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"Holo"er than thou: Chromosome segregation and kinetochore function in *C. elegans*

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Abstract

Kinetochores are proteinaceous organelles that assemble on centromeric DNA to direct chromosome segregation in all eukaryotes. While many aspects of kinetochore function are conserved, the nature of the chromosomal domain upon which kinetochores assemble varies dramatically between different species. In monocentric eukaryotes, kinetochores assemble on a localized region of each chromosome. In contrast, holocentric species such as the nematode *Caenorhabditis elegans* have diffuse kinetochores that form along the entire length of their chromosomes. Here, we discuss the nature of chromosome segregation in *C. elegans*. In addition to reviewing what is known about kinetochore function, chromosome structure, and chromosome movement, we consider the consequences of the specialized holocentric architecture on chromosome segregation.

The diffuse kinetochore of holocentric organisms

The centromere of eukaryotic chromosomes was first defined as the site of the primary constriction of a chromosome that was visible by light microscopy (Flemming 1880). Following molecular and cytological analyses of these regions, the centromere is now defined as the chromosomal domain that directs the formation of a kinetochore, a proteinaceous organelle that functions as the primary chromosomal attachment site for spindle microtubules. However, in a large number of eukaryotes, a primary constriction is not observed on chromosomes, and spindle microtubules attach along the entire length of the chromosome. Such chromosomes are referred to as holocentric or holokinetic, and the kinetochores are described as diffuse. Holocentricity appears to have arisen multiple times during evolution since it is present in independent eukaryotic lineages including all nematode species, as well as insects and diverse plants (Pimpinelli & Goday 1989). The existence of related plant species with either holocentric or monocentric chromosome architectures (Pazy & Plitmann 2002) offers the possibility for investigating the evolution of holocentricity, but at present there is little information on why these two distinct chromosome architectures are prevalent in the eukaryotic kingdom.

One potential advantage associated with a holocentric chromosome architecture is related to double-strand DNA breaks. In monocentric organisms, unrepaired double-stranded breaks result in acentric DNA fragments that cannot be maintained by a cell due to their inability to attach to spindle microtubules. In contrast, since holocentric chromosomes have microtubule attachments sites

along their entire length, chromosome fragments formed from breakage events have a higher probability of being stably maintained. As noted above, all nematode species examined to date contain holocentric chromosomes (Pimpinelli & Goday 1989). Nematode development is typically characterized by a fixed lineage, where a single inappropriate cell death can have severe consequences. Therefore, it has been suggested that holocentricity in nematodes helps avoid the disastrous consequences of unrepaired chromosome breakage events (Pimpinelli & Goday 1989). However, the wide prevalence of monocentric organisms suggests that the advantages gained with a holocentric chromosome architecture are counterbalanced by disadvantages, possibly including complications related to the segregation of recombined meiotic chromosomes (see below).

The nature of centromeric DNA varies dramatically between species, even in organisms with localized centromeres. In budding yeast, the centromere is a short sequence that is highly conserved between the different chromosomes (McAinsh *et al.* 2003). In humans, there is not a strict dependence on a specific DNA sequence. However, centromeric chromatin is distinct from bulk chromatin in that it typically contains numerous α-satellite repeats (Sullivan *et al.* 2001). The only holocentric chromosomes that have been characterized in molecular detail are those of the nematode *Caenorhabditis elegans*, a popular experimental organism. In *C. elegans*, specific DNA sequences that direct kinetochore assembly have not been identified. Strikingly, diffuse kinetochores in *C. elegans* can assemble on and direct the segregation of extrachromosomal arrays formed from prokaryotic DNA (Dernburg 2001, Howe *et al.* 2001). This observation suggests that specific DNA sequences are not required for the assembly of a functional kinetochore. However, it remains possible that degenerate repetitive sequences present throughout *C. elegans* chromosomes contribute to directing the formation of kinetochores.

The ultrastructure of diffuse kinetochores

While the unique chromosome architecture of holocentric organisms suggests that their diffuse kinetochores would be very different from the localized kinetochores of monocentric species, ultrastructural studies have demonstrated a high degree of similarity. In holocentric species, kinetochores form paired lines or plates on opposite faces of condensed mitotic chromosomes. Each line represents the diffuse kinetochore of a single chromatid. Transmission electron microscopy of *C. elegans* chromosomes (Albertson & Thomson 1982) and of holocentric chromosomes from the hemipteran insect *Oncopeltus faciatus* (Comings & Okada 1972) fixed using glutaraldehyde revealed the presence of an electron-dense plaque extending along the entire poleward face of each chromatid. These plaques have a trilaminar structure with electron-dense inner and outer layers that are both ~20 nm thick separated by a ~30-nm-wide low-electron-density region. The chromatin directly underlying the kinetochore is more electron dense than the rest of the chromosome. This structure is very similar to the classical trilaminar kinetochore structure defined by electron microscopy following chemical fixation of monocentric organisms (Comings & Okada 1971, Rieder 1982). Recent analysis of kinetochore ultrastructure in holocentric and monocentric organisms using high-pressure freezing and freeze substitution, which preserve structures better than chemical fixation, further supports their similarity. Using this method, instead of a trilaminar structure, a clear zone of ~150 nm which excludes ribosomes and other cytoplasmic components is observed next to centromeric chromatin (McEwen *et al.* 1998, Howe *et al.* 2001, O'Toole *et al.* 2003). Taken together, the EM studies suggest that, despite the apparent differences in chromosomal architecture, kinetochores are structurally very similar between holocentric and monocentric organisms.

Kinetochore specification in *C. elegans*

Given the remarkable diversity of centromere sequences between species, a major unanswered question is how a specific chromosomal region directs kinetochore assembly. Interestingly, while the nature of centromeric DNA varies dramatically, many kinetochore proteins are conserved.

