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Review

Evolutionary Turnover of Kinetochore Proteins: A Ship of Theseus?

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The kinetochore is a multiprotein complex that mediates the attachment of a eukaryotic chromosome to the mitotic spindle. The protein composition of kinetochores is similar across species as divergent as yeast and human. However, recent findings have revealed an unexpected degree of compositional diversity in kinetochores. For example, kinetochore proteins that are essential in some species have been lost in others, whereas new kinetochore proteins have emerged in other lineages. Even in lineages with similar kinetochore composition, individual kinetochore proteins have functionally diverged to acquire either essential or redundant roles. Thus, despite functional conservation, the repertoire of kinetochore proteins has undergone recurrent evolutionary turnover.

Trends

CenH3 plays an essential role in chromosome segregation of most eukaryotes.

At least three CenH3-independent chromosome segregation systems have evolved: in trypanosomes, insects, and during meiosis in nematodes.

The inner kinetochore protein CENP-T is essential in some organisms but dispensable in others.

CENP-B-like proteins have arisen multiple times in evolution.

> The ship wherein Theseus and the youth of Athens returned from Crete had thirty oars, and was preserved by the Athenians. . .. . they took away the old planks as they decayed, putting in new and stronger timber in their places, in so much that this ship became a standing example among the philosophers, for the logical question of things that grow; one side holding that the ship remained the same, and the other contending that it was not the same.
>
> –Plutarch, Theseus

Kinetochores Mediate Chromosome Segregation in Eukaryotes

Chromosome segregation is an essential, conserved process that ensures faithful transmission of genetic information to daughter cells during cell division in all eukaryotes. Specialized chromosomal regions called centromeres coordinate this process of chromosome segregation. Centromeres were first cytologically described as primary constrictions on chromosomes where spindle microtubules attach to pull the sister chromatids apart during the mitotic and meiotic divisions [1]. Two different types of centromere arrangements are found in eukaryotes: monocentric or holocentric (Box 1). In both types of centromeres, the physical connection between the spindle microtubules and the centromeric DNA is provided by a large proteinaceous structure termed the kinetochore [2]. Micrographic images of the kinetochore reveal a bipartite structure consisting of an inner complex that connects to the centromeric DNA and an outer complex that connects to the spindle microtubules. Detailed biochemical studies resolved this bipartite structure into many protein subunits that function in a coordinated manner to enable dynamic assembly on centromeric DNA [3]. Perhaps the best elucidation of the kinetochore and its biophysical mechanism comes from budding yeast, where *in vivo* isolation of intact kinetochores has led to unprecedented insights into its structure and regulation [4,5].

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Box 1. Dramatic Variation of Number and Arrangement of Attached Spindle Microtubules

Most eukaryotic chromosomes are monocentric, that is, the centromere is restricted to one region of the chromosome. Although monocentromeres are commonly visible as primary constrictions of condensed chromosomes, the actual length of the centromeric region can vary over several orders of magnitude across different eukaryotes [14]. The simplest monocentromere is the budding yeast point centromere, which measures approximately 100 bp in length and captures one spindle microtubule [88] (Figure IA). In contrast to this, 'regional' centromeres found in most other eukaryotes can span up to a few megabases [89] (Figure IB) and capture several microtubules [90].

Several lineages have evolved holocentromeres, in which microtubule attachment sites extend along large portions or even the entire length of the chromosome [91] (Figure IC). Based on the phylogenetically dispersed pattern of holocentric species among eukaryotes, it appears likely that holocentricity repeatedly evolved from monocentric ancestors [11,91]. Holocentromeres have been best characterized in *Caenorhabditis elegans*. Profiling of inner kinetochore components revealed both discrete sites along the *C. elegans* chromosomes that recruit kinetochore components, as well as enrichment over transcriptionally silent regions [92,93]. Although this arrangement is likely to be conserved in other holocentric nematodes, it is unclear whether such a 'polycentric' architecture also applies to other independently derived holocentric species. Indeed, a recent study in holocentric plants revealed that centromeric sites are composed of dispersed satellite DNA along the chromosomes [94]. Despite these dramatic differences in the number and spatial arrangement of attached spindle microtubules, none of those changes have been found to correlate with changes in kinetochore composition with the exception of holocentric insects (see Figure 1D in main text) [11]. In fact, most monocentric and holocentric species use the histone variant CenH3 as the chromatin determinant enabling kinetochore assembly.

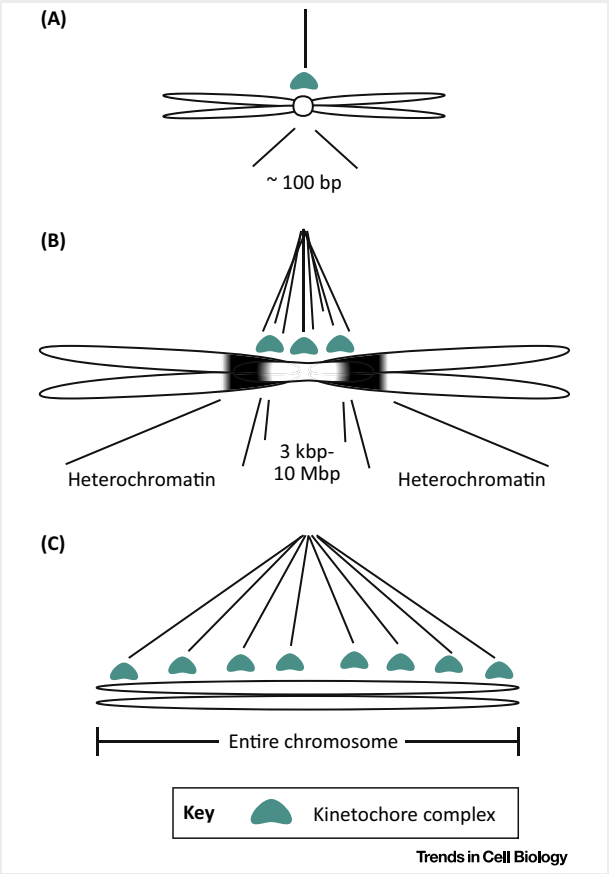

Figure I. Dramatic Variation in Kinetochore Architectures. We highlight three types of kinetochore arrangements to illustrate their range, from the simplest 'point' monocentromere in budding yeast (A), to regional monocentromeres (B), and to holocentromeres seen in many lineages including nematodes (C).

The basic organization of the kinetochore is highly conserved in all eukaryotes. Early studies of kinetochore function and composition were mainly performed in yeast and vertebrate cell lines as model systems. Not only did these studies reveal fundamental principles of kinetochore function but they also uncovered a high degree of similarity between yeast and vertebrate kinetochores, especially of the inner complex, despite millions of years of divergence between the two lineages [6] (Figure 1A,B, Key Figure). However, analysis of kinetochore composition in other eukaryotes revealed an unexpected degree of variation. For instance, the *Drosophila melanogaster* kinetochore, which has also been well dissected via biochemical and genetic means [7–10], is missing many of the inner kinetochore components found in vertebrate and budding yeast cells (Figure 1C). Similarly, the kinetochores of the holocentric *Bombyx mori* are distinct from those in vertebrate and budding yeast [11] (Figure 1D). Finally, a recent proteomic study of the kinetochore in trypanosomes, an early branching lineage of eukaryotes, revealed a completely different set of kinetochore proteins, with no detectable homology to fungal or vertebrate kinetochores [12,13]. In addition to their different repertoires, animal and plant kinetochore proteins have undergone rapid protein evolution despite conservation of kinetochore function [14,15].

