromeres must function both smoothly and stably. However, centromeric DNA sequences are poorly conserved between species despite common functions and similar centromeric protein composition, which leads us to the question: how are centromeres established and maintained? In this review, we summarize the recent progress in deciphering the mechanisms of centromere function. Specifically, we focus our attention on mechanisms closely-related to centromeric DNA and chromatin. By gathering such information, we hope to reveal a new dimension to the true nature of centromeres.

Key words: centromere, chromosome segregation, kinetochore

INTRODUCTION

Since its discovered by Walter Flemming through his pioneering microscopic observations, the centromere has bewitched cell biologists for more than a century (Sullivan et al., 2001). Up to now, centromeres have been identified in a wide variety of organisms (Allshire and Karpen, 2008; Vagnarelli et al., 2008), and appear to affect various cellular activities, including asymmetric cell division (Chang et al., 2006), genome elimination (Cui and Gorovsky, 2006; Ravi and Chan, 2010), genotoxic stress responses (Major et al., 1999), ageing (Maehara et al., 2010) and speciation (Ventura et al., 2007). This may be due to the fact that centromeres are so fundamental that they invariably affect different cellular aspects. However, our current understanding of the centromere itself is far from complete.

Centromeres are required for faithful chromosome segregation. Upon entry into mitosis, centromere maturation occurs in duplicated chromosomes and promotes functional kinetochore assembly. A mature centromere is a prerequisite for the elaborate association between chromosomes and the spindle microtubules, which leads to equal segregation of duplicated sister chromatids (Cheeseman and Desai, 2008). Kinetochore malfunction often results in chromosomal aneuploidy in the daughter cells and may cause tumorigenesis and chromosome instability (Holland and Cleveland, 2009). Therefore, kinetochores are broadly recognized as the key structure in chromosome transmission. However, centromeric DNA sequences are not well conserved among eukaryotes (Allshire and Karpen, 2008; Vagnarelli et al., 2008; Henikoff and Furuyama, 2010). So, how did centromere sequences become so diverse despite a common function and indispensable properties? What kind of mechanism is responsible for this diversity? What enables the smooth and stable operation of centromeres? To address these questions, we will start by summarizing the growing body of knowledge about how kinetochores are assembled on centromeric DNA. Specifically, we focus our attention on selected kinetochore factors that act in proximity to centromeric DNA and chromatin and hope to gain clues about how centromeres establish their extraordinary structure and how they function on chromosomal DNA.

CENTROMERE CLASSIFICATION

Based on the length of centromeric DNA, chromosomes are broadly classified into two types: monocentric chromosomes and holocentric chromosomes. The centromeres of monocentric chromosomes are morphologically distinct from the other parts of the chromosome and mostly recognized as thinner chromatid regions, referred to as primary constrictions, whereas the centromeres of holocentric chromosomes are not. Importantly, monocentric chromosomes can be further subdivided into two groups based on their dependency on the DNA sequence; namely, point centromeres and regional centromeres, (Sullivan et al., 2001; Allshire and Karpen, 2008; Vagnarelli et al., 2008).

At point centromeres, kinetochores are assembled in a...
DNA sequence-specific manner. First example of the point centromeres was given from the early studies in the budding yeast *Saccharomyces cerevisiae*. The point centromere was identified as a 125 bp sequence, which is conserved in all 16 chromosomes of the organism (Fitzgerald-Hayes et al., 1982). Similar sequence-specific point centromeres have been identified in other budding yeast species in the same phylogenetic branch as *S. cerevisiae* (Meraldi et al., 2006).