The kinetochores of all eukaryotes examined to date are built upon centromeric chromatin containing specialized nucleosomes with the histone H3 variant CENP-A. CENP-A containing chromatin provides the structural foundation of the kinetochore and is required for targeting of all other tested kinetochore proteins (Howman *et al.* 2000, Oegema *et al.* 2001, Van Hooser *et al.* 2001). As expected from the holocentric nature of their chromosomes, the *C. elegans* CENP-A homologue HCP-3 localizes along the length of mitotic chromosomes and is visible as two paired lines on opposite faces of these chromosomes during prometaphase and metaphase (Figure 1 A; Buchwitz *et al.* 1999).

During mitosis in *C. elegans*, CENP-A nucleosomes are not present in the bulk of the chromatin and are restricted to the poleward chromosomal faces (Figure 1). Therefore, CENP-A^{HCP-3} nucleosomes must be interspersed with histone H3 nucleosomes along linear DNA (Figure 1B). The same is also true in monocentric organisms. Artificial stretching of *Drosophila* chromosomes demonstrated that regions of DNA containing CENP-A nucleosomes are separated by intervening regions containing histone H3 nucleosomes (Blower *et al.* 2002). Upon entry into mitosis, formation of higher-order chromosome structure must generate distinct domains in which CENP-A nucleosomes predominate. Therefore, monocentric and holocentric chromosomes must both organize CENP-A containing chromatin so that it is presented as a unit, while packaging histone H3 chromatin into the inner-centromeric region between the outward-facing sister kinetochores (Figure 1B).

While the central role of CENP-A in kinetochore specification is clear in multiple organisms, the molecular machinery that selectively incorporates CENP-A instead of histone H3 has not been identified. Currently, little is known about the regulation of CENP-A deposition in either holocentric or monocentric organisms. One possibility is that CENP-A deposition is directed by pre-existing CENP-A nucleosomes and never occurs *de novo*. In this model, random distribution of nucleosomes during DNA replication would partition existing CENP-A nucleosomes to the two sister chromatids. This inherited CENP-A nucleosome population would then direct deposition of new CENP-A nucleosomes in its proximity (Sullivan 2001). However, preliminary data suggests that CENP-A is absent from *C. elegans* gametes (our unpublished observations), so a newly fertilized zygote must have additional mechanisms for

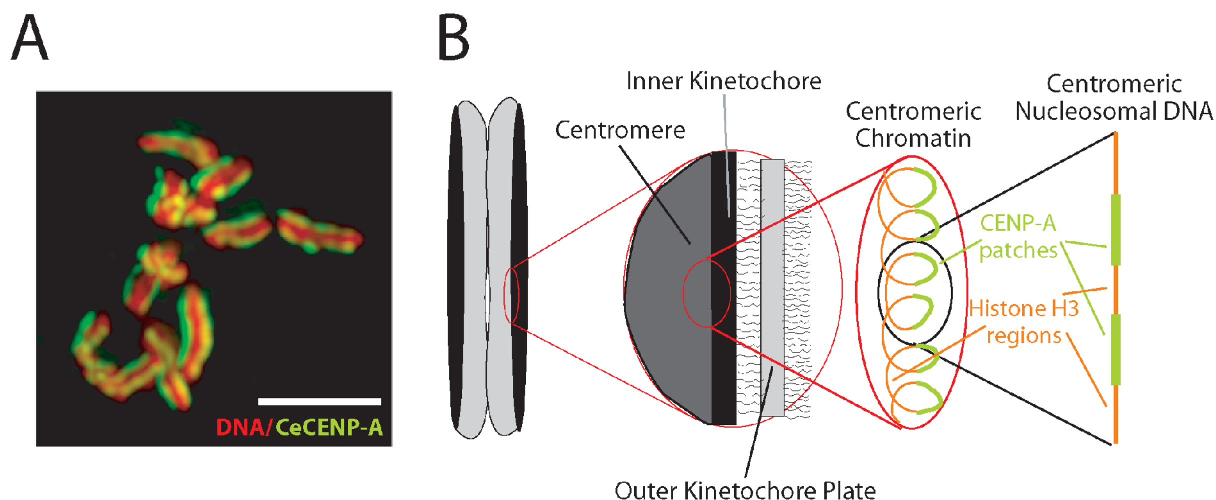


Figure 1. CENP-A is an essential determinant of kinetochore specification. (**A**) CENP-A^{HCP-3} localizes to the diffuse *C. elegans* kinetochores. Immunofluoresence showing CENP-A staining in yellow and DNA in red. Scale bar is 5 μm. (**B**) Model for formation of physically juxtaposed CENP-A-containing chromatin domains on a condensed chromatid. Interspersed regions of histone H3-containing nucleosomes and CENP-A-containing nucleosomes are organized such that CENP-A-containing domains face outward and direct formation of the outer domains of the kinetochore, while histone H3 domains are internal and constitute the bulk of chromosomal chromatin.

regulating CENP-A deposition. Alternatively, CENP-A may incorporate randomly throughout the chromosome, and then patches of CENP-A could self associate to form the specialized chromatin that is the basis for the diffuse kinetochores. To distinguish between these models or identify additional possibilities, functional studies of CENP-A deposition will be required. Genome-wide screens for factors that affect CENP-A distribution should yield new insights into how kinetochores are specified by formation of CENP-A containing chromatin.