The evolutionary dynamics of the kinetochore complex is reminiscent of a thought experiment called the 'Ship of Theseus' recorded by the Greek philosopher Plutarch. This paradox raises the question: does a ship that has all its wooden parts replaced one-by-one still remain the same? During the evolutionary process, the kinetochore 'ship' has maintained its conserved function in chromosome segregation, despite apparently replacing many or all of its parts. We summarize three signatures of evolutionary turnover, focusing on inner kinetochore composition – loss of kinetochore components that are essential in some species, recurrent innovation of novel kinetochore components, and differential importance of certain kinetochore components for the essential function of chromosome segregation.

Loss of an 'Essential' Chromatin Determinant of Kinetochore Assembly

In most eukaryotes, a specialized centromeric histone H3 variant called CenH3 [16] is found predominantly and ubiquitously at centromeres. In these eukaryotes, CenH3 is the major chromatin determinant enabling kinetochore assembly on centromeric regions. The first discovery of CenH3 (called CENP-A in mammals) hinged serendipitously on an observation made in 1985; antibodies present in the serum of patients with an autoimmune disease were found to react with kinetochore proteins [17]. Subsequent studies in other species of animals, fungi, plants, and protists showed that the incorporation of CenH3 homologs is a common feature of their centromeres [18–21], thereby establishing CenH3 as the chromatin marker associated with all active centromeres, and likely responsible for their inheritance (Box 2).

Box 2. Genetic versus Epigenetic Centromeres

Although the meaning of 'epigenetic' has been widely debated [95], there is general agreement that epigenetic inheritance is characterized by a lack of DNA sequence determinants. Epigenetic centromeres would then be defined as centromeres that lack specific DNA sequence requirements. The first characterized centromeres were the genetically defined 'point' centromeres of the budding yeast *Saccharomyces cerevisiae*, which are specified by a tripartite ~120-bp sequence that wraps a single CenH3 (called Cse4 in budding yeast) nucleosome [88]. In contrast to this, regional centromeres in fission yeast and *Candida* are not associated with specific DNA sequences, supporting the model that they are epigenetically defined.

In most eukaryotes, centromeres are embedded in megabases of tandemly repeated DNA 'satellite' sequences, which strongly suggest that satellite DNA specifies centromeres. However, the discovery that a human neocentromere lacks alpha-satellite sequences suggested that human centromeres could be entirely epigenetic [96]. Despite this evidence for very rare epigenetic centromeres, human artificial centromeres require alpha-satellite arrays with a binding site for CENP-B protein (CENP-B box) to be propagated in culture [97]. Moreover, most human CENP-A was found to be enriched over two classes of highly homogeneous CENP-B box-containing alpha-satellite dimers [98], suggesting that stable centromeres are consistently associated with particular alpha-satellite repeats. The stable inheritance of the centromere-defining chromatin determinant over sequence-defined alpha-satellite repeat dimers supports the distinction between epigenetically defined neocentromeres and genetically defined alpha-satellite centromeres. Such a genetic definition might apply to other regional centromeres and even to holocentromeres that are composed of particular satellite DNAs [94].

Key Figure

Variation in Kinetochore Composition in Four Eukaryotic Taxa

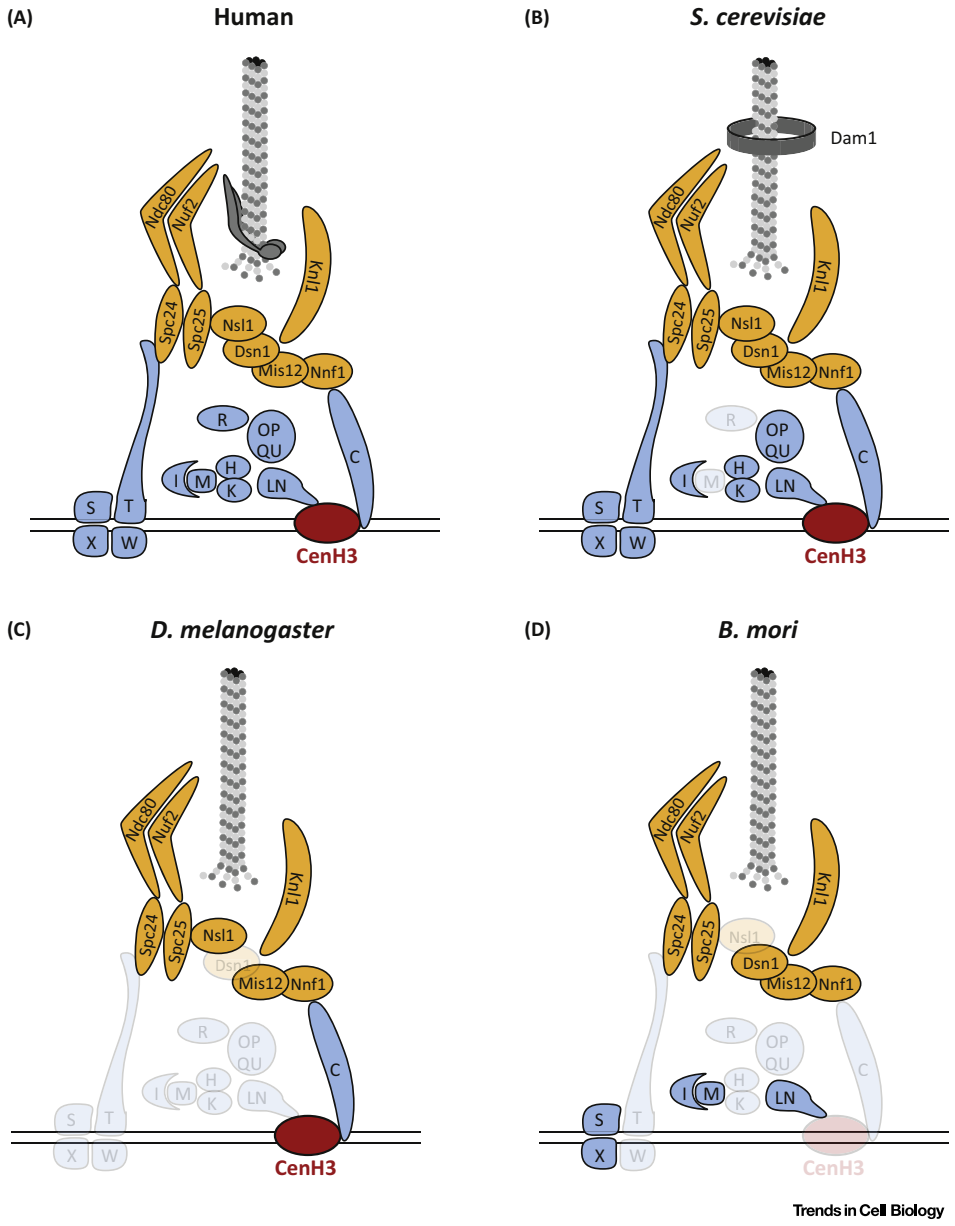

Figure 1. Kinetochores have been best-dissected biochemically in vertebrates (A) and budding yeast (B). These two kinetochores are remarkably similar in composition, with the most notable difference being the absence of CENP-M in yeast (grayed out) and the fungal-specific origin of the Dam1 complex, the functional counterpart of the Ska1 complex. However, the kinetochores in *Drosophila melanogaster* (C) and holocentric *Bombyx mori* (D) are very distinct from those in vertebrate and budding yeast. The conclusions that certain CCAN components are missing in both (C) and (D) are tempered by the possibility that rapid evolution might have impaired homology searches; these conclusions await further confirmation via proteomic or other experimental means. Abbreviation: CCAN, constitutive centromere-associated network.