Regional centromeres were discovered later than point centromeres, but are present in a wide variety of organisms ranging from humana *Homo sapiens* to the dimorphic fungus *Candida albicans* (Allshire and Karpen, 2008). This type of centromere is generally larger than point centromeres, although their actual sizes vary from 3–4.5 kb in *C. albicans* to a few Mb in human. Most of these centromeres comprise repetitive DNA elements, yet the primary sequences of these elements show almost no conservation across species. In addition, the intriguing phenomena of centromere inactivation and ectopic kinetochore formation have been observed in organisms bearing regional centromeres (Sullivan et al., 2001; Allshire and Karpen, 2008; Vagnarelli et al., 2008). In particular, ectopic kinetochore formation is also referred to as neocentromere formation, and has been observed in humans (Voullaire et al., 1993), fruit flies *Drosophila melanogaster* (Maggert and Karpen, 2001), barley *Hordeum vulgare* (Nasuda et al., 2005), maize *Zea mays* (Topp et al., 2009) and 2 types of yeasts, *Schizosaccharomyces pombe* and *C. albicans* (Ishii et al., 2008; Ketel et al., 2009). The above observations collectively reinforce the idea that kinetochore assembly in organisms with regional centromeres occurs epigenetically in a manner independent of the primary DNA sequence (Henikoff and Furuyama, 2010).

By contrast, holocentric kinetochores are assembled broadly along the entire length of the chromosome (Sullivan et al., 2001; Allshire and Karpen, 2008; Vagnarelli et al., 2008). Kinetochore assembly occurs irrespective of the primary DNA sequence, likely in a manner similar to that observed for regional centromeres (Maddox et al., 2004; Cheeseman and Desai, 2008). Such linearly-shaped kinetochores are used for mitotic chromosome segregation in organisms such as nematodes *Caenorhabditis elegans* and arachnids *Tityus bahiensis* (Sullivan et al., 2001; Maddox et al., 2004; Schvarzeinstein et al., 2010). The holocentric organisms also undergo sexual reproduction, implying that such kinetochore plates may somehow also support reductional chromosome segregation. Nematodes, however, adopt a different segregation mechanism during meiosis, in which the telomere ends distal to the chiasma of each bivalent are selectively attached by the spindle microtubules of opposite sides without any obvious kinetochore plates (Maddox et al., 2004; Schvarzeinstein et al., 2010). Thus, recombination and chiasma formation between homologous chromosomes does not cause any problem in the subsequent meiotic segregation of holocentric chromosomes (Pimpinelli and Goday, 1989; Albertson and Thomson, 1993).

**CENP-A AND ITS REGULATORS**

The kinetochore structure is highly conserved between yeast and humans, regardless of whether it is a point- or regional-type centromere, or whether it is assembled on a monocentric or holocentric chromosome (Cheeseman and Desai, 2008). Consistent with this notion, the protein constituents are also highly conserved (Table 1). Of these, CENP-A is thought to be one of the most important factors that defines kinetochore identity (Sullivan et al., 2001; Allshire and Karpen, 2008; Vagnarelli et al., 2008) (Fig. 1). Human CENP-A was originally isolated as one of three different proteins (CENP-A, -B, -C) that are specifically recognized by antibodies produced by patients with CREST syndrome (Moroi et al., 1980). CENP-A encodes a centromere-specific variant of histone H3, which replaces the canonical histone H3 within nucleosomes located at centromeres (Earnshaw and Rothfield, 1985; Palmer et al., 1987). Loss of CENP-A by experimental manipulation results in the disappearance of many kinetochore components, whereas the loss of many kinetochore components does not significantly affect the centromere deposition of CENP-A (Takahashi et al., 2000; Blower and Karpen, 2001; Hori et al., 2008). Therefore, CENP-A forms the bedrock of the multiprotein kinetochore complex. In fruit fly cells, ectopic kinetochore formation was observed when the CENP-A gene encoded by *CID* (CENP-A<sup>CID</sup>, see Table 1) was overexpressed, suggesting that CENP-A<sup>CID</sup> is indeed a direct determinant of kinetochore assembly (Heun et al., 2006). The crucial role of CENP-A in kinetochore assembly was further demonstrated by the recent analysis of artificial CENP-A<sup>CID</sup>, targeting experiments in fruit flies (Mendiburo et al., 2011) and by *in vitro* reconstitution analysis using *Xenopus laevis* egg extracts (Guse et al., 2011). In human cells, however, CENP-A overexpression does not result in the ectopic formation of a complete and functional kinetochore (Van Hooser et al., 2001). Thus, deposition of CENP-A may be important, but not sufficient, for kinetochore assembly in human cells. A mechanism that bypasses the requirement for CENP-A has also been proposed based on the analysis of synthetic kinetochore assembly (Gascoigne et al., 2011).