Using *C. elegans* as a metazoan model to study kinetochore assembly and function

Kinetochores play an essential role during every cell division, which has considerably limited their molecular analysis in metazoans. In *C. elegans*, it is possible to analyse the function of essential gene products in single cells, making it uniquely suited for studying kinetochores. The ability to generate newly fertilized *C. elegans* embryos depleted of a targeted gene product results from a combination of RNA interference (RNAi) and the architecture of the syncitial gonad. Introduction of double-stranded RNA efficiently prevents expression of a specific gene by targeting its mRNA for degradation. Continued formation of new oocytes facilitates the removal of the pre-existing gene product present in the gonad. Within 36–48 h after introduction of the double-stranded RNA, newly formed oocytes are completely (>95%) depleted of the targeted gene product. Fertilization of these oocytes results in embryos that initiate the first mitosis in the absence of the target protein. Therefore, by analysing this first cell division, it is possible to determine the consequences of specifically removing a protein on chromosome segregation. Consistent with a fundamental role for CENP-A in kinetochore function, depletion of CENP-A$^{HCP\text{-}3}$ by RNAi results in catastrophic defects in mitotic chromosome segregation (Oegema *et al.* 2001, Desai *et al.* 2003).

To exploit the above technical paradigm for analysing kinetochore function, three different assays have been developed in the *C. elegans* embryo (Figure 2; Oegema *et al.* 2001, Desai *et al.* 2003). First, time lapse imaging of embryos stably expressing GFP-histone H2B (to mark the chromosomes; Figure 2 A, arrow head) and GFP-γ-tubulin (to mark spindle poles; Figure 2 A, arrows) provides a powerful visual assay for chromosome segregation. In CENP-A$^{HCP\text{-}3}$ depleted embryos, following nuclear envelope breakdown (NEBD), the DNA from the oocyte and sperm pronuclei remains as two distinct chromatin masses (Figure 2 A). Chromosomes never align at a metaphase plate, and no anaphase chromosome segregation is observed. Second, it is possible to monitor the mechanical strength of the kinetochore–microtubule interface by measuring the kinetics of spindle pole separation. In wild-type embryos, strong astral forces act to position the spindle poles in the one cell embryo (Figure 2B; Grill *et al.* 2001). These forces are resisted by bipolar kinetochore–microtubule attachments to paired sister chromatids, generating tension in the spindle (Figure 2B). If kinetochore–microtubule attachments are disrupted (such as in depletion of CENP-A$^{HCP\text{-}3}$), the spindle is unable to resist the astral forces resulting in rapid spindle pole separation (Figure 2B). Finally, examining the localization of other known kinetochore proteins when one kinetochore protein is depleted provides a readout for effects on kinetochore assembly. Work thus far has demonstrated mostly linear dependency relationships between pairs of kinetochore proteins. For example, depletion of CENP-A$^{HCP\text{-}3}$ results in the inability of all known kinetochore proteins to localize to chromosomes (Figure 2 C). In contrast, depletion of other kinetochore proteins, such as the conserved kinetochore structural protein CENP-C$^{HCP\text{-}4}$, does not affect CENP-A$^{HCP\text{-}3}$ localization (Figure 2 C). In all three of these assays, analysis of CENP-A$^{HCP\text{-}3}$ depleted embryos suggests that there is a failure in the formation of a functional kinetochore, indicating that depletion of CENP-A results in a 'kinetochore null' phenotype (KNL; Figure 2B).

Beyond CENP-A: molecular composition of the *C. elegans* kinetochore

In addition to CENP-A$^{HCP\text{-}3}$, more than 30 different *C. elegans* kinetochore proteins have