Box 3. No Designated Driver for CenH3 Delivery

The deposition of CenH3 into chromatin is under tight control. Failure to deposit adequate amounts of CenH3 could lead to functionally acentric chromosomes, whereas ectopic incorporation of CenH3 can cause dicentric chromosomes [99]; both outcomes are detrimental to accurate chromosome segregation. The deposition of CenH3 occurs during a defined window during the cell cycle, although this window varies among species [100]. Indeed, constitutive incorporation of CenH3 throughout the entire cell cycle results in mitotic defects [101]. Based on the requirement for tight temporal and spatial control of CenH3 incorporation, one might assume that the deposition machinery of CenH3 would be conserved across eukaryotes. This is not the case.

In vertebrates and budding yeast, homologs of the specialized histone chaperone HJURP/Scm3 mediate the deposition of CenH3 into centromeric chromatin [102–105]. HJURP/Scm3 probably arose in a common ancestor of fungi and animals. However, it has been apparently lost in several animal lineages including nematodes and flies, raising the question of how these lineages compensated for the loss of this essential gene [106]. In flies, the loss of HJURP/Scm3 was perhaps facilitated by the evolution of the non-orthologous chaperone CAL1 that deposits *Drosophila melanogaster* CenH3/CID onto centromeric chromatin [107–109]. Intriguingly, the CenH3-interacting domains of CAL1 and Scm3 show similarity in both sequence and structure, which could be a result of convergent evolution [110]. The similar emergence of new chaperones in other lineages may have led to the loss of HJURP/Scm3. Alternatively, an existing histone chaperone might have acquired the additional function of CenH3 incorporation. In that context, it is worth noting that the histone chaperone DAXX was recently found to be involved in ectopic incorporation in human cells overexpressing CENP-A [111]. Thus, CenH3 incorporation can also be mediated by chaperones that also deliver other histone variants.

Although it is clear that HJURP/Scm3 and CAL1 represent *bona fide* CenH3 assembly factors, other proteins are also needed to coordinate CenH3 deposition. For instance, proteins of the Mis18 complex (Mis18α, Mis18β, and Mis18BP1/KNL2) and Mis16 are also required for CenH3 deposition in HJURP-containing (e.g., human, fission yeast) and HJURP-lacking (e.g., *C. elegans*, *Arabidopsis*) lineages [112–118]. Outside animals and fungi, even less is known about CenH3 incorporation in plants or protists, where no designated CenH3 chaperones have been identified.

Despite its functional conservation across most eukaryotes, the evolutionary history of CenH3 remains unresolved. Owing to its conserved presence at the eukaryotic centromere, we favor the more parsimonious model of a single origin for CenH3 in an early eukaryotic ancestor [22]. However, CenH3 homologs in different species are far less conserved compared to H3 and thus we cannot rule out the possibility of multiple independent origins of CenH3 in the H3 family [23,24]. Even less conserved is the machinery that mediates the deposition of CenH3 at centromeres (Box 3).

Not only is CenH3 a marker of centromeres but it is also a key player in the kinetochore assembly pathway. In species as diverse as fungi, worms, plants, and humans, CenH3 is required to anchor the kinetochore to centromeric chromatin [2]. These findings led to the expectation that CenH3 would be found at all centromeres. However, this expectation was challenged by multiple discoveries of CenH3-independent kinetochore assembly pathways in eukaryotes. The first completely CenH3-independent chromosome segregation pathway was discovered in kinetoplastids [12]. Not only do these species lack CenH3 but detailed biochemical studies revealed a kinetoplastid kinetochore complex, whose components shared no detectable homology to other eukaryotic kinetochores [12]. This may reflect the fact that kinetoplastids branched early during eukaryotic evolution from the lineages that gave rise to plants, fungi, and animals [25], which compromises the power of remote homology detection programs. It is formally possible that the CenH3-independent kinetoplastid kinetochore represents a system ancestral to the origin of the more well-studied kinetochore complex. However, the discovery of CenH3 in other early branching eukaryotes such as *Giardia lamblia* [19] and *Trichomonas vaginalis* [26], together with recently revised models of eukaryotic evolution [27], suggest instead that CenH3-independent kinetochores in kinetoplastids represent a derived rather than ancestral state. Nevertheless, the discovery and characterization of the unconventional kinetochores in kinetoplastids provides unique insights into the origin of chromosome segregation machineries and the general design principles crucial for kinetochore function.

A second CenH3-independent chromosome segregation pathway was recently described in several orders of insects using genomic and transcriptomic surveys [11]. Unlike in the case of

kinetoplastids, it is unambiguous that CenH3 absence in insects is due to at least four independent loss events, some of which occurred as long as 300 million years ago. In insects, CenH3 losses occurred in lineages that underwent dramatic changes in their centromeric architecture, from monocentricity to holocentricity (Box 1). This perfect correlation in insects suggests a causal relationship between the two events, that is, transition to holocentric chromosomes in insects might have facilitated the loss of CenH3, or vice versa. Notably, other kinetochore components are still present in CenH3-deficient insects (see Figure 1D, for example), which implies that these species utilize CenH3-independent ways of initiating kinetochore assembly using at least some of the same components used in CenH3-encoding insects. Outside of insects, other holocentric species with sequenced genomes such as *Caenorhabditis elegans* do encode CenH3, where it carries out essential functions in mitotic chromosome segregation [11,18,28]. This suggests that the association of CenH3 loss with holocentricity might be unique to the insect lineage.

Despite its essential mitotic role in *C. elegans*, CenH3 is not required for meiotic divisions, thus representing a third case of a CenH3-independent chromosome segregation pathway [29]. Although the outer kinetochore complex is required for proper chromosome alignment during early meiotic metaphase, even this complex disassembles before chromosomes separate during *C. elegans* meiosis. In this instance, lateral attachments to microtubule bundles along the sides of chromosomes and microtubule motor proteins may provide the major driving force [30,31]. Intriguingly, neocentromeres formed on maize knob chromosomal elements during meiosis also do not require CenH3 for mediating attachments to the meiotic spindle [32].

The three cases of CenH3 loss we have highlighted here have different evolutionary origins; these species likely accomplish chromosome segregation by distinct means. Together these examples emphasize the fact that even kinetochore proteins that are otherwise considered most fundamental to centromere function and kinetochore assembly can be lost.

A Variable Requirement of CENP-T for Kinetochore Function

There are two primary modes of connecting the centromeric DNA and the outer kinetochore [2,33,34] (Figure 1). The first of these is mediated by CENP-C, which is a large, unstructured protein [35] that directly interacts with CenH3 to connect the inner kinetochore to the Mis12 outer kinetochore complex [36–38]. The interaction of CENP-C with CenH3 is mediated by the carboxy-terminus of CenH3 and a conserved carboxy-terminal motif in the CENP-C protein, termed the CENP-C motif [39,40]. In mammals, CENP-C additionally binds to CenH3 via a central region; however, this central region is only conserved in mammals [40]. Despite the essentiality of the CENP-C protein and the conservation of the CENP-C motif, the cognate interaction sites at the C-termini of CenH3 proteins are surprisingly not well conserved. Instead, CenH3 C-termini share the common feature of being more hydrophobic than those of the canonical H3. This has led to the suggestion that the hydrophobicity of the C-terminal tail of CenH3, rather than a specific amino acid sequence, is the key determinant for CENP-C recognition [40]. CENP-C and CenH3 are interdependent at centromeres: incorporation of CenH3 is necessary for CENP-C recruitment, binding of CENP-C stabilizes CenH3 nucleosomes at centromeres, and CENP-C depletion leads to rapid loss of CenH3 from centromeres [41]. Consistent with the interdependence of CenH3 and CENP-C, CENP-C proteins are absent in holocentric insects that lack CenH3 [11].