There are several structural models of non-canonical CENP-A-containing nucleosomes (Henikoff and Furuyama, 2010). However, recent crystallographic analysis of the human CENP-A-nucleosome, together with its cognate DNA, revealed that the overall structure of CENP-A-nucleosomes resembles that of the canonical H3-nucleosome, suggesting the ability of CENP-A-nucleosomes to form a regular nucleosomal array (Tachiwana et al., 2010).
Centromere specification and regulation

2011). On the other hand, the same structural analysis also revealed clear differences in the conformation of DNA wrapped around the CENP-A-nucleosome; the entrance and exit paths of the nucleosomal DNA showed extraordinary flexibility (Tachiwana et al., 2011). In native kinetochores, the flexible nature of CENP-A-associated DNA may be used by the DNA-binding components in the kinetochore, such as CENP-B and CENP-C (Carroll et al., 2010; Tachiwana et al., 2011). Other potential differences have also been proposed based on other CENP-A structural analyses (Sekulic et al., 2010; Hu et al., 2011).

In addition, structural domain analysis of CENP-A revealed that a specific portion of its histone-fold domain, called CATD (CENP-A Targeting Domain), is required for the centromeric deposition of CENP-A (Black et al., 2004). The 22 amino acids of CATD are specific for the CENP-A protein and are conserved across species, whereas they are not present in the canonical histone H3. A chimeric protein consisting of histone H3 and CATD is actively targeted to the centromere, and is able to restore kinetochore function in cells lacking endogenous CENP-A (Black et al., 2004, 2007). CATD in the prenucleosomal form of CENP-A is directly recognized by HJURP, a CENP-A-specific histone chaperone (Foltz et al., 2009). Furthermore, CATD promotes the interaction between CENP-A and CENP-N, as well as recognition by the protein degradation machinery (see below). Therefore, CATD is a key domain of CENP-A, which regulates its deposition.

Deposition timing of CENP-A during the cell cycle varies depending on the organism. However, in most organisms, CENP-A deposition occurs after canonical histone H3 is deposited during S-phase; i.e. mitotic telophase to G1-phase in human cells (Jansen et al., 2007), mitotic anaphase in fruit flies (Schuh et al., 2007) and S-phase and G2-phase in fission yeast S. pombe (Takayama et al., 2008). In human cells, a minimum subcomplex consisting of CENP-A and histone H4 initially binds to HJURP.

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<th>H. sapiens</th>
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Table 1. Major protein constituents of the centromere/kinetochore of organisms
through the CATD, and is subsequently incorporated into the centromeric nucleosome (Dunleavy et al., 2009; Foltz et al., 2009) (Fig. 2). This HJURP-mediated CENP-A incorporation is also dependent on the Mis18 complex (Barnhart et al., 2011). Human Mis18 was originally isolated as a homolog of fission yeast Mis18, which forms a complex with Mis16, a counterpart of RbAp46/48, and is implicated in the histone acetylation pathway (Hayashi et al., 2004; Fujita et al., 2007). The localization of human Mis18 complex to centromeres only occurs during certain cell cycle phases: from mitotic telophase to G1 phase (Fujita et al., 2007). Intriguingly, the timing of Mis18 complex recruitment to active centromeres was recently shown to be determined solely by the CDK-mediated phosphorylation of M18BP1, a component of the Mis18 complex (Silva et al., 2012). The competency of centromere chromatin to deposit CENP-A may be defined by M18BP1 phosphorylation in each cell cycle, and cell cycle-specific CENP-A deposition may be established in conjunction with a yet-to-be-defined interplay between the Mis18 complex and HJURP (Fig. 2).