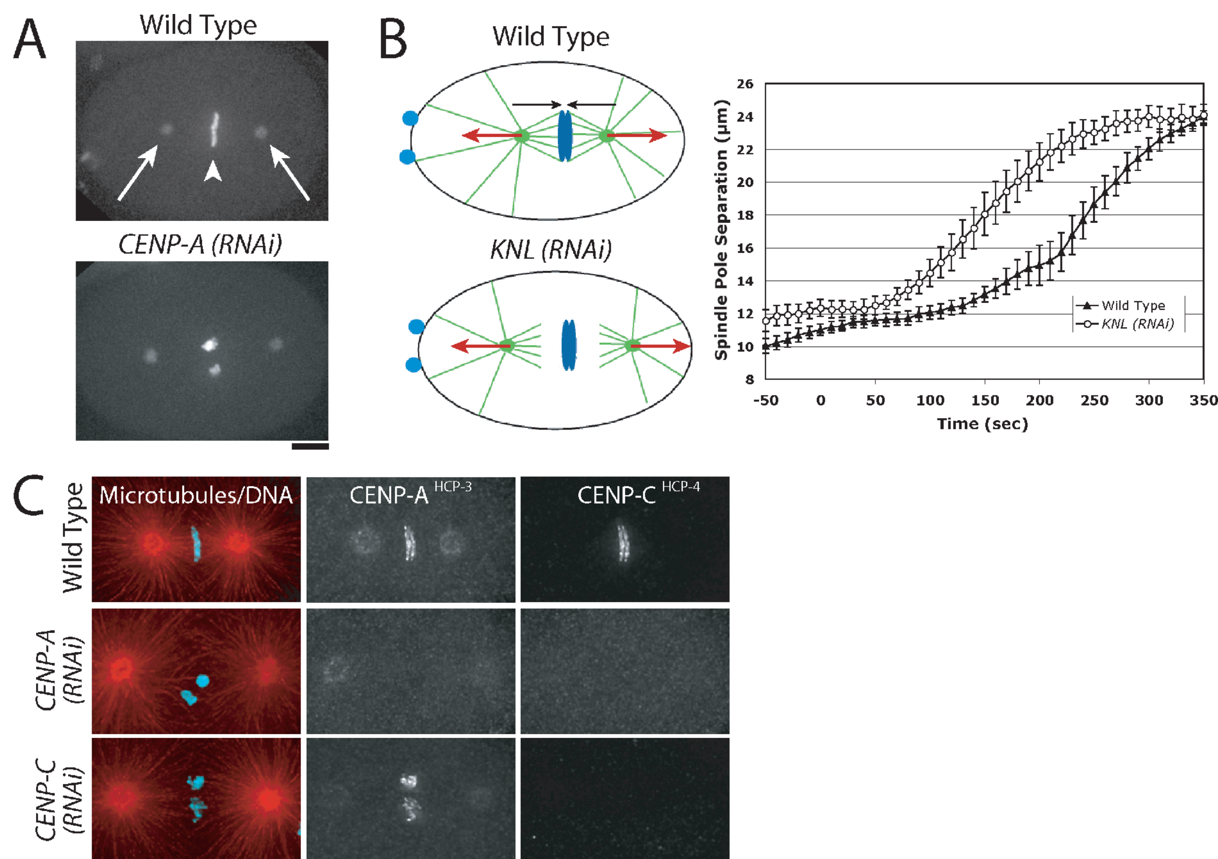


Figure 2. Assays for studying kinetochore function in *C. elegans*. (**A**) Time lapse imaging of the first mitotic cell division provides a qualitative analysis of chromosome segregation defects. Embryos expressing GFP-gamma-tubulin (arrows) and GFP-histone H2B (arrow head) allows the visualization of chromosome movements with respect to spindle poles. Depletion of CENP-A$^{HCP\text{-}3}$ results in a failure to distribute chromosomes to form a metaphase plate and no anaphase chromosome segregation (Oegema *et al.* 2001). Both embryos are from identical time points after nuclear envelope breakdown (NEBD). (**B**) Astral forces in the *C. elegans* embryo provide an indirect readout for the mechanical stability of the kinetochore–microtubule interface. *Left*, diagram showing the outward forces on spindle poles. In wild type, these forces are resisted by bipolar attachments of paired sister chromatids. When kinetochore function is disrupted, there is no resistance to these forces and spindle poles separate prematurely. *Right*, graph showing spindle pole separation in wild-type embryos and embryos in which kinetochore function is severely impaired. (**C**) Pair-wise depletion/targeting analysis provides insight into the mechanism of kinetochore assembly. Immunofluoresence showing microtubule, DNA, CENP-A$^{HCP\text{-}3}$, and CENP-C$^{HCP\text{-}4}$ localization in wild type, CENP-A-depleted embryos, and CENP-C-depleted embryos. The results clearly indicate that CENP-A$^{HCP\text{-}3}$ is required for CENP-C$^{HCP\text{-}4}$ localization, but not *vice versa*. Scale bars are 10 µm.

been identified to date (Figure 3). As expected, many of these proteins are visibly conserved throughout eukaryotes. Approximately half of these proteins were first identified on the basis of their homology with kinetochore proteins found in other organisms (Buchwitz *et al.* 1999, Moore & Roth 2001, Oegema *et al.* 2001, Scaerou *et al.* 2001, Desai *et al.* 2003). Additional *C. elegans* kinetochore proteins have been identified using classical genetic screens for chromosome segregation defects (Howe *et al.* 2001), large-scale loss of function screening using RNAi (Gonczy *et al.* 2000, Desai *et al.* 2003), biochemical purification of native kinetochore complexes (our unpublished results), and antibody screening to identify proteins which localize to the diffuse kinetochores (Moore *et al.* 1999).

In addition to CENP-A$^{HCP\text{-}3}$, two other proteins have been identified which exhibit a KNL

Centromeric Chromatin

Centromeric Nucleosome		
HCP-3	Cenp-A	2, 10
	Histone H4	
	Histone H2A	
	Histone H2B	
HCP-4	Cenp-C	8, 10, 14

Regulatory Factors

Spindle Assembly Checkpoint		
MDF-1	Mad1	3
MDF-2	Mad2	3, 16
SAN-1	Mad3/BubR1	16
BUB-1	Bub1	10, 14
BUB-3	Bub3	
Aurora B Complex		15
AIR-2	Aurora B	5, 6, 7
ICP-1	INCENP	6, 10
BIR-1	Survivin	7
CSC-1		15

Outer Kinetochore

KNL-1	AF15q14	14
MIS-12	hMis12	17
NDC-80	Ndc80/HEC	14
HIM-10	hNuf2	9, 14
HCP-1/HCP-2	CENP-F ??	4, 14
CLS-2	CLASP2/Orbit	14
KLP-7	MCAK kinesin	10
CZW-1	ZW10	1, 11
F55G1.4	ROD	11

Cohesin Complex		18
SCC-1	Scc1/Rad21	20
SCC-3	Scc3	19, 21
SMC-1	Smc1	
SMC-3	Smc3	
TIM-1	Timeless	18

Condensin Complex		22
MIX-1	Smc2/XCAP-E	13
SMC-4	Smc4/XCAP-C	13
HCP-6	XCAP-D3	12
F55C5.4	XCAP-G2	
C29E4.2	Barren/XCAP-H2	

1. Starr et al., 1997
2. Buchwitz et al., 1999
3. Kitagawa and Rose, 1999
4. Moore et al., 1999
5. Hsu et al, 2000
6. Kaitna et al, 2000
7. Speliotes et al., 2000
8. Moore and Roth, 2001
9. Howe et al., 2001
10. Oegema et al., 2001
11. Scaerou et al., 2001
12. Stear and Roth, 2002
13. Hagstrom et al., 2002
14. Desai et al., 2003
15. Romano et al., 2003
16. Nystul et al., 2003
17. Goshima et al., 2003
18. Chan et al., 2003
19. Pasierbek et al., 2003
20. Mito et al, 2003
21. Wang et al., 2003
22. Ono et al., 2003

Figure 3. Proteins required for chromosome segregation in *C. elegans*. *C. elegans* proteins and their corresponding homologues are shown for each functional category. Numbers indicate a reference for the most recent molecular characterization of that protein. References not present in text: Hsu *et al.* 2000, Kaitna *et al.* 2000, Speliotes *et al.* 2000, Goshima *et al.* 2003, Pasierbek *et al.* 2003, Wang *et al.* 2003.