The CENP-T protein provides a second mode of connecting the centromeric DNA and the outer kinetochore. Tethering CENP-T to ectopic sites is sufficient for kinetochore assembly in chicken cells [33]. The N-terminus of CENP-T interacts with the Ndc80 outer kinetochore complex [10,33,42]. A defining feature of CENP-T is a histone fold at the C-terminus followed by a conserved amino acid extension of unknown function [43,44]. Its cognate histone fold

interaction partner is CENP-W; a CENP-T/W dimer structurally resembles an H2A/H2B dimer [44]. The CENP-T/W dimer can be paired with a CENP-S/X dimer, completing a nucleosome-like complex that is found at centromeres [44]. This resemblance to nucleosomes led to suggestions that CENP-T-W-S-X may also wrap centromeric DNA similar to centromeric nucleosomes [45]. Although this might suggest that all four proteins are equally important for kinetochore function, phenotypes of CENP-S and CENP-X deletions are rather mild compared to CENP-T and CENP-W deletions in chicken DT40 cells [43,46]. Furthermore, although recruitment of CENP-S and CENP-X to kinetochores is dependent on CENP-T and CENP-W, some species containing CENP-S and CENP-X appear not to have CENP-T and CENP-W based on computational predictions [6,11]. In those species, CENP-S and CENP-X might be retained as a result of their role in DNA repair [47], which is independent of their CENP-T/W-dependent function at the kinetochore.

In contrast to CENP-C, CENP-T is only sporadically conserved across eukaryotes. While CENP-T homologs are essential in vertebrates and fission yeast [43,48,49], no CENP-T homologs have been identified in *D. melanogaster* or *C. elegans* [6]. Thus, it appears that in some species, one axis of connecting the centromere to the outer kinetochore (via CenH3 and CENP-C) is sufficient for kinetochore function, whereas in others, additional recruitment of CENP-T is required for proper kinetochore formation. Yet, CENP-T homologs display extensive protein sequence divergence, which complicates homology-based detection of CENP-T homologs from genomic sequences even in well-sequenced taxa such as arthropods, nematodes, or plants. Thus, it is difficult to conclusively rule out the absence of CENP-T based purely on homology searches; such conclusions should be confirmed by additional experimental approaches when possible. Indeed, combining experimental approaches with remote homology predictions successfully enabled the identification of a budding yeast CENP-T homolog, which also binds to the Ndc80 complex as does in vertebrate CENP-T [10]. However, whereas CENP-T is essential in all other species tested, the budding yeast CENP-T is not essential for chromosome segregation [10]. This finding, among others, suggests that the functional composition of the fission yeast kinetochores more closely resemble vertebrate kinetochores, than those from budding yeast.

New Genes for Old Functions: The Enigma of Recurrent Domestications of CENP-B-like Proteins

Another dramatic example of the rapid turnover of kinetochore proteins that emerges is the recurrent domestication of CENP-B DNA-binding proteins for centromere functions (Figure 2). CENP-B proteins are derived from transposases of ancient pogo-like DNA transposons and have originated independently in mammals, fission yeast [50], *Lepidoptera* [51], and other metazoans [52]. In both mammals and fission yeast, CENP-B proteins localize to centromeres. CENP-B-like proteins in fission yeast are involved in heterochromatin formation surrounding the centromeric region [53,54], but also serve additional roles in silencing of transposable elements [55,56]. By contrast, the role of mammalian CENP-B proteins at centromeres is less understood. Thus, although all CENP-B proteins are homologous whether they perform the same function is unclear.

Mammalian CENP-B proteins have DNA-binding sites (called CENP-B boxes) in satellite repeat families present in megabase-long centromeric arrays [57], except on the Y chromosome [58]. Furthermore, CENP-B has been conserved through most of mammalian evolution. Despite this massive abundance of its recognition motif and its evolutionary conservation, CENP-B is not essential for centromere function and CENP-B boxes have not been strictly conserved during mammalian evolution [59]. This suggests that CENP-B conservation might be attributable to a non-centromeric function such as the silencing of transposable elements as described in fission yeast [55,56]. The most direct demonstration of the role of CENP-B in kinetochore formation has come out of a recent study in which human CENP-B was shown to interact with the N-termini of

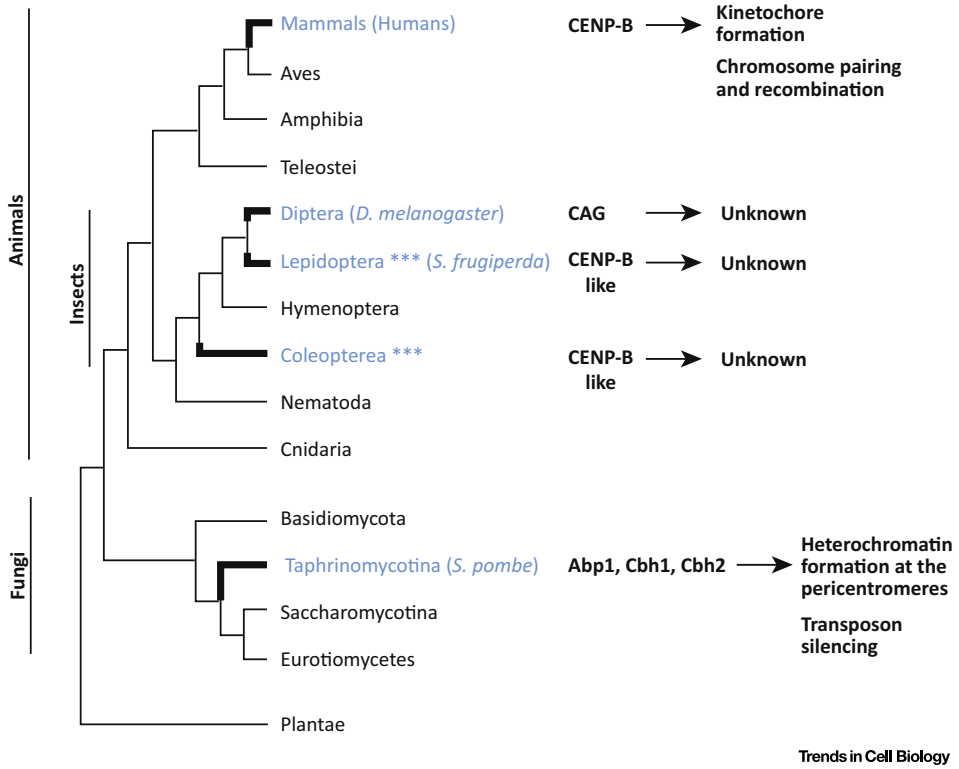

Figure 2. Recurrent Domestication of Pogo-like Transposases to Give Rise to CENP-B-like DNA-Binding Proteins. CENP-B-like proteins have been domesticated multiple independent times in animals and fungi. Three domesticated CENP-B-like proteins were found in fission yeast (highlighted in blue). Blue asterisks indicate potential domestications of additional CENP-B-like proteins in insects (often multiple instances per genome) predicted from bioinformatics analyses [52] that have yet to be confirmed experimentally.

CenH3 and CENP-C [60]. CENP-B mutations in human cells led to depletion of both CENP-C levels as well as defects in cell cycle-dependent loading of CenH3. This suggested a model in which CENP-B represents a parallel pathway for CENP-C recruitment and CenH3 stabilization at human centromeres [60].