The fission yeast HJURPScm3 also localizes to centromeres in a cell cycle-specific manner; it dissociates from centromeres when cells enter mitosis, but re-accumulates during late anaphase to G1 phase, which coincides with localization of the Mis16/Mis18 complex to the centromere (Pidoux et al., 2009; Williams et al., 2009). In addition, centromere-targeting of HJURPScm3 requires proper localization of both Mis6 and the Mis16/Mis18 complex to centromeres (Hayashi et al., 2004; Pidoux et al., 2009; Williams et al., 2009). Taken together, these studies suggest that HJURP-mediated nucleosomal deposition of CENP-A appears to be widely conserved.

Meanwhile, the CATD of budding yeast CENP-A Cse4 is recognized by an E3 ubiquitin ligase, Psh1, which targets CENP-A Cse4 for degradation (Hewawasam et al., 2010; Ranjitkar et al., 2010). This CATD-mediated degradation of CENP-A Cse4 by Psh1 directly competes with HJURPScm3 recognition of, and association with, CATD in budding yeast. This regulation serves to clear excess amounts of CENP-A Cse4 that fail to bind to HJURP Scm3, which consequently prevents CENP-A Cse4 misincorporation and stabilizes centromere identity (Collins et al., 2004). However, deleting the PSH1 gene and mutating the ubiquitination sites of CENP-A Cse4 does not completely block the degradation of CENP-A Cse4 (Hewawasam et al., 2010; Collins et al., 2004). These data suggest that additional protein degradation pathways exist in this organism. Like Psh1, the fruit fly F-box protein, Ppa, recognizes the CATD of CENP-A CID and promotes its proteasome-dependent degradation, suggesting that Ppa contributes to the maintenance of stable CENP-A CID at a defined locus (Moreno-Moreno et al., 2011). CENP-A degradation-mediated kinetochore site surveillance may be a universal system conserved among divergent species.

**DNA-PROXIMAL MEMBERS OF CCAN**

The kinetochore is structurally subdivided into two...
parts: the outer kinetochore and the inner kinetochore. A distinguishing feature of the outer kinetochore is that it is assembled only during mitosis, whereas the inner kinetochore structure, or at least its chromosomal association, is maintained throughout the cell cycle (Cheeseman and Desai, 2008). This is mainly due to the mitosis-specific targeting of outer kinetochore proteins, such as the KMN network composed of the Mis12 complex, the Ndc80 complex, and KNL-1/Blinkin, to the inner kinetochore protein-marked region (Maddox et al., 2004; Cheeseman and Desai, 2008). This outer kinetochore assembly provides a proper binding interface for microtubules (Fig. 1).

The inner kinetochore structure, mostly specified by its constitutive association with centromeric chromatin, is comprised of evolutionarily conserved proteins that are collectively referred to as CCAN (Constitutive Centromere-Associated Network) (Fig. 1). The CCAN components have been identified through extensive biochemical studies and cellular localization analyses (Obuse et al., 2004; Foltz et al., 2006; Izuta et al., 2006; Okada et al., 2006). They are represented by CENP-C, -H, -I, -K to -U, -W and -X, which can be further categorized into subgroups based on interaction studies performed in chicken and human cell lines (Cheeseman and Desai, 2008; Perpelescu and Fukagawa, 2011). Most CCAN proteins are conserved in fission yeast, but surprisingly no CCAN constituent, apart from CENP-C, has been identified in fruit flies or nematodes (Perpelescu and Fukagawa, 2011) (Table 1).

By purifying CENP-A from an oligo-nucleosomal fraction of HeLa cell chromatin extract, many proteins that interact specifically with CENP-A or CENP-A-surrounding nucleosomes have been isolated (Obuse et al., 2004; Foltz et al., 2006; Izuta et al., 2006; Okada et al., 2006). Of these, the CENP-C, -H, -M, -N, -T and -U proteins were named CENP-A NAC (Nucleosome Associated Complex) after they were found in the vicinity of CENP-A (Foltz et al., 2006). These proteins are characterized as CCAN members that are more intimately related to CENP-A-nucleosomes. A number of other factors can also be found in close proximity to CENP-A, including CENP-B, HJURP, nucleophosmin and a general nucleosome remodeler FACT (Cheeseman and Desai, 2008; Perpelescu and Fukagawa, 2011).