phenotype. These include CENP-C$^{HCP\text{-}4}$, a highly conserved structural component of the inner kinetochore (Moore & Roth 2001, Oegema *et al.* 2001), and KNL-1, which provides a scaffold for the assembly of the outer domains of the kinetochore (Desai *et al.* 2003). Analysis of the functional relationships between these proteins indicates that CENP-A$^{HCP\text{-}3}$ and CENP-C$^{HCP\text{-}4}$ act to establish the specialized chromatin domains of the kinetochore. CENP-C$^{HCP\text{-}4}$ in turn interacts with KNL-1 which assembles the outer domains of the kinetochore. These outer domains are composed of a variety of additional proteins that contribute to forming the interface with spindle microtubules (Moore *et al.* 1999, Howe *et al.* 2001, Oegema *et al.* 2001, Desai *et al.* 2003).

Components of the mitotic checkpoint (also referred to as the spindle assembly checkpoint) also localize to *C. elegans* kinetochores. These proteins were first identified in budding yeast based on their ability to arrest cell-cycle progression in the presence of defective kinetochore–microtubule attachments (reviewed in Cleveland *et al.* 2003). In yeast, these proteins primarily play a watchdog role and are not essential for mitotic progression. In vertebrate somatic cells, these proteins play an essential role in ensuring the proper timing of every mitotic division. In contrast, components of the mitotic checkpoint do not appear to play either an essential or watchdog role during mitosis in the early *C. elegans* embryo. Disruption of kinetochore–microtubule attachments does not result in a significant delay in cell cycle timing during the early cell divisions (Desai *et al.* 2003). However microtubule depolymerization does cause a checkpoint-dependent mitotic arrest in the somatic *C. elegans* gonad (Kitagawa & Rose 1999). In addition, the mitotic checkpoint proteins Mad1$^{MDF\text{-}1}$ and Mad2$^{MDF\text{-}2}$ are essential to maintain viable and fertile *C. elegans* strains (Kitagawa & Rose 1999). In the absence of oxygen, *C. elegans* embryos exhibit a remarkable process of suspended animation in which development arrests until oxygen is once again available. This process requires Mad3$^{SAN\text{-}1}$ and Mad2$^{MDF\text{-}2}$, suggesting that an oxygen-sensing pathway triggers a checkpoint-mediated mitotic arrest (Nystul *et al.* 2003). In addition, several components of the mitotic checkpoint have an essential role in kinetochore function. Depletion of BUB-1, ZW10$^{CZW\text{-}1}$, or ROD results in embryonic lethality (Starr *et al.* 1997, Scaerou *et al.* 2001,

Desai *et al.* 2003), suggesting that these proteins play essential roles at kinetochores independently of their checkpoint function.

Contribution of *C. elegans* chromosome structure to mitotic segregation

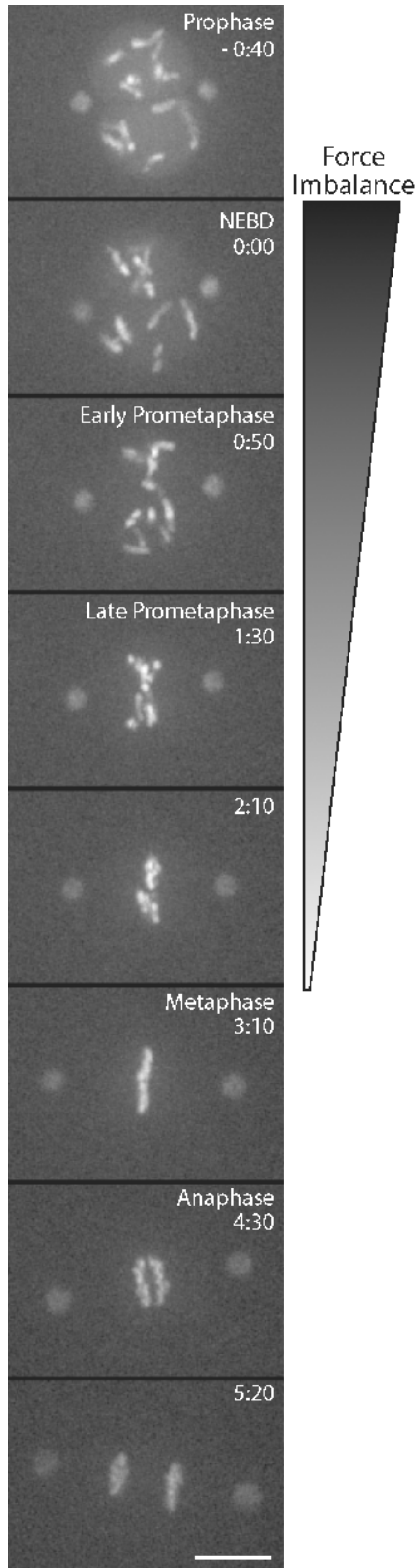
In interphase, chromosomal DNA is dispersed throughout the nucleoplasm. For mitotic segregation to occur, chromosomal DNA must be highly compacted into discrete units that can be physically separated within the spatial confines of a cell. Upon entry into mitosis, chromosomes undergo structural changes to become more compact and structurally rigid (Marshall *et al.* 1997, Swedlow & Hirano 2003). In addition, sister chromatids must be held together to prevent premature segregation and ensure equal distribution of the genome in anaphase. The large target area for microtubule attachment on holocentric chromosomes creates an important requirement for condensation and cohesion to ensure proper bipolar attachment of sister chromatids.