Although CENP-B has been lost in some mammalian lineages, it is difficult to reconcile this dispensability with the observation that it may mediate some important, although nonessential, function at the kinetochore. We speculate that this discrepancy could result from the specific DNA-binding properties of CENP-B and the unique battle waged between centromeres during female meiosis. Under the 'centromere drive' hypothesis, chromosomes compete via their centromeric satellites in female meiosis for inclusion into the oocyte [14,61]. Greater recruitment of centromeric proteins might directly translate to meiotic success [62]. In this regard, recruiting CENP-B proteins by virtue of CENP-B boxes may be one means by which certain centromeres could outcompete others 'selfishly' for meiotic dominance [63]. However, centromere drive can result in deleterious effects on male fertility [64,65] and other functions [66]. To counter these deleterious effects, suppressor alleles are predicted to arise. Some of these suppressors could result in centromere strength (and kinetochore function) being less dependent on CENP-B recruitment, thereby negating the 'selfish' advantage of some centromeric satellite repeats. Under this model, the loss of CENP-B could be a result of the transition between 'centromere drive' in which specific DNA sequences are selected by competition (a 'genetic' state), and 'suppression' in which DNA specificity is ignored for centromere specification (an 'epigenetic' state) [67]. This 'centromere

drive' hypothesis might especially apply to kinetochore proteins such as CENP-B with DNA-binding specificity, but only in lineages that undergo female meiosis.

Centromere drive and its suppression can also explain the signatures of rapid evolution observed even in essential kinetochore components such as CenH3 and CENP-C in multiple animal and plant lineages [14,15,35]. The relentless need to innovate at the centromere–kinetochore interface as a result of centromere drive has also been proposed to lead to the rapid acquisition of essential roles for centromere function on an evolutionary young gene in *Drosophila* [68]. It is currently unclear to what extent such lineage-specific, evolutionarily young genes have further diversified kinetochore repertoires across animals and plants.

Evolutionary Turnover of the Kinetochore Complex

In the previous sections, we used the examples of three inner kinetochore proteins to highlight three facets of plasticity of kinetochore repertoires: loss of components essential in some lineages (CenH3), differential essentiality for kinetochore function (CENP-T), and invention of novel components (CENP-B). These facets can be extended to other kinetochore proteins of the inner kinetochore (collectively termed the constitutive centromere-associated network, or CCAN), the outer kinetochore, and even to components of the mitotic checkpoint, which acts to safeguard against premature chromosome segregation.

The CCAN component CENP-M represents another example for the nonuniform presence of a kinetochore component. CENP-M homologs are easily recognizable as catalytically inactive members of the GTPase superfamily. At the kinetochore, CENP-M forms a tight complex with CENP-I in a way that is structurally reminiscent of a small GTPase bound to importin beta [6,69]. The CENP-M/CENP-I complex associates with CENP-H and CENP-K and localization of the entire complex is interdependent with CENP-T in human cells [69]. Notably, although CENP-M is essential in vertebrates, it appears to be absent in fungi [10,49,70,71]. It is still unclear whether this is because CENP-M is a vertebrate- or animal-specific invention or whether it was lost in fungi. In the latter case, it will be interesting to test if another GTPase-like protein performs the same function in fungi as CENP-M does in vertebrates [69], or whether they have adopted a completely non-homologous means to substitute for CENP-M.

The CENP-O complex is another example of a CCAN complex of proteins that is essential for kinetochore function in some species but not others [49,70,72,73]. The CENP-O complex includes CENP-O, CENP-P, CENP-Q, CENP-U, and CENP-R (CENP-R is not present in fungi). Two components of the CENP-O complex, CENP-Q and CENP-U, are essential in budding yeast and have been shown to interact with the Mis12 complex along with CENP-C. Budding yeast centromeres consist of only one CenH3 and CENP-C, but several Mis12 complexes. The CENP-U/Q-mediated interaction with the Mis12 complex has therefore been proposed to provide additional bridges to the outer kinetochore [72]. In contrast to budding yeast, CENP-U is not required for viability in chicken DT40 and mouse embryonic fibroblasts. Yet, CENP-U mutant mice die during early embryonic development and CENP-U is essential in undifferentiated murine embryonic stem cells [74]. To explain this difference, it has been proposed that kinetochores are weaker in all cell types in the absence of CENP-U. This effect might lead to more detrimental consequences in rapidly dividing embryonic cells resulting from weaker mitotic checkpoints, which would otherwise allow cells to correct inappropriate kinetochore attachments [74].

With the exception of CENP-C, most CCAN components have only been found in vertebrates and fungi based on sequence homology [10,75]. It is possible that CCAN components are present in other species but homology detection is challenged by a high degree of divergence and lack of predefined protein domains. If CCAN proteins are truly absent in some lineages, it will

be interesting to determine whether their kinetochore function is replaced by other proteins, or whether the kinetochore composition in those lineages is less complex. To explore these ideas, it would be useful to study the composition of kinetochores in additional lineages such as *Dictyostelium*, ciliates, and plants that are accessible to genomics and proteomics.

Although we have focused in this review on inner kinetochore proteins, some outer kinetochore components also exhibit features of evolutionary lability. The function of the outer kinetochore KNL1 proteins in microtubule attachment and the mitotic checkpoint is highly conserved across eukaryotes. Yet, repeat arrays that recruit BUB1–BUB3 signaling proteins exhibit recurrent episodes of rapid evolution in both number and sequence [76]. It is unclear what drives these changes and whether these episodes affect the strength or fidelity of the signaling. Similar to the lineage-specific invention of some inner kinetochore proteins, computational predictions based on homology suggest that the Dam1 outer kinetochore complex arose in an early fungal ancestor. The Dam1 complex forms oligomers around spindle microtubules, thereby contributing to the strength of microtubule attachment [77] (Figure 1A). In humans and other species such as nematodes and plants, the Ska1 complex might be the functional equivalent of the Dam1 complex in fungi in terms of coupling chromosome movements to microtubule dynamics [78–80], although both complexes do not share sequence or even structural similarity.

In addition to evolutionary turnover within the kinetochore complex, recent studies have found significant turnover within components of the mitotic checkpoint pathway [81]. For example, although the mitotic checkpoint kinase Mps1 is essential in fungi and several animal lineages [82,83], it is lost in *C. elegans* [81]. Indeed, a recent study has confirmed that a different kinetochore-localized kinase, the Polo-like kinase 1, may have replaced Mps1 in nematodes [84]. Evolutionary turnover is even seen in protein complexes involved in the recruitment and removal of signaling proteins involved in the mitotic checkpoint. This turnover includes both evolutionary adaptation of existing proteins (RZZ complex components Rod and ZW10) [85] as well as invention of novel proteins (Spindly, RZZ complex component Zwilch) [81,86,87]. The fact that some of these components cannot be found outside the opisthokonts suggest that a more ancient mechanism for checkpoint regulation in eukaryotes may have been replaced by RZZ/Spindly in opisthokonts. Identification of these ancestral mechanisms will require studies beyond the traditional model organisms, which have been largely limited to within the opisthokonts thus far.

Concluding Remarks

In this review, we have highlighted the evolutionary dynamics of the kinetochore complex. Despite accomplishing the same essential function of connecting centromeric DNA to spindle microtubules in all eukaryotes, kinetochore composition is unexpectedly evolutionarily labile. What is the effect of this lack of evolutionary conservation on core functions of the kinetochore, including kinetochore strength and regulation? While one kinetochore–microtubule attachment is sufficient to segregate budding yeast chromosomes, many kinetochore–microtubule attachments work in concert to segregate a human monocentric chromosome or a nematode holocentric chromosome. This suggests that the relative demands on kinetochore strength might be different among different species; whether this is a cause or consequence of changes in kinetochore composition remains unexplored. Similarly, it is unknown how the number of kinetochore–microtubule interactions affects stringency of kinetochore regulation, which may explain the rapid evolution of kinetochore proteins such as KNL1 [76].