CENP-N is a CENP-A NAC member that binds directly to CENP-A (Carroll et al., 2009). However, it binds simultaneously to another CCAN member, CENP-L. Thus, CENP-N acts as a direct linker between the CENP-A-nucleosome and CCAN (Fig. 1). Consistently, CENP-N depletion leads to a drastic loss of centromere-associating CENP-H, -I, -K proteins in addition to a moderate decline in CENP-A and -C protein levels (Carroll et al., 2009). It was reported recently that CENP-N localizes to the kinetochore only in S and G2 phase, but not in M and G1 phase (Hellwig et al., 2011). Thus, CENP-N is thought to be involved in the replication fidelity of centromeric chromatin, although direct evidence is still lacking. Fission yeast CENP-N$^{N_{Mis15}}$ also physically interacts with CENP-P$^{Mis6}$ and CENP-M$^{Mis17}$, and is required for localization of CENP-A$^{Cnp1}$, CENP-P$^{Mis6}$ and CENP-M$^{Mis17}$ to the centromere (Hayashi et al., 2004). Moreover, CENP-N$^{N_{Mis14}}$ is required for kinetochore assembly in budding yeast (Mythreye and Bloom, 2003). CENP-N of both yeasts, however, are reported to be localized to centromeres throughout the cell cycle (Mythreye and Bloom, 2003; Hayashi et al., 2004).

CENP-C binds to both DNA and nucleosomes, and is also thought to act as a scaffold for versatile interacting proteins (Przewloka et al., 2011; Screpanti et al., 2011) (Fig. 1). In chicken cells, CENP-C binds to the canonical histone H3 proteins, which are localized to centromere regions (Hori et al., 2008). By contrast, direct binding of CENP-C to the C-terminus of CENP-A has been reported in human cells (Carroll et al., 2010). It was also reported recently that CENP-C directly interacts with M18BP1 of the Mis18 complex and contributes to the cell cycle-specific deposition of CENP-A in mouse and Xenopus cell-free extracts (Moree et al., 2011; Dambacher et al., 2012). Structural analysis of CENP-C further revealed that its conserved N-terminal domain is required for the proper localization of outer kinetochore proteins such as Mis12 and KNL-1 (Gascoigne et al., 2011; Przewloka et al., 2011; Screpanti et al., 2011). These observations suggest that CENP-C functions as a structural connector between the inner and outer kinetochore during mitosis (Fig. 1). Interestingly, CENP-C$^{Cnp3}$ in fission yeast is not essential for viability, which is in marked contrast with the necessity of the other CCAN proteins in this organism (Tanaka et al., 2009). Nonetheless, a similar scaffolding role for CENP-C$^{Cnp3}$ has been proposed, based on the fact that CENP-C$^{Cnp3}$ interacts with the CCAN member CENP-L$^{F31}$, the condensin recruiter complex Psc1/Mde4 (see below), and the meiosis-specific regulator, Moe1 (Tanaka et al., 2009). In fact, CENP-L$^{F31}$ is required for targeting of the essential CENP-D$^{Mis1}$-CENP-K$^{N_{Mis4}}$ complex to the centromere. However, CENP-L$^{F31}$ targeting is also mediated by CENP-T$^{Cnp20}$, which may account for the dispensable nature of CENP-C$^{Cnp3}$ in fission yeast (Tanaka et al., 2009). In contrast, CENP-C is essential for kinetochore assembly and cell cycle progression in fruit flies (Orr and Sunkel, 2011). This could be due to the absence of CCAN members other than CENP-C in this organism (Table 1). The interaction between the outer kinetochore and the inner kinetochore, or with the CENP-A$^{Cnp3}$-nucleosome, may rely solely on CENP-C, and other proteins may not be able to fulfill such a connector-like role (Fig. 1).