Chromosome compaction during mitosis requires the function of the condensin complex, a set of highly conserved proteins found in all eukaryotes. In vertebrates, the condensin complex is divided into two functional types, termed condensin I and II (Ono *et al.* 2003). Both forms of condensin share two invariant subunits (SMC-4 and SMC-2) but have divergent forms of the remaining components. The different constituents of condensin I and II allow the two complexes to function in complementary chromosome condensation roles. Chromosomes lacking either condensin I or II display distinct morphologies (Ono *et al.* 2003). The absence of condensin I results in chromosomes that appear swollen and poorly condensed. In contrast, in the absence of condensin II, chromosomes appear curly but generally condensed. These observations led to the hypothesis that condensin I facilitates general condensation of chromatin, while condensin II creates a rigid chromosome structure.

Examination of the *C. elegans* genome revealed that only components of condesin II complex are present (Ono *et al.* 2003), suggesting that *C. elegans* chromosomes are more rigid than chromosomes of other species. While the stiffness of *C. elegans* chromosomes has not been measured directly, several pieces of evidence are consistent with this expectation. In monocentric organisms, forces generated by kinetochore microtubules stretch the chromosomal region between sister kinetochores to over double its rest length (Maddox *et al.* 2003, Waters *et al.* 1998). In contrast, observation of holocentric chromosomes during mitosis shows that sister kinetochores are separated by a constant distance along the length of chromosomes and that this distance does not dramatically increase when bipolar attachments are formed (our unpublished observations; Desai *et al.* 2003). In addition, twisting of chromosomes (as observed by kinetochore staining in fixed embryos) is rare, suggesting the presence of a stiff chromosome architecture (Stear & Roth 2002). Finally, chromosomes congress in prometaphase as bars and do not appear to move in a manner that would imply fluidity along the length of the chromosome (see Figure 4).

Depletion of condesin components in *C. elegans* results in both meiotic and mitotic defects. Depletion of SMC-4 or CAP-D3$^{HCP\text{-}6}$ results in connections between separating chromatin masses (termed chromatin bridges) in meiosis and mitosis (Hagstrom *et al.* 2002, Stear & Roth 2002). The meiotic defects are probably due to a number of problems, beginning with a failure to form proper synaptonemal complexes and culminating with incomplete segregation of the meiotic chromatin. The mitotic defects are related to chromosome twisting which results in attachment of one kinetochore to both spindle poles (termed merotelic attachment). Interestingly, depletion of the *C. elegans* condensin complex does not completely abolish chromosome condensation suggesting the existence of an alternate pathway for chromosome condensation (Hagstrom *et al.* 2002).

Sister chromatid cohesion is accomplished by a separate complex of conserved proteins, termed cohesin, that is comprised of the core subunits Scc3, Smc1, Smc3 and Scc1. *C. elegans* has a single homologue of each of the first three components, but four homologues of Scc1 (COH-1, SCC-1, COH-3 and REC-8) which appear to function in distinct aspects of sister chromatid cohesion. For example, REC-8 appears to function specifically



during meiosis (Pasierbek *et al.* 2001). Loss of cohesin function results in premature segregation and aneuploidy. Depletion of SCC-3, SMC-1, SMC-3 or SCC-1 by RNAi produces mitotic figures with chromatin bridges as well as abnormally shaped interphase nuclei in multicellular embryos (Mito *et al.* 2003). Recent biochemical investigation of *C. elegans* cohesin has identified TIM-1 (homologue of Drosophila Timeless; a circadian rhythm regulator) as an unexpected player in cohesion. Depletion of TIM-1 results in similar phenotypes to those listed above for conserved cohesion complex subunits; however the precise role of TIM-1 is as yet unknown (Chan *et al.* 2003).

The combined activities of cohesin and condensin function to facilitate accurate chromosome segregation and prevent merotelic attachments. Merotelic attachments present an especially complex problem in holocentric species where the large surface area of the diffuse kinetochores increases the likelihood of a single kinetochore forming attachments to microtubules emanating from opposite spindle poles. To prevent these incorrect attachments, stiff chromosomes (from the condensin II complex) are held back to back (by the cohesin complex), restricting the attachment of a given kinetochore to a single spindle pole and ensuring that the sister kinetochore attaches to the opposite spindle pole. However, in addition to chromosomal architecture, regulatory mechanisms function to ensure the formation of bipolar attachments during mitosis. A conserved complex, which in *C. elegans* consists of the Aurora B kinase AIR-2, INCENP$^{ICP\text{-}1}$, Survivin$^{BIR\text{-}1}$, and an additional subunit called CSC-1 (Romano *et al.* 2003), has been shown to function in other eukaryotes to inactivate kinetochore–microtubule attachments until bi-orientation is achieved (reviewed in Cheeseman & Desai 2004). The high fidelity of chromosome segregation in *C. elegans*,

Figure 4. Chromosome movements during mitosis in *C. elegans*. Images from a time lapse movie of the first mitosis in an embryo expressing GFP-gamma-tubulin and GFP-histone H2B. Following nuclear envelope breakdown (NEBD), prometaphase chromosomes movements are rapid. As mitosis progresses, these movements (and the corresponding imbalance in forces that gives rise to them) decrease until the chromosomes are aligned at the metaphase plate. During anaphase, no chromosome to pole movement (anaphase A) is observed. Scale bar is 5 μm.

despite the holocentric chromosome architecture, makes it likely that future studies will provide insights into how merotelic attachments are avoided/corrected to prevent aneuploidy.

Holocentric chromosome dynamics

The presence of a diffuse kinetochore along the entire length of each chromosome has important implications for the mechanics of chromosome segregation in holocentric species. In addition to facilitating the attachment of chromosomes to spindle microtubules, kinetochores play an essential role in translating this attachment into chromosome movement. In particular, kinetochores must function to: (a) facilitate chromosome congression in prometaphase, (b) generate tension on paired sister chromatids during metaphase to satisfy the spindle assembly checkpoint and ensure bi-orientation, and (c) segregate sister chromatids to opposite poles in anaphase.

