

Review



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Biochemical evidence for diverse strategies in the inner kinetochore

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The kinetochore is a complex structure whose function is absolutely essential. Unlike the centromere, the kinetochore at first appeared remarkably well conserved from yeast to humans, especially the microtubule-binding outer kinetochore. However, recent efforts towards biochemical reconstitution of diverse kinetochores challenge the notion of a similarly conserved architecture for the constitutively centromere-associated network of the inner kinetochore. This review briefly summarizes the evidence from comparative genomics for interspecific variability in inner kinetochore composition and focuses on novel biochemical evidence indicating that even homologous inner kinetochore protein complexes are put to different uses in different organisms.

1. Introduction

Centromeres are one of the last great enigmas of the genome. Although specialized sites of microtubule attachment on chromosomes were first described by Flemming over a century ago [1], the linear assemblies of most human centromeres remain elusive to this day, nearly two decades after the official completion of the Human Genome Project [2–5]. Curiously, despite the fact that all centromeres share a common function—recruitment of kinetochore proteins, which mediate chromosome-microtubule attachments essential for cell division—they differ radically in size and sequence.

Some organisms, including the model organism *Caenorhabditis elegans*, have holocentric chromosomes, which bind microtubules along their entire lengths through widespread kinetochore activity [6–8]. But in most organisms, kinetochore activity is restricted to a single, relatively small region of each chromosome, termed a regional centromere [9]. The simplest centromeric sequences are found in *Saccharomyces cerevisiae* and related budding yeast; these centromeres are defined by a minimal sequence of only about 125 base pairs containing three conserved centromere-determining elements (CDEI, CDEII and CDEIII) [10–12]. Such ‘point’ centromeres are exceptional for both their diminutive size and sequence conservation [13,14]. In general, centromeric DNA is highly repetitive and AT-rich, though the size and sequence of regional centromeres vary tremendously between species; regional centromeres can be as small as a few kilobases, or they can span megabases [15,16]. Centromere sequence and location can even vary at the population level within species [4,17–19]. There is tremendous variation in the DNA sequences on which kinetochores assemble.

However, it should be noted that this traditional definition of the centromere excludes the more recently recognized contributions of ‘pericentric’ chromatin, which forms specialized, conserved structures with important biophysical properties (reviewed in [20]). Lawrimore & Bloom [20] postulate that highly looped, ‘bottlebrush’ structures may be formed either by a single regional centromere [21] or a clustered ensemble of point centromeres and their pericentromeric chromatin [22,23]. Although there are different models of the exact structure of pericentromeric chromatin [24,25], there is a growing consensus that such structures are significant and may represent a conserved centromeric architecture that belies the genetic variety of centromere sequence [20,24].

Furthermore, genetic sequence alone does not a centromere make. Both genetic and epigenetic factors contribute to centromere identity, and their relative importance varies between organisms [16]. Budding yeast rely heavily on genetic determinants of centromere identity [11,16,26]. But in the vast majority of organisms studied thus far,¹ centromeres are primarily specified in an epigenetic manner by the presence of a centromere-specific histone H3 variant termed CENP-A [31,32] (table 1). CENP-A positioning is epigenetically propagated, because existing CENP-A nucleosomes template new CENP-A deposition by recruiting necessary factors such as HJURP, CENP-C and the Mis18 complex in vertebrates [33–39]. Indeed, in higher eukaryotes, centromere inheritance appears minimally affected by DNA sequence; centromeres can migrate into neighbouring regions, a phenomenon known as ‘centromere drift’ [16,40]. Experimentally mistargeted CENP-A can incorporate into non-centromeric chromatin (including both heterochromatin and actively transcribed euchromatin) and recruit kinetochore proteins, creating ‘neocentromeres’ that can be transmitted mitotically [33,41,42]. Neocentromeres also form on non-repetitive DNA without experimental manipulation—over 100 different neocentromeres have been identified in living humans [43,44]. In several such cases, the original centromeres were permanently silenced without rearrangement or deletion of formerly centromeric repeat DNA sequences, by poorly understood epigenetic mechanisms [45,46]. In other words, in humans, the presence CENP-A at a given chromosomal locus, unlike the underlying DNA sequence, is both necessary and sufficient for mitotic centromere function.