Like CENP-C, CENP-T in chicken cells also binds to canonical histone H3-nucleosomes at the centromeres, but not to CENP-A-nucleosomes (Hori et al., 2008). In
addition, the N-terminal domain of CENP-T binds directly to the Ndc80 complex. These observations suggest another physical connection between the outer kinetochore and inner kinetochore that acts in parallel with CENP-C (Gascoigne et al., 2011) (Fig. 1). The CENP-T-Ndc80 interaction is regulated by CDK-mediated phosphorylation at the CENP-T N-terminus. Importantly, ectopic tethering of the CENP-T N-terminus and the CENP-C N-terminus was sufficient for recruiting outer kinetochore proteins and for proper kinetochore function in human and chicken cells, even in the absence of CENP-A (Gascoigne et al., 2011). This observation further stresses the significance of the connector-like factors CENP-C and CENP-T in centromere-concentrated CCAN when a functional kinetochore is assembled in vertebrates.

In addition to the N-terminal outer kinetochore-interaction domain, CENP-T possesses another intriguing domain, a histone-fold domain, in its C-terminus (Hori et al., 2008). A similar histone-fold motif was identified in CENP-W, -S and -X (Hori et al., 2008; Amano et al., 2009) (Table 1). Recently, the crystal structure of the CENP-T-W complex, the CENP-S-X complex, and the CENP-T-W-S-X tetrameric complex, was solved, and a unique nucleosome-like DNA binding mode was proposed for the T-W-S-X heterotetramer (Nishino et al., 2012). Expression of a tetramer formation-defective mutant of CENP-T or CENP-S resulted in CENP-T-W-S-X deprivation at the centromere and consequent kinetochore disassembly. Therefore, the nucleosome-like contact between CENP-T-W-S-X and the centromere DNA plays an integral role in functional kinetochore assembly. Intriguingly, CENP-S-X alone also forms a tetramer and binds to DNA, but in a different mode (Nishino et al., 2012), suggesting that it has a completely different function at non-centromeric chromosome arm regions. Consistent with this, CENP-S and -X were independently isolated as the FANCN (Fanconi Anemia Complementation group M)-binding proteins MHF-1 and -2 (Singh et al., 2010; Yan et al., 2010) (Table 1).

**COMMON CHROMATIN FACTORS IMPLICATED AT KINETOCHORES**

Until now, we have mainly discussed factors that localize and perform functions exclusively at the kinetochore. However, general chromatin factors exist at the kinetochore and also contribute to kinetochore performance.

Blocks of CENP-A-nucleosome arrays alternate with blocks of H3-nucleosome arrays in fly and human centromeres (Blower et al., 2002). The interspersed arrays of canonical nucleosomes serve as binding targets for CENP-C and CENP-T (Hori et al., 2008). Intriguingly, such nucleosomes exhibit a distinct histone modification pattern, such as hypoacetylation of histone H3 and H4, low methylation of histone H3-K9, and increased di-methylation of histone H3-K4 (Sullivan and Karpen, 2004). The role of these modifications remains unclear, but H3-K4 di-methylation at synthetic kinetochores was recently shown to be a prerequisite for HJURP recruitment and newly-synthesized CENP-A deposition (Bergmann et al., 2011). Di-methylated H3-K4 is a hallmark of active chromatin and, thus, cryptic RNA transcription at centromeres may play a role in kinetochore function and/or propagation. Consistent with this, mitotic inhibition of RNA polymerase II transcription at the centromere leads to chromosome mis-segregation and delocalization of CENP-C from the inner kinetochore (Chan et al., 2012).