Sister kinetochores of holocentric chromosomes must act in a co-ordinated fashion to accomplish congression, as is also the case for monocentric chromosomes. During prometaphase, kinetochores attach end-on to dynamic microtubule plus ends. As more microtubules attach, differential movement of sister kinetochores results in congression to the metaphase plate (reviewed in Rieder & Salmon 1998). Net force imbalances on the sister kinetochores are necessary to lead to directed movements of the chromosome (Skibbens *et al.* 1995). In *C. elegans*, prometaphase chromosome movements are very rapid (Figure 4; time from nuclear envelope breakdown to metaphase is $\sim$2 min). During congression, one end of a chromosome is often observed leading the rest of the chromosome. However, different ends of a single holocentric chromosome have never been observed moving towards opposite poles, indicating a high level of co-ordination (our unpublished observations). This observation suggests that forces acting on one end of a diffuse kinetochore on a holocentric chromosome are either physically or biochemically transmitted to the other end, ensuring that all microtubule attachment sites on a single kinetochore exhibit the same behaviour. Likewise, sister kinetochore movements are highly co-ordinated. Failure of a kinetochore to sense and respond to changes in the movement of its sister kinetochore would prevent congression and also result in improper stretching and ultimately chromosome breakage.

The observations above indicate that holocentric kinetochore dynamics in prometaphase are fundamentally similar to those of monocentric kinetochores. In contrast, metaphase chromosome behaviour in *C. elegans* appears different from that observed in vertebrates. As *C. elegans* chromosomes line up at the metaphase plate, oscillation frequency and amplitude reduce (Figure 4). Interestingly, after all chromosomes achieve metaphase alignment, oscillations cease completely, resulting in a very tight plate (Figure 4). This contrasts with observations in mammalian tissue culture cells, where metaphase chromosome oscillations are common (Skibbens *et al.* 1995). In other experimental systems including *Xenopus* extracts (Maddox *et al.* 2003), metaphase oscillations are not observed. However, prometaphase congression in *Xenopus* extracts is relatively slow in contrast to the very rapid congression seen in *C. elegans*. The absence of metaphase oscillations could either indicate that the forces on chromosomes are carefully balanced at metaphase or that a change in forces occurs after chromosomes become aligned. In vertebrates, forces on chromosomes are based on interactions with microtubules both at kinetochores and along the chromosome arms. Kinetochore-based forces provide directional motility, while arm forces are generally thought to produce a repulsive force from the spindle poles (ejection force; reviewed in Rieder & Salmon 1998). It is not known how the ejection force mechanism would work in holocentric organisms, where chromosomal arms do not exist. It is possible that ejection forces are very minor in *C. elegans*. Alternatively, a cell cycle change may regulate mitotic forces on chromosomes. For example, a biochemical switch could reduce or turn off microtubule-generated forces at kinetochores when the full complement of microtubules is achieved.

While a regulated change in kinetochore forces has not been identified in other organisms, such a hypothesis is supported by observations of chromosome dynamics during anaphase in *C. elegans*. At anaphase onset, sister chromosomes separate and move away from each other at a high rate.

This rate is identical to the rate of spindle pole separation (anaphase B). In fact, chromosome to pole movement (anaphase A) is not observed in *C. elegans* early embryos (Oegema *et al.* 2001). To our knowledge, this is the only example of cell division in which anaphase A does not occur. Thus, studies of chromosome dynamics in *C. elegans* could reveal an unidentified biochemical switch regulating force production by kinetochores.

Holocentric chromosomes in meiosis

As discussed above, the holocentric nature of *C. elegans* chromosomes results in unique mitotic behaviour. However, during meiosis, chromosomes face a different set of challenges. In particular, during meiosis I, both sister chromatids on a single chromosome must be segregated to the same spindle pole. While the stiff rod-like mitotic chromosomes force sister kinetochores to face opposite directions and help prevent merotely during mitosis, this orientation of sister kinetochores would severely disrupt meiosis I chromosome segregation. In addition, homologous chromosomes are held together by recombination during meiosis I. In *C. elegans*, a single meiotic cross-over event occurs between chromatids of homologous chromosomes (Hillers & Villeneuve 2003). Thus, DNA flanking this recombination event that was originally from a single chromatid must now move to opposite poles (see Figure 5). This would not be possible if kinetochore–microtubule attachments were present along the entire chromatid length, as is the case during mitosis. Therefore, holocentric organisms have localized chromosome–microtubule attachments during meiosis.

Electron microscopic analyses of meiosis in holocentric organisms indicated that spindle microtubules are embedded directly into chromatin at the ends of chromosomes and no trilaminar structure is visible at these interaction sites (Comings & Okada 1972, Pimpinelli & Goday 1989, Albertson & Thomson 1993). However, the specific end of the chromosome that spindle microtubules attach to during meiosis I appears random. In contrast to the holokinetic chromosomes observed during mitosis, it appears that there are two potential localized meiotic centromeres which can function to mediate chromosome–spindle interactions. One of these, chosen at random, is active during meiosis I. Following completion of meiosis I, the chromosome end that directed poleward movement is now located at the spindle equator (Figure 5) and thus the second potential meiotic centromere is active during meiosis II.