Most importantly, our survey reveals the effects of a long, historical bias of studying kinetochore compositions in vertebrates, fungi, and a handful of model invertebrate animals. Although genome sequencing and new proteomic approaches are helping to correct this bias, additional experimental approaches and remote homology predictions will be needed to determine

Outstanding Questions

Are the architectural principles of kinetochores and their regulation during cell division preserved in divergent organisms such as trypanosomes despite lack of conservation of kinetochore components?

What were the intermediate evolutionary steps by which a previously essential centromeric protein was lost in certain lineages (e.g., CenH3 in holocentric insects) or rendered nonessential in others (e.g., CENP-T in budding yeast)?

What has replaced CenH3 function in trypanosomes and holocentric insects?

How do changes in kinetochore architecture (e.g., holocentric versus monocentric, chromosome fusions) or strength mediate such transitions?

What is the effect of evolutionary turnover of kinetochore proteins on kinetochore strength and fidelity of chromosome segregation?

Is the composition of meiotic kinetochores identical to mitotic kinetochores? If not, what are the meiosis-specific components, how do they function, and how well are they conserved in evolution especially in taxa that do not undergo female or male meiosis?

What is the effect of evolutionary turnover on checkpoint proteins and even proteins that 'moonlight' at the kinetochore (e.g., some nuclear pore complex proteins) on kinetochore function?

kinetochore composition in additional major eukaryotic lineages including plants. Only then will a true picture of component fluidity and general design principles of kinetochores begin to emerge (see Outstanding Questions).

In spite of the evolutionary turnover of kinetochore repertoires, we favor Aristotle's resolution of the paradox of the 'Ship of Theseus': although the matter (composition) of the kinetochore varies over time, the formal cause – or design – remains the same.

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References

1. Flemming, W. (1882) *Zellsubstanz, Kern und Zelltheilung*, F.C.W. Vogel
2. Cheeseman, I.M. (2014) The kinetochore. *Cold Spring Harb. Perspect. Biol.* 6, a015826
3. Rieder, C.L. (1982) The formation, structure, and composition of the mammalian kinetochore and kinetochore fiber. *Int. Rev. Cytol.* 79, 1–58
4. Gonen, S. *et al.* (2012) The structure of purified kinetochores reveals multiple microtubule-attachment sites. *Nat. Struct. Mol. Biol.* 19, 925–929
5. Akiyoshi, B. *et al.* (2010) Tension directly stabilizes reconstituted kinetochore–microtubule attachments. *Nature* 468, 576–579
6. Westermann, S. and Schleiffer, A. (2013) Family matters: structural and functional conservation of centromere-associated proteins from yeast to humans. *Trends Cell Biol.* 23, 260–269
7. Barth, T.K. *et al.* (2014) Identification of novel *Drosophila* centromere-associated proteins. *Proteomics* 14, 2167–2178
8. Przewloka, M.R. *et al.* (2007) Molecular analysis of core kinetochore composition and assembly in *Drosophila melanogaster*. *PLoS ONE* 2, e478
9. Przewloka, M.R. *et al.* (2009) Searching for *Drosophila* Dsn1 kinetochore protein. *Cell Cycle* 8, 1292–1293
10. Schleiffer, A. *et al.* (2012) CENP-T proteins are conserved centromere receptors of the Ndc80 complex. *Nat. Cell Biol.* 14, 604–613
11. Drinnenberg, I.A. *et al.* (2014) Recurrent loss of CenH3 is associated with independent transitions to holocentricity in insects. *Elife* 3, e03676
12. Akiyoshi, B. and Gull, K. (2014) Discovery of unconventional kinetochores in kinetoplastids. *Cell* 156, 1247–1258
13. Akiyoshi, B. and Gull, K. (2013) Evolutionary cell biology of chromosome segregation: insights from trypanosomes. *Open Biol.* 3, 130023
14. Malik, H.S. and Henikoff, S. (2009) Major evolutionary transitions in centromere complexity. *Cell* 138, 1067–1082
15. Roach, K.C. *et al.* (2012) Rapid evolution of centromeres and centromeric/kinetochore proteins. In *Evolution in the Fast Lane: Rapidly Evolving Genes and Genetic Systems* (Rama Singh, J.X. R.K., ed.), pp. 83–93, Oxford University Press
16. Palmer, D.K. *et al.* (1991) Purification of the centromere-specific protein CENP-A and demonstration that it is a distinctive histone. *Proc. Natl. Acad. Sci. U.S.A.* 88, 3734–3738
17. Earnshaw, W.C. and Rothfield, N. (1985) Identification of a family of human centromere proteins using autoimmune sera from patients with scleroderma. *Chromosoma* 91, 313–321
18. Buchwitz, B.J. *et al.* (1999) A histone-H3-like protein in *C. elegans*. *Nature* 401, 547–548
19. Dawson, S.C. *et al.* (2007) The cenH3 histone variant defines centromeres in *Giardia intestinalis*. *Chromosoma* 116, 175–184
20. Stoler, S. *et al.* (1995) 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.* 9, 573–586
21. Henikoff, S. *et al.* (2000) Heterochromatic deposition of centromeric histone H3-like proteins. *Proc. Natl. Acad. Sci. U.S.A.* 97, 716–721
22. Talbert, P.B. and Henikoff, S. (2010) Histone variants – ancient wrap artists of the epigenome. *Nat. Rev. Mol. Cell Biol.* 11, 264–275
23. Malik, H.S. and Henikoff, S. (2003) Phylogenomics of the nucleosome. *Nat. Struct. Biol.* 10, 882–891
24. Postberg, J. *et al.* (2010) The evolutionary history of histone H3 suggests a deep eukaryotic root of chromatin modifying mechanisms. *BMC Evol. Biol.* 10, 259
25. Baldauf, S.L. (2003) The deep roots of eukaryotes. *Science* 300, 1703–1706
26. Zubacova, Z. *et al.* (2012) Histone H3 variants in *Trichomonas vaginalis*. *Eukaryot. Cell* 11, 654–661
27. Derelle, R. *et al.* (2015) Bacterial proteins pinpoint a single eukaryotic root. *Proc. Natl. Acad. Sci. U.S.A.* 112, E693–E699
28. Heckmann, S. *et al.* (2011) Holocentric chromosomes of *Luzula elegans* are characterized by a longitudinal centromere groove, chromosome bending, and a terminal nucleolus organizer region. *Cytogenet. Genome Res.* 134, 220–228
29. Monen, J. *et al.* (2005) Differential role of CENP-A in the segregation of holocentric *C. elegans* chromosomes during meiosis and mitosis. *Nat. Cell Biol.* 7, 1248–1255
30. Dumont, J. *et al.* (2010) A kinetochore-independent mechanism drives anaphase chromosome separation during acentrosomal meiosis. *Nat. Cell Biol.* 12, 894–901
31. Muscat, C.C. *et al.* (2015) Kinetochore-independent chromosome segregation driven by lateral microtubule bundles. *Elife* 4, e06462
32. Zhong, C.X. *et al.* (2002) Centromeric retroelements and satellites interact with maize kinetochore protein CENH3. *Plant Cell* 14, 2825–2836
33. Gascoigne, K.E. *et al.* (2011) Induced ectopic kinetochore assembly bypasses the requirement for CENP-A nucleosomes. *Cell* 145, 410–422
34. Rago, F. *et al.* (2015) Distinct organization and regulation of the outer kinetochore KMN network downstream of CENP-C and CENP-T. *Curr. Biol.* 25, 671–677
35. Talbert, P.B. *et al.* (2004) Adaptive evolution of centromere proteins in plants and animals. *J. Biol.* 3, 18
36. Milks, K.J. *et al.* (2009) Dissection of CENP-C-directed centromere and kinetochore assembly. *Mol. Biol. Cell* 20, 4246–4255
37. Screpanti, E. *et al.* (2011) Direct binding of Cenp-C to the Mis12 complex joins the inner and outer kinetochore. *Curr. Biol.* 21, 391–398