In addition, most regional centromeres are associated with heterochromatin structure (Allahire and Karpen, 2008). Heterochromatin is distributed at regions distinct from the above-mentioned blocks of alternating CENP-A nucleosomes and di-methylated H3-K4, known as pericentric heterochromatin (Blower et al., 2002; Sullivan and Karpen, 2004). Notably, several reports suggest that pericentric heterochromatin promotes de novo kinetochore formation. In fission yeast, neighboring heterochromatin is both necessary and sufficient for the establishment of CENP-A<sup>Cnp1</sup> chromatin on plasmid DNA, although it is dispensable for the maintenance of CENP-A<sup>Cnp1</sup> chromatin (Folco et al., 2008; Kagansky et al., 2009). Heterochromatin is also associated with the artificial kinetochore assembled on synthetic human centromeric DNA (Nakashima et al., 2005; Okada et al., 2007). Furthermore, neocentromeres are formed at regions adjacent to heterochromatin in fission yeast (Ishii et al., 2008) and in fruit fly cells that overexpress CENP-A<sup>cd</sup> (Olszak et al., 2011). However, de novo kinetochore formation on DNA injected into *C. elegans* embryos occurs independently of heterochromatin (Yuen et al., 2011). In addition, neocentromere formation was frequently observed in the absence of heterochromatin in *C. albicans* (Ketel et al., 2009). Thus, pericentric heterochromatin plays an important role in kinetochore formation, but its extent may differ between species.

**THE CONTRIBUTION OF CHROMATIN TO PROPER KINETOCHORE BI-ORIENTATION**

The ultimate purpose of kinetochore assembly is the faithful segregation of chromosomes. This can be achieved by proper microtubule attachment to kinetochores and organized chromosome movement. Inaccurate kinetochore-microtubule attachments are mostly monitored and dealt with by the SAC (Spindle Assembly Checkpoint), which delays the onset of anaphase until attachments are corrected (Musacchio and Salmon, 2007). However, SAC only senses unattached kinetochores or a lack of tension between the sister kinetochores. Hence,
it is unable to recognize a peculiar kinetochore error, referred to as merotelic attachment, where one of either sister kinetochores is captured by microtubules emanating from both poles (Musacchio and Salmon, 2007; Gregan et al., 2011). Aurora B kinase and the CPC (Chromosome Passenger Complex) (Ruchaud et al., 2007) deal with merotelic attachment by specifically removing merotically-attached microtubules (Gregar et al., 2011). However, the chromosomal configuration that differentiates the kinetochore domain from the rest of the chromosome and puts the kinetochores to the proper orientation is also important for reducing erroneous attachments and attaining faithful chromosome segregation. These geometric issues appear to be handled by common regulators of chromosome structure, such as the cohesin and condensin complexes, and by histone modifications in addition to CPC (Yamagishi et al., 2010; Tada et al., 2011) (Fig. 3).

The cohesin complex physically connects the duplicated sister chromatids and plays a crucial role at the centromeres. However, recent analyses indicate that cohesin is also involved in the recruitment of CPC (Yamagishi et al., 2010). Targeting of CPC to chromatin is achieved, in part, by its interaction with phosphorylated T3 of histone H3 (Kelly et al., 2010; Wang et al., 2010; Yamagishi et al., 2010). The conserved kinase, haspin, is responsible for this phosphorylation (Dai et al., 2005), and cohesin contributes to CPC recruitment by tethering the haspin kinase to its cognate sites through a direct protein-protein interaction (Yamagishi et al., 2010) (Fig. 3). Notably, H3-T3 phosphorylation is not necessarily restricted to centromeres (Kelly et al., 2010; Nonaka et al., 2002). However, another histone modification, the phosphorylation of histone H2A at its C-terminus, allows centromeric chromatin to be differentially-recognized and contributes to CPC loading (Kawashima et al., 2010). H2A phosphorylation is catalyzed by Bub1, a kinase implicated in the SAC pathway, and is localized to the outer kinetochore in human cells through its interaction with KNL-1/Blinkin (Kiyomitsu et al., 2007; Musacchio and Salmon, 2007). Bub1-directed H2A phosphorylation at the kinetochore was recently shown to recruit the shugoshin protein, which in turn recruits CPC, both of which occur via physical interactions (Kawashima et al., 2010; Tsukahara et al., 2010) (Fig. 3). Shugoshin was originally isolated as a conserved PP2A-associated protector of centromeric cohesin (Kitajima et al., 2006; Riedel et al., 2006); it is also recruited to pericentric heterochromatin through direct interaction with the heterochromatin protein, HP1 (Yamagishi et al., 2008). However, shugoshin functions concurrently as a local CPC adaptor when loaded at the inner kinetochore via H2A phosphorylation (Kawashima et al., 2010). Intriguingly, inactivation of haspin alone reduces centromeric CPC and compromises kinetochore function, even in the presence of the Bub1-shugoshin pathway (Dai et al., 2006; Wang et al., 2010; Yamagishi et al., 2010). It is proposed that phosphorylation of H3-T3 and the C-terminus of H2A act synergistically to target CPC to the inner kinetochore, perhaps by differentiating chromatin (Yamagishi et al., 2010) (Fig. 3).