Given the difference in kinetochore structure observed during meiosis, it remains unclear which proteins are required for meiotic chromosome segregation and how their functions are restricted to chromosome ends. At least some of the mitotic kinetochore proteins also localize to meiotic chromosomes (Howe *et al.* 2001), although the localization pattern does appear distinct from that of mitotic chromosomes. In addition, meiotic defects have been identified for proteins that play essential roles in mitotic kinetochore function. The mitotic kinetochore protein Nuf2^{HIM-10} was first identified in a genetic screen for strains with a 'high incidence of male' progeny. Hermaphrodite *C. elegans* are genetically XX, while males are XO and arise from rare non-disjunction events during normal meiosis. Since mutation of *him-10* enhances these non-disjunction events, this suggests that Nuf2 $^{HIM-10}$ does function in meiotic chromosome segregation. Future work will examine whether the remaining components of the mitotic kinetochore additionally function during mitosis, and how their activities are altered to generate a localized kinetochore at chromosome ends.

Conclusion

Kinetochores in holocentric and monocentric species perform the same essential cellular function by generating a physical linkage between spindle microtubules and chromosomes. Here, we have reviewed studies on chromosome segregation and kinetochore function in the holocentric nematode *C. elegans*, which reveal striking similarities as well as occasional differences to monocentric organisms. The similarities make *C. elegans* an attractive system in which to study general kinetochore function, as well as examine the unique features that are required to generate a holocentric chromosome. Future studies on diffuse kinetochore function should lead to new

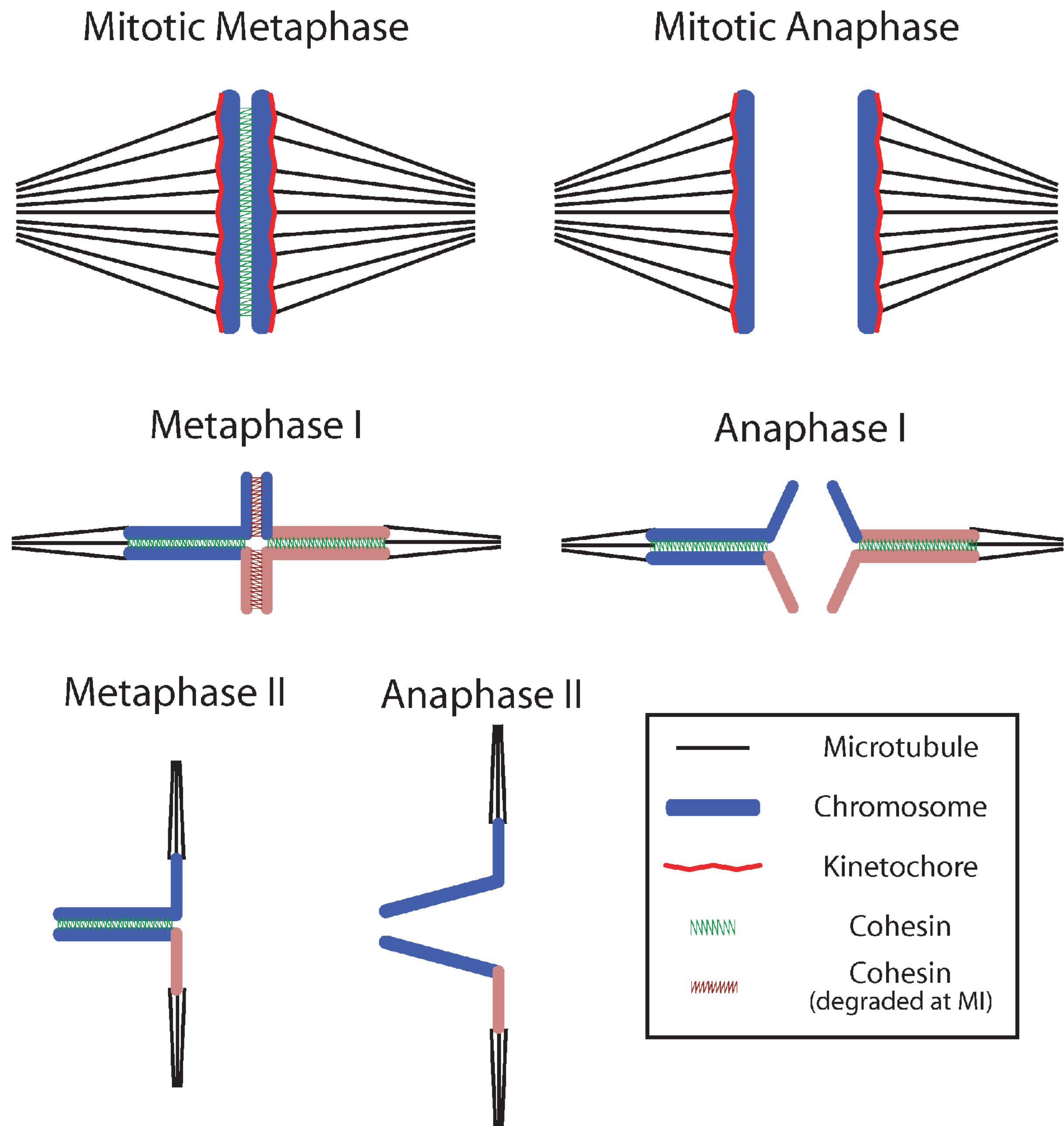


Figure 5. Mitotic vs. meiotic segregation in holocentric species. Diagram showing chromosome segregation during mitosis and meiosis. During mitosis, chromosomes are holocentric with a diffuse kinetochore along the length. During meiosis, microtubules are directly embedded in the chromosome ends, but the specific chromosome end is chosen randomly. DNA originally from one homologue is shown in either blue or pink. Chromosomes composed of two colours represent the resolved products of the single meiotic recombination event (see Hillers & Villeneuve 2003). At meiosis I, cohesion between homologues is degraded allowing the separation of homologous chromosomes to opposite poles. During meiosis II, the opposite end of the chromosome is used to attach to microtubules. At the transition to anaphase, cohesion between paired sister chromatids is eliminated allowing their segregation to opposite poles.

insights into the mechanisms of chromosome segregation.

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