38. Przewloka, M.R. *et al.* (2011) CENP-C is a structural platform for kinetochore assembly. *Curr. Biol.* 21, 399–405
39. Carroll, C.W. *et al.* (2010) Dual recognition of CENP-A nucleosomes is required for centromere assembly. *J. Cell Biol.* 189, 1143–1155
40. Kato, H. *et al.* (2013) A conserved mechanism for centromeric nucleosome recognition by centromere protein CENP-C. *Science* 340, 1110–1113
41. Falk, S.J. *et al.* (2015) Chromosomes. CENP-C reshapes and stabilizes CENP-A nucleosomes at the centromere. *Science* 348, 699–703
42. Nishino, T. *et al.* (2013) CENP-T provides a structural platform for outer kinetochore assembly. *EMBO J.* 32, 424–436
43. Hori, T. *et al.* (2008) CCAN makes multiple contacts with centromeric DNA to provide distinct pathways to the outer kinetochore. *Cell* 135, 1039–1052
44. Nishino, T. *et al.* (2012) CENP-T-W-S-X forms a unique centromeric chromatin structure with a histone-like fold. *Cell* 148, 487–501
45. Takeuchi, K. *et al.* (2014) The centromeric nucleosome-like CENP-T-W-S-X complex induces positive supercoils into DNA. *Nucleic Acids Res.* 42, 1644–1655
46. Amano, M. *et al.* (2009) The CENP-S complex is essential for the stable assembly of outer kinetochore structure. *J. Cell Biol.* 186, 173–182
47. Singh, T.R. *et al.* (2010) MHF1–MHF2, a histone-fold-containing protein complex, participates in the Fanconi anemia pathway via FANCM. *Mol. Cell* 37, 879–886
48. Tanaka, K. *et al.* (2009) CENP-C functions as a scaffold for effectors with essential kinetochore functions in mitosis and meiosis. *Dev. Cell* 17, 334–343
49. Foltz, D.R. *et al.* (2006) The human CENP-A centromeric nucleosome-associated complex. *Nat. Cell Biol.* 8, 458–469
50. Casola, C. *et al.* (2008) Convergent domestication of pogo-like transposases into centromere-binding proteins in fission yeast and mammals. *Mol. Biol. Evol.* 25, 29–41
51. d'Alencon, E. *et al.* (2011) Characterization of a CENP-B homolog in the holocentric Lepidoptera *Spodoptera frugiperda*. *Gene* 485, 91–101
52. Mateo, L. and Gonzalez, J. (2014) Pogo-like transposases have been repeatedly domesticated into CENP-B-related proteins. *Genome Biol. Evol.* 6, 2008–2016
53. Nakagawa, H. *et al.* (2002) Fission yeast CENP-B homologs nucleate centromeric heterochromatin by promoting heterochromatin-specific histone tail modifications. *Genes Dev.* 16, 1766–1778
54. Clarke, L. (1998) Centromeres: proteins, protein complexes, and repeated domains at centromeres of simple eukaryotes. *Curr. Opin. Genet. Dev.* 8, 212–218
55. Zaratiegui, M. *et al.* (2011) CENP-B preserves genome integrity at replication forks paused by retrotransposon LTR. *Nature* 469, 112–115
56. Cam, H.P. *et al.* (2008) Host genome surveillance for retrotransposons by transposon-derived proteins. *Nature* 451, 431–436
57. Masumoto, H. *et al.* (1989) A human centromere antigen (CENP-B) interacts with a short specific sequence in alphoid DNA, a human centromeric satellite. *J. Cell Biol.* 109, 1963–1973
58. Miga, K.H. *et al.* (2014) Centromere reference models for human chromosomes X and Y satellite arrays. *Genome Res.* 24, 697–707
59. Goldberg, I.G. *et al.* (1996) Surprising deficiency of CENP-B binding sites in African green monkey alpha-satellite DNA: implications for CENP-B function at centromeres. *Mol. Cell. Biol.* 16, 5156–5168
60. Fachinetti, D. *et al.* (2015) DNA sequence-specific binding of CENP-B enhances the fidelity of human centromere function. *Dev. Cell* 33, 314–327
61. Henikoff, S. *et al.* (2001) The centromere paradox: stable inheritance with rapidly evolving DNA. *Science* 293, 1098–1102
62. Chmatal, L. *et al.* (2014) Centromere strength provides the cell biological basis for meiotic drive and karyotype evolution in mice. *Curr. Biol.* 24, 2295–2300
63. Marshall, O.J. and Choo, K.H. (2012) Putative CENP-B paralogues are not present at mammalian centromeres. *Chromosoma* 121, 169–179
64. Fishman, L. and Saunders, A. (2008) Centromere-associated female meiotic drive entails male fitness costs in monkeyflowers. *Science* 322, 1559–1562
65. Daniel, A. (2002) Distortion of female meiotic segregation and reduced male fertility in human Robertsonian translocations: consistent with the centromere model of co-evolving centromere DNA/centromeric histone (CENP-A). *Am. J. Med. Genet.* 111, 450–452
66. Fishman, L. and Kelly, J.K. (2015) Centromere-associated meiotic drive and female fitness variation in Mimulus. *Evolution* 69, 1208–1218
67. Dawe, R.K. and Henikoff, S. (2006) Centromeres put epigenetics in the driver's seat. *Trends Biochem. Sci.* 31, 662–669
68. Ross, B.D. *et al.* (2013) Stepwise evolution of essential centromere function in a *Drosophila* neogene. *Science* 340, 1211–1214
69. Basilico, F. *et al.* (2014) The pseudo GTPase CENP-M drives human kinetochore assembly. *Elife* 3, e02978
70. Okada, M. *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, 446–457
71. Izuta, H. *et al.* (2006) Comprehensive analysis of the ICEN (Interphase Centromere Complex) components enriched in the CENP-A chromatin of human cells. *Genes Cells* 11, 673–684
72. Hornung, P. *et al.* (2014) A cooperative mechanism drives budding yeast kinetochore assembly downstream of CENP-A. *J. Cell Biol.* 206, 509–524
73. Hori, T. *et al.* (2008) CENP-O class proteins form a stable complex and are required for proper kinetochore function. *Mol. Biol. Cell* 19, 843–854
74. Kagawa, N. *et al.* (2014) The CENP-O complex requirement varies among different cell types. *Chromosome Res.* 22, 293–303
75. Perpelescu, M. and Fukagawa, T. (2011) The ABCs of CENPs. *Chromosoma* 120, 425–446
76. Tromer, E. *et al.* (2015) Widespread recurrent patterns of rapid repeat evolution in the kinetochore scaffold KNL1. *Genome Biol. Evol.* 7, 2383–2393
77. Umbreit, N.T. *et al.* (2014) Kinetochores require oligomerization of Dam1 complex to maintain microtubule attachments against tension and promote biorientation. *Nat. Commun.* 5, 4951
78. Welburn, J.P. *et al.* (2009) The human kinetochore Ska1 complex facilitates microtubule depolymerization-coupled motility. *Dev. Cell* 16, 374–385
79. Buttrick, G.J. and Millar, J.B. (2011) Ringing the changes: emerging roles for DASH at the kinetochore–microtubule interface. *Chromosome Res.* 19, 393–407
80. Schmidt, J.C. *et al.* (2012) The kinetochore-bound Ska1 complex tracks depolymerizing microtubules and binds to curved protofilaments. *Dev. Cell* 23, 968–980
81. Vleugel, M. *et al.* (2012) Evolution and function of the mitotic checkpoint. *Dev. Cell* 23, 239–250
82. Abrieu, A. *et al.* (2001) Mps1 is a kinetochore-associated kinase essential for the vertebrate mitotic checkpoint. *Cell* 106, 83–93
83. Lauze, E. *et al.* (1995) Yeast spindle pole body duplication gene MPS1 encodes an essential dual specificity protein kinase. *EMBO J.* 14, 1655–1663
84. Espeut, J. *et al.* (2015) Natural loss of Mps1 kinase in nematodes uncovers a role for polo-like kinase 1 in spindle checkpoint initiation. *Cell Rep.* 12, 58–65
85. Civril, F. *et al.* (2010) Structural analysis of the RZZ complex reveals common ancestry with multisubunit vesicle tethering machinery. *Structure* 18, 616–626
86. Griffis, E.R. *et al.* (2007) Spindly, a novel protein essential for silencing the spindle assembly checkpoint, recruits dynein to the kinetochore. *J. Cell Biol.* 177, 1005–1015
87. Williams, B.C. *et al.* (2003) Zwilch, a new component of the ZW10/ROD complex required for kinetochore functions. *Mol. Biol. Cell* 14, 1379–1391