In contrast to cohesin and shugoshin, condensin plays a more direct role in establishing chromosome bi-orientation (Gerlich et al., 2006; Samoshkin et al., 2009; Tada et al., 2011). In particular, merotelic attachment and defective chromosome segregation are frequently observed during mitosis and the 2nd nuclear division of meiosis in fission yeast when either Pcs1 or Mde4 are mutated (Gregar et al., 2007). Pcs1 and Mde4 are the functional homologs of

![Fig. 3. CPC recruitment and its effect. (left) Haspin phosphorylates histone H3-T3 and recruits CPC. Bub1 kinase phosphorylates histone H2A and recruits shugoshin, which recruits CPC. These two pathways act synergistically to target CPC to the inner kinetochore. (right) Merotelic attachment caused by the defective action of condensin at the kinetochore is corrected by the Aurora B activity accumulated at the inner kinetochore.](image-url)
budding yeast monocistron subunits (Rabitsch et al., 2003; Gregan et al., 2007), and were recently shown to be directly recruited to centromeres (Tada et al., 2011). In the absence of centromere-loaded condensin, which is normally promoted by the Pse1/Mde4 complex, the clamping of several microtubule binding sites within a single kinetochore may become defective. This is most likely due to the loss of intra-chromatid crosslinks, which could cause merotelic attachment (Gregan et al., 2011; Tada et al., 2011) (Fig. 3). More importantly, even though centromere localization specificity is provided by the Pse1/Mde4 complex, chromatin association with condensin itself is mediated through the interaction with nucleosomal histone H2A, and is enabled by direct Aurora B phosphorylation of the CAP-H subunit of condensin (Tada et al., 2011). Therefore, Aurora B/CPC not only corrects merotelic attachment, but also suppresses it by regulating condensin (Fig. 3). A study showed that a Pse1 mutant became lethal in the absence of Bub1 or HP1\textsuperscript{ Sty6} in fission yeast (Rabitsch et al., 2003), suggesting that the condensin-mediated merotely-suppression pathway works in parallel with the cohesin/heterochromatin-mediated merotely-correction pathway, both of which are under the control of CPC (Fig. 3).

CONCLUSION AND PERSPECTIVES

This review summarizes some of the recent advances regarding the composition and regulation of the centromere and/or kinetochore. The original aim was to determine how common kinetochore structure and functions can be established on diverse centromeric DNA. We focused our attention on studies related to centromeric DNA. Therefore, the subtopics in this review have been chosen in a cross-sectoral manner. However, based on our selection, it appears that both centromere-specific components and global chromatin components contribute to kinetochore assembly. The kinetochore-specific CCAN members often bind to the canonical histone H3 nucleosome, whereas post-translational histone modifications provide specificity for the kinetochore domain. CENP-A is a centromere-specific histone variant and is most likely the central kinetochore determinant, but its specificity is largely due to its surrounding partners, regardless of whether they are centromere-specific or global.

Thus, specificity and universality both represent the nature of the centromere/kinetochore; it is reminiscent of DNA sequence-independent neocentromere formation and epigenetic centromere inactivation (Sullivan et al., 2001). By balancing plasticity and elasticity, centromeres function both smoothly and stably. Then, a next challenge could be to elucidate how the balance is exquisitely controlled. Artificial recapitulation of the centromere plasticity, such as \textit{de novo} centromere activation and inactivation at will, would become a powerful tool to tackle with otherwise evolutionary issue in laboratory. In future, yet-undiscovered rule might be manifested which governs the chromosomal identity of the centromeres and persists despite speciation and evolution.

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