Despite a shared method of epigenetic centromere specification and potential structural commonalities in the pericentromere, the sheer diversity of centromere size and sequence indicates that these loci are rapidly evolving [47,48].

The paradox of absolutely essential, yet poorly conserved centromeres defied explanation until it was placed in the intellectual framework of meiotic drive [49]. This novel view of centromeric chromatin as selfish genetic elements, competing with their homologues for inclusion in gametes during asymmetrical meiotic divisions, explained the rapid evolution of centromeric DNA, and the influential theory became known as ‘centromere drive’ [49–51].

The kinetochore, too, is a complex structure whose function is absolutely essential. Unlike the centromere, the kinetochore at first appeared remarkably well conserved from yeast to humans, especially the microtubule-binding outer kinetochore. However, recent efforts towards biochemical reconstitution of diverse kinetochores challenge the notion of a similarly conserved architecture for the constitutively centromere-associated network (CCAN) of the inner kinetochore. This review briefly summarizes the evidence from comparative genomics for interspecific variability in inner kinetochore composition and focuses on novel biochemical evidence indicating that even homologous inner kinetochore protein complexes are put to different uses in different organisms. We confine our discussion to the mitotic functions of inner kinetochore proteins, because their contributions to meiosis are much less well understood [52–54].

2. Genetic evidence

Two types of genetic evidence suggest an evolutionarily labile inner kinetochore: evidence that CCAN proteins are rapidly

evolving on the sequence level and evidence that different genomes contain different subsets of kinetochore proteins.

There is ample evidence that many kinetochore proteins are rapidly evolving. Comparing the rate of nonsynonymous substitutions (dN) to synonymous ones (dS) is a widely used metric for quantification of selection pressure on a given gene. Kinetochore protein genes have an average dN/dS value four times greater than that of anaphase-promoting complex proteins [12,30]. Although ascomycete kinetochore proteins are largely conserved with those of metazoans (hence the tremendous value of *S. cerevisiae* as a model organism in the field of kinetochore biology), even within the fungal kingdom inner kinetochore proteins are poorly conserved at the primary sequence level [55]. CENP-A, the foundation of the kinetochore, is also rapidly evolving [51,56,57], whereas conventional histones are among the best-conserved proteins in eukaryotic genomes.

Comparative genomics confirm that, throughout eukaryotic evolution, many inner kinetochore proteins have been lost as a group numerous times [30]. But major components of the outer kinetochore, including the Ndc80 complex (Ndc80c), Mis12c^{MIND} and the Spc105 complex (Spc105c), have been largely conserved throughout eukaryotic evolution [12,30,58].

Ndc80c, the primary microtubule-binding element of the kinetochore, is the most striking example of outer kinetochore conservation. It is present in nearly every eukaryotic genome surveyed [30], with the possible exception of kinetoplastids [28,58].

An important caveat to all of these genomic studies is that while the ‘hits’ can be informative, the ‘misses’ must be viewed sceptically. Failure to detect a homologue could indicate that it is indeed absent from the queried genome, but it could also indicate that a divergent primary sequence has allowed the homologue to escape detection. Much more persuasive are biochemically validated similarities and differences between kinetochores from different lineages.

3. Biochemical evidence

Biochemical and biophysical characterization of the kinetochore began with its microtubule-binding elements, where remarkable conservation was observed. All kinetochores that have been robustly characterized thus far couple to microtubules through a combination of Ndc80c and either Dam1c or the Ska complex [59–64], functional analogues with inversely correlated phylogenetic distributions [65]. Together, Ndc80c and either Dam1c or the Ska complex perform the most fundamental function of the kinetochore: harnessing the force of depolymerizing microtubules [60,64,66–70]. To date, every well-characterized kinetochore relies on a homologue of Ndc80c for microtubule binding (with the possible exception of kinetoplastids [28,71]).