88. Furuyama, S. and Biggins, S. (2007) Centromere identity is specified by a single centromeric nucleosome in budding yeast. *Proc. Natl. Acad. Sci. U.S.A.* 104, 14706–14711

89. Sullivan, L.L. *et al.* (2011) Genomic size of CENP-A domain is proportional to total alpha satellite array size at human centromeres and expands in cancer cells. *Chromosome Res.* 19, 457–470

90. McEwen, B.F. *et al.* (1997) Kinetochore fiber maturation in PtK1 cells and its implications for the mechanisms of chromosome congression and anaphase onset. *J. Cell Biol.* 137, 1567–1580

91. Melters, D.P. *et al.* (2012) Holocentric chromosomes: convergent evolution, meiotic adaptations, and genomic analysis. *Chromosome Res.* 20, 579–593

92. Gassmann, R. *et al.* (2012) An inverse relationship to germline transcription defines centromeric chromatin in *C. elegans*. *Nature* 484, 534–537

93. Steiner, F.A. and Henikoff, S. (2014) Holocentromeres are dispersed point centromeres localized at transcription factor hotspots. *Elife* 3, e02025

94. Marques, A. *et al.* (2015) Holocentromeres in Rhynchospora are associated with genome-wide centromere-specific repeat arrays interspersed among euchromatin. *Proc. Natl. Acad. Sci. U.S.A.* 112, 13633–13638

95. Deans, C. and Maggert, K.A. (2015) What do you mean, 'epigenetic'? *Genetics* 199, 887–896

96. Voullaire, L.E. *et al.* (1993) A functional marker centromere with no detectable alpha-satellite, satellite III, or CENP-B protein: activation of a latent centromere? *Am. J. Hum. Genet.* 52, 1153–1163

97. Masumoto, H. *et al.* (2004) The role of CENP-B and alpha-satellite DNA: de novo assembly and epigenetic maintenance of human centromeres. *Chromosome Res.* 12, 543–556

98. Henikoff, J.G. *et al.* (2015) A unique chromatin complex occupies young alpha-satellite arrays of human centromeres. *Sci. Adv.* 1, e1400234

99. Heun, P. *et al.* (2006) Mislocalization of the *Drosophila* centromere-specific histone CID promotes formation of functional ectopic kinetochores. *Dev. Cell* 10, 303–315

100. Muller, S. and Almouzni, G. (2014) A network of players in H3 histone variant deposition and maintenance at centromeres. *Biochim. Biophys. Acta* 1839, 241–250

101. McKinley, K.L. and Cheeseman, I.M. (2014) Polo-like kinase 1 licenses CENP-A deposition at centromeres. *Cell* 158, 397–411

102. Foltz, D.R. *et al.* (2009) Centromere-specific assembly of CENP-a nucleosomes is mediated by HJURP. *Cell* 137, 472–484

103. Stoler, S. *et al.* (2007) Scm3, an essential *Saccharomyces cerevisiae* centromere protein required for G2/M progression and Cse4 localization. *Proc. Natl. Acad. Sci. U.S.A.* 104, 10571–10576

104. Camahort, R. *et al.* (2007) Scm3 is essential to recruit the histone h3 variant cse4 to centromeres and to maintain a functional kinetochore. *Mol. Cell* 26, 853–865

105. Dunleavy, E.M. *et al.* (2009) HJURP is a cell-cycle-dependent maintenance and deposition factor of CENP-A at centromeres. *Cell* 137, 485–497

106. Sanchez-Pulido, L. *et al.* (2009) Common ancestry of the CENP-A chaperones Scm3 and HJURP. *Cell* 137, 1173–1174

107. Mellone, B.G. *et al.* (2011) Assembly of *Drosophila* centromeric chromatin proteins during mitosis. *PLoS Genet.* 7, e1002068

108. Schittenhelm, R.B. *et al.* (2010) Detrimental incorporation of excess Cenp-A/Cid and Cenp-C into *Drosophila* centromeres is prevented by limiting amounts of the bridging factor Cal1. *J. Cell Sci.* 123, 3768–3779

109. Chen, C.C. *et al.* (2014) CAL1 is the *Drosophila* CENP-A assembly factor. *J. Cell Biol.* 204, 313–329

110. Phansalkar, R. *et al.* (2012) Evolutionary insights into the role of the essential centromere protein CAL1 in *Drosophila*. *Chromosome Res.* 20, 493–504

111. Lacoste, N. *et al.* (2014) Mislocalization of the centromeric histone variant CenH3/CENP-A in human cells depends on the chaperone DAXX. *Mol. Cell* 53, 631–644

112. Maddox, P.S. *et al.* (2007) Functional genomics identifies a Myb domain-containing protein family required for assembly of CENP-A chromatin. *J. Cell Biol.* 176, 757–763

113. Hayashi, T. *et al.* (2004) Mis16 and Mis18 are required for CENP-A loading and histone deacetylation at centromeres. *Cell* 118, 715–729

114. Subramanian, L. *et al.* (2014) Eic1 links Mis18 with the CCAN/Mis6/Ctf19 complex to promote CENP-A assembly. *Open Biol.* 4, 140043

115. Fujita, Y. *et al.* (2007) Priming of centromere for CENP-A recruitment by human hMis18alpha, hMis18beta, and M18BP1. *Dev. Cell* 12, 17–30

116. An, S. *et al.* (2015) Mis16 independently recognizes histone H4 and the CENP-A(Cnp1)-specific chaperone Scm3sp. *J. Mol. Biol.* 427, 3230–3240

117. Moree, B. *et al.* (2011) CENP-C recruits M18BP1 to centromeres to promote CENP-A chromatin assembly. *J. Cell Biol.* 194, 855–871

118. Lermontova, I. *et al.* (2013) Arabidopsis kinetochore null2 is an upstream component for centromeric histone H3 variant cenH3 deposition at centromeres. *Plant Cell* 25, 3389–3404