But there are several different strategies by which the inner kinetochore may recruit Ndc80c and transmit force to the centromere. Thus far, three types of inner kinetochore have been described biochemically (figure 1). There are CENP-C-dependent inner kinetochores (e.g. *Drosophila melanogaster*), CENP-QU-dependent inner kinetochores (e.g. *S. cerevisiae*) and CENP-T-dependent inner kinetochores. This last category can be subdivided into those that contain CENP-A homologues (e.g. *Gallus gallus*) and those that do not (e.g. *Bombyx mori*). The authors do not mean to suggest that these three categories encompass all the extant diversity

Table 1. Distribution and importance (if known) of inner kinetochore proteins in organisms with relatively well-characterized kinetochores. Grey shading indicates that no homologue has yet been identified in this organism. Blue shading indicates that a homologue has been identified, but not yet been shown to be essential. Green shading indicates that a homologue is both present and essential for viability. Orange shading indicates the primary path of outer kinetochore recruitment. Yellow shading indicates a secondary path(s) of outer kinetochore recruitment. Future biochemical characterization of diverse inner kinetochores will necessitate updating this table with novel findings about each protein's homologues, essentiality and function.

CENP-	fungus		nematode	insect	mammal	bird	insect	amphibian	mammal	plant
	<i>S. cerevisiae</i>	<i>S. pombe</i>								
A	Cse4	Cnp1			CENP-A	CenpA	CID	CENP-A	Cenpa	cenH3/HTR12
C	Mif2	Cnp3	HCP-4		CENP-C	CENPC	CENP-C	CENP-C	Cenpc1	atCENP-C
H	Mcm16	Fta3		GSSPFG00011797001?	CENP-H	CENPH		CENP-H	Cenph	
I	Ctf3	Mis6		KWMTBOM002221	CENP-I	CENPI		CENP-I	Cenpi	
K	Mcm22	Sim4		LOC101741561	CENP-K	CENPK		CENP-K	Cenpk	
L	Imi3	Fta1		KWMTBOM011447	CENP-L	CENPL		CENP-L	Cenpl	
M				LOC101745870	CENP-M	CENPM		CENP-M	Cenpm	
N	Chl4	Mis15		KWMTBOM006206	CENP-N	CENPN		CENP-N	Cenpn	
O	Mcm21	Mal2		KWMTBOM014835?	CENP-O	CENPO		CENP-O	Cenpo	NP_568235.1
P	Ctf19	Fta2		KWMTBOM009290?	CENP-P	CENPP		CENP-P	Cenpp	
Q	Okp1	Fta7			CENP-Q	CENPQ			Cenpq	
U	Ame1	Mis17			CENP-U	CENPU		CENP-U	Cenpu	
R					CENP-R			CENP-R	Cenpr	
S	Mhf1	Mhf1	Y48E1C.1		CENP-S	CENPS		CENP-S	Cenps	NP_199906.1
T	Cnn1	Cnp20		CENP-T	CENPT	CENPT		CENP-T	Cenpt	
W	Wip1	Wip1			CENP-W	CENPW		CENP-W	Cenpw	
X	Mhf2	Mhf2	F35H10.5		CENP-X	CENPX		CENP-X		NP_001323102
—	Nkp1	Fta4								
—	Nkp2	Cnl2								

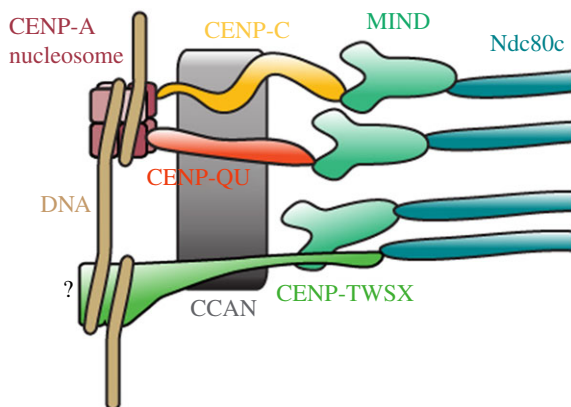


Figure 1. Three strategies of Ndc80c recruitment have been described biochemically in different organisms. Some organisms, notably ascomycetes, recruit Ndc80c primarily through CENP-QU homologues. In others, such as *D. melanogaster*, the CENP-C-based recruitment pathway dominates. Another set of organisms, including *G. gallus*, relies most heavily on CENP-TW for Ndc80c recruitment. CENP-QU and CENP-C bind centromeric nucleosomes directly and recruit Ndc80c through Mis12c^{MIND}. CENP-TW, on the other hand, can bind Ndc80c both directly and through Mis12c^{MIND}. CENP-TW is recruited to the kinetochore by upstream components of the CCAN (although it has been posited that CENP-TWSX could bind DNA directly in some organisms).

of inner kinetochore architecture, merely those that have been described biochemically to date. For instance, the divergent kinetochores of kinetoplastids likely represent still a fourth type of inner kinetochore, but a biochemical dissection of that system is still ongoing.

3.1. CENP-C-based inner kinetochores

Identifying the most critical component of the highly simplified *D. melanogaster* inner kinetochore was relatively easy—CENP-C is the only candidate. The *D. melanogaster* genome lacks homologues to all other inner kinetochore proteins except CENP-A [30,72–74]. Although several insect lineages have lost their CENP-A homologues, *D. melanogaster* is not one of them [27].

Kinetochore formation in *Drosophila* cells depends absolutely on the presence of CENP-C [75]. It has also been experimentally validated both *in vivo* and *in vitro*, that CENP-C bridges CENP-A and Mis12c^{MIND} [76–78]. Targeting the N-terminus of CENP-C to centromeres is sufficient to recruit Mis12c^{MIND}, Ndc80c and other outer kinetochore components to these ectopic loci [77]. Using both FRET- and talin-vinculin-based force sensors, it has been demonstrated that force is transmitted through CENP-C in mitotic *D. melanogaster* cells [79].

Drosophila melanogaster is not the only organism to have lost most of the CCAN. Examining the evolutionary dynamics of the kinetochore, van Hooff *et al.* [30] found that most of the CCAN evolved as an evolutionary unit that has been lost from many lineages. The exceptions were CENP-R, a recent invention in animals, CENP-S and -X, which have non-kinetochore functions in DNA repair [80,81] and CENP-C. Because CENP-C is the only widely retained CCAN component without a non-kinetochore function, the simplified, CENP-C-based CCAN may be a widespread inner kinetochore architecture. Kinetochore assembly on the holocentric chromosomes of *C. elegans* relies absolutely upon the CENP-C

homologue HCP-6; its depletion phenocopies depletion of CENP-A [82,83].

3.2. CENP-QU-based inner kinetochores

A pioneering model organism in the field of kinetochore biology, *S. cerevisiae* is also an interesting case in terms of its relationship to the theory of centromere drive. There is conflicting evidence as to whether or not budding yeast undergo asymmetrical meiotic or postmeiotic divisions [84,85], so it is unclear if budding yeast centromeres and inner kinetochores have been shaped by this type of meiotic intragenomic conflict. Centromere sequences in closely related yeast species are rapidly evolving [86–88], but it is unknown if this is the result of centromere drive.

Budding yeast have 16 inner kinetochore proteins, of which only three are essential [89]. These essential proteins are CENP-Q^{Okp1} and CENP-U^{Ame1} [90], which form a dimeric subcomplex, and CENP-C^{Mif2} [91–93]. CENP-QU^{Okp1/Ame1} and CENP-C^{Mif2} bind both DNA and Mis12c^{MIND} [94], although they cannot bind to the same Mis12c^{MIND} simultaneously [95]. It has recently been demonstrated that CENP-QU^{Okp1/Ame1} and CENP-C^{Mif2} are independently capable of transmitting mitotically relevant forces between Mis12c^{MIND} and a centromeric nucleosome [96], but while the Mis12c^{MIND}-binding residues of CENP-U^{Ame1} are essential, the Mis12c^{MIND}-binding N-terminus of CENP-C^{Mif2} is not [94]. The association of CENP-QU^{Okp1/Ame1} with centromeric nucleosomes is regulated by post-translational modifications to the N-terminus of CENP-A^{Cse4} [97]. The CENP-QU-based inner kinetochore appears to be conserved among ascomycetes, as both CENP-QU homologues Fta7 and Mis17 are essential in *Schizosaccharomyces pombe*, which is quite phylogenetically distant from *S. cerevisiae* [98,99].

The role of CENP-QU in other organisms is less clear. Human CENP-Q was reported to bind microtubules, but not Mis12c^{MIND} [100,101]. Although CENP-U is essential during mammalian embryogenesis [102], its essential function could be something other than Mis12c^{MIND} recruitment. For example, CENP-U is reported to recruit the mitotic kinase Plk1 to kinetochores [103,104], and CENP-Q is required for loading the kinesin CENP-E onto kinetochores [105].

The diverse roles of CENP-QU in different organisms reflect this subcomplex's unique evolutionary history. In many lineages, the inner kinetochore has been simplified by the loss of genes over evolutionary time [30,73]. CENP-QU is the rare exception to that rule: an inner kinetochore subcomplex essential in some organisms, yet inferred to have been absent from the last eukaryotic common ancestor [30]. CENP-QU (and inessential inner kinetochore components Nkp1 and Nkp2) likely arose through the duplication of an ancestral Mis12c^{MIND} complex [106]. Thus CENP-QU is a relatively recent addition to the inner kinetochore. Although it is essential in organisms like *S. cerevisiae*, CENP-QU has been lost recurrently throughout the fungal kingdom [29].

In budding yeast, CENP-T is an inessential protein [107–110] whose deletion causes only a mild defect in chromosome transmission fidelity [111]. Kinetochore localization of CENP-T depends on nearly every other inner kinetochore component [112,113]. Intriguingly, CENP-T becomes essential when interactions between Mis12c^{MIND} and the inner kinetochore are disrupted [112], suggesting that it might serve as a backup pathway for recruitment of the outer kinetochore in

budding yeast. This stands in sharp contrast to the essential role of CENP-T in several other organisms.

3.3. CENP-T-based inner kinetochores

The third class of inner kinetochores that has been described to date is the CENP-T-based inner kinetochore. Whereas CENP-QU and CENP-C interact with both CENP-A and DNA [94], CENP-T interacts directly with the latter, but not the former [114]. CENP-T and CENP-W form a dimer of histone fold domains, and together CENP-TWSX forms a stable tetramer that can induce supercoils into DNA through a DNA-binding surface resembling that of a canonical nucleosome [115]. There are two types of CENP-T-based inner kinetochores: those which contain CENP-A (e.g. *G. gallus*) and those that lack CENP-A (e.g. *B. mori*), perhaps having replaced it with CENP-T.

3.3.1. CENP-T-based kinetochores that contain CENP-A

Although *G. gallus* is not a traditional model organism, its kinetochore is relatively well studied, thanks in large part to work by the Fukagawa Lab. In chicken DT40 cells, both CENP-C and CENP-T can recruit the outer kinetochore to ectopic locations when artificially tethered to a LacO array [116]. But although the N-terminus of CENP-C is essential for CENP-C's interaction with Mis12c^{MIND} [116], it is not essential for viability or normal levels of Ndc80c recruitment to mitotic kinetochores [117], (just as the N-terminus of CENP-C^{Mif2} is not essential in budding yeast [94]). By contrast, the association of CENP-T with Ndc80c is essential for mitotic progression and recruitment of the outer kinetochore [117]. Using a talin sensor system, Hara *et al.* have even demonstrated that in native *G. gallus* kinetochores, pulling force from microtubules is exerted primarily on the CENP-T pathway [117]. In short, the CENP-C pathway is dispensable in *G. gallus*, while the CENP-T pathway is essential.

Intriguingly, forced binding of CENP-C to Mis12c^{MIND} (by removing the autoinhibitory basic motif of Dsn1) can rescue the growth defects observed in cells expressing a CENP-T mutant lacking its Ndc80c-binding domain [117]. This suggests that CENP-C might represent a supernumerary, backup Ndc80c recruitment pathway in the CENP-T-based inner kinetochore of *G. gallus*.

3.3.2. CENP-T-based kinetochores that lack CENP-A

Until relatively recently, the centromere-specific histone variant CENP-A was thought to be the universal epigenetic determinant of centromere identity. Even this strategy is not, however, universal. CENP-A, although inferred to be present in the last eukaryotic common ancestor [30], has been independently lost in several lineages, including certain holocentric insects [27], kinetoplastids [118] and some fungi [29].

The Drinnenberg Lab has pioneered characterization of the CENP-A-independent *B. mori* kinetochore. Like *D. melanogaster*, *B. mori* has an inner kinetochore simplified by the loss of many CCAN components, but unlike fruit flies, whose spartan inner kinetochore is based on CENP-C, the silkworm appears to lack any CENP-C homologue [73]. Indeed, proteomic analysis in cell lines from holocentric Lepidoptera identified probable homologues only to CENP-I, -K, -L, -M, -N and -T in the inner kinetochore [119]. The identity of these

potential kinetochore components was corroborated by the finding that their depletion caused severe mitotic defects. (Proteins with very remote homology to CENP-O, -P and -H were also identified, but not validated experimentally.) Curiously, the depletion of CENP-I, a protein that is not known to bind DNA in other organisms, caused the most severe phenotype, perhaps because it sits upstream of CENP-T in a tentative recruitment hierarchy [119]. Such a hierarchy would mirror the situation in budding yeast, where CENP-T^{Cnn1} is recruited to the kinetochore in a CENP-I^{Ctf3}-dependent manner [113]. In *B. mori*, as in *G. gallus* cells [120], ectopic CENP-T tethered to a LacO array was sufficient to recruit outer kinetochore components, including Ndc80c [119].

The phenomenon of kinetochores lacking CENP-A is not confined to the order Lepidoptera, nor to holocentric organisms. Navarro-Mendoza and colleagues [29] have recently described the curious case of the early-diverging fungus *Mucor circinelloides*. This opportunistic human pathogen possesses clear homologues to nearly all the kinetochore components of *S. cerevisiae*, with the notable exceptions of CENP-A, -C and -QU [29]. Homologues of CENP-T and Mis12c^{MIND} components localize to kinetochores, but none of the histone H3 variants in *M. circinelloides* exhibit similar localization patterns, indicating that none of them has replaced CENP-A [29]. As CENP-A, -C and -QU appear to be missing from the entire order Mucorales, we speculate that this clade may represent an independently evolved, monocentric lineage in which CENP-T has functionally replaced CENP-A as the foundation of the kinetochore. Further biochemical characterization of Mucorales kinetochores will be needed to support this theory.

3.4. Hybrid inner kinetochores

Where do humans fall in this classification scheme? Genetic and biochemical evidence indicates that the human inner kinetochore is a 'hybrid' of two of the types outlined above, dependent on *both* CENP-T and CENP-C (but not CENP-QU) for outer kinetochore recruitment.

Neither CENP-Q nor CENP-U is essential for viability in HeLa cells [121], although the latter *is* essential in embryonic cells [102]. These data, combined with the finding that human CENP-QU is incapable of binding Mis12c^{MIND} [101], strongly suggest that CENP-QU does not recruit the outer kinetochore in human cells.

By contrast, both the CENP-T and CENP-C genes are essential for growth and proliferation of human cancer cell lines [122], and there is excellent biochemical evidence for the dual roles of CENP-C and CENP-T. Both can recruit outer kinetochore proteins in human cells [123,124], and co-depletion of CENP-C and CENP-T in HeLa cells causes mislocalization of all other kinetochore components tested [120]. Conversely, simultaneous—but not individual—targeting of CENP-C and CENP-T to ectopic foci is sufficient to recruit outer kinetochore components at levels stoichiometrically proportional to the amount of CENP-C and -T present at the foci relative to kinetochores [124]. These foci are then able to interact with microtubules and perturb the segregation of chromosomes that also contain an endogenous kinetochore [124]. In short, *both* CENP-C and CENP-T are required for outer kinetochore assembly in human cells.

Intriguingly, using super-resolution fluorescence microscopy, Suzuki *et al.* [125] found that the mean position of the N-terminus of CENP-C in HeLa cells was surprisingly

distant from Mis12c^{MIND}, leading the authors to conclude that only a small minority of CENP-C binds Mis12^{MIND}. Depletion of CENP-C increases intrakinetochore stretch of CENP-T [125], suggesting that the latter protein might shoulder more responsibility for force transmission when the CENP-C pathway is compromised.

Xenopus laevis is a model organism that also seems to rely on both CENP-C and CENP-T for Ndc80c recruitment. Immunodepletion of CENP-C from egg extracts prevents kinetochore formation on sperm chromatin [126]. *X. laevis* CENP-C binds directly to CENP-A nucleosomes and mediates Mis12^{MIND} recruitment to kinetochore [126,127]. These centromeres lack a CENP-Q homologue [30], but the CENP-T pathway also clearly contributes to outer kinetochore recruitment in *X. laevis*. Although depletion of CENP-C from *Xenopus* cell-free extracts reduces the kinetochore localization of CENP-TW, both Mis12c^{MIND} and Ndc80c are still recruited; importantly, this is not the case when CENP-T and CENP-C are co-depleted [128]. Although both pathways contribute to kinetochore recruitment, it could be argued that the contribution of CENP-C to kinetochore localization of CENP-TW indicates the primary importance of the former pathway.

As novel kinetochores are characterized at the biochemical level, it seems likely that other 'hybrid' inner kinetochores will be discovered.

4. Conclusion

Though only a few model organisms' kinetochores have been biochemically characterized, at least three distinct strategies for bridging the centromere and outer kinetochore have emerged. *S. cerevisiae* relies primarily on CENP-QU^{Okp1/Ame1}; *D. melanogaster* relies primarily on CENP-C; some organisms, including *G. gallus*, rely primarily on CENP-T. Intriguingly, there are now examples of multiple lineages that lack a canonical centromeric histone variant in which CENP-T may have taken its place as the foundation of the kinetochore. Compared with the near ubiquity of Ndc80c as the major microtubule coupler in every kinetochore that has been characterized

biochemically, the diversity of these stratagems underscores an intriguing contrast between the evolutionarily labile inner kinetochore and highly conserved outer kinetochore.

Why such a diversity of inner kinetochore architectures? We hypothesize that it may be a consequence of centromere drive. Perhaps diverse inner kinetochore architectures have arisen over evolutionary time because inner kinetochore proteins must coevolve with rapidly changing centromeric DNA in order to retain their essential functions. This could even be framed as a corollary of the centromere drive theory: inner kinetochores, which mediate interaction with rapidly evolving centromeric DNA, should vary more in their composition and organization than outer kinetochores, which mediate interaction with microtubules, highly conserved elements of the cytoskeleton.

As more kinetochores are characterized biochemically, we predict that a still broader diversity of inner kinetochore architectures will be revealed. We are especially curious about the kinetochores of kinetoplastids, which lack known homologues to any CCAN proteins, and may also represent an exception to the universality of outer kinetochore architecture [28,71,129].

We also look forward to the characterization of more inner kinetochores from holocentric organisms. Holocentricity has been proposed as an adaptive mechanism to overcome the fitness costs of meiotic drive. It will therefore be of great interest whether or not the breakneck speed of inner kinetochore diversification has slowed in such species after the transition to holocentricity.

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Endnote

¹The exceptions are certain insects [27], kinetoplastids [28] and early-diverging fungi [29,30].

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