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SPOTLIGHT

Chromosome segregation during female meiosis in *C. elegans*: A tale of pushing and pulling

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The role of the kinetochore during meiotic chromosome segregation in *C. elegans* oocytes has been a matter of controversy. Danlasky et al. (2020. *J. Cell. Biol.* https://doi.org/10.1083/jcb.202005179) show that kinetochore proteins KNL-1 and KNL-3 are required for early stages of anaphase during female meiosis, suggesting a new kinetochore-based model of chromosome segregation.

Meiosis consists of two consecutive chromosome segregation events preceded by a single round of DNA replication. Homologous chromosomes are separated in meiosis I, which is followed by sister chromatid separation in meiosis II to produce haploid gametes. Both of these stages require chromosomes/chromatids to align during metaphase before separating to opposite poles during anaphase. During mitosis, microtubules emanating from centrosomes at opposite poles of the cell bind chromosomes through a multiprotein complex called the kinetochore, allowing chromosomes to be pulled apart (1, 2). This segregation event takes place in two stages: anaphase A, where chromosomes are pulled toward spindle poles due to microtubule depolymerization, and anaphase B, where spindle poles themselves move farther apart, taking the attached chromosomes with them (3, 4). In many organisms, including mammals, oocytes lack centrosomes, and it has been of great interest to clarify the mechanisms used to ensure chromosomes are properly segregated during female meiosis (5, 6). *Caenorhabditis elegans* has served as a model for studying both mitosis and meiosis, but the mechanisms operating during female meiosis have been a matter of debate and controversy.

In 2010, Dumont et al. showed that the kinetochore is required for chromosome alignment and congression during metaphase (7). However, they suggested that chromosome segregation was the result of microtubule polymerization between the segregating chromosomes (Fig. 1), resulting in a pushing force exerted onto chromosomes toward the spindle poles in a largely kinetochore-independent manner (7). This mechanism was also supported by the finding that CLIP-associated protein (CLASP)–dependent microtubule polymerization between the segregating chromosomes is essential for chromosome separation (8). An alternative model suggested that chromosomes are transported through microtubule-free channels toward the spindle poles by the action of dynein (9). Later evidence put in doubt a role for dynein and favored a model in which chromosomes initially separate when the spindle shortens and the poles overlap with chromosomes in an anaphase A–like mechanism. This is then followed by separation of chromosome-bound poles by outward microtubule sliding in an anaphase B–like fashion (10). However, because microtubules emanating from the spindle poles are not required to separate the homologous chromosomes but microtubules between the separating chromosomes are (8), this model is unlikely, at least as an explanation for mid-/late-anaphase movement. Furthermore, although lateral microtubule interactions with chromosomes predominate during metaphase of *C. elegans* oocyte meiosis, cryo-electron tomography data described end-on attachments between the separating chromosomes as anaphase progresses (11). This led to the suggestion that lateral microtubule interactions with chromosomes are responsible for the initial separation, but microtubule polymerization between the separating chromosomes is required for the later stages of segregation (11). The mechanisms involved in this initial separation have remained obscure. In this issue, Danlasky et al. show that the kinetochore is in fact required for the initial stages of chromosome segregation during female meiosis—an important step forward in our understanding of the mechanisms governing acentrosomal chromosome segregation (12).

By simultaneously depleting kinetochore proteins KNL-1 and KNL-3 in *C. elegans*, Danlasky et al. observed the meiotic chromosome congression and alignment defects described in previous studies (7). However, this double-depletion phenotype displayed three key characteristics that suggested a role for kinetochores in chromosome segregation, which are discussed below.

The kinetochore is required for bivalent stretching. It was previously shown that the bivalent chromosomes stretch before the initiation of segregation (10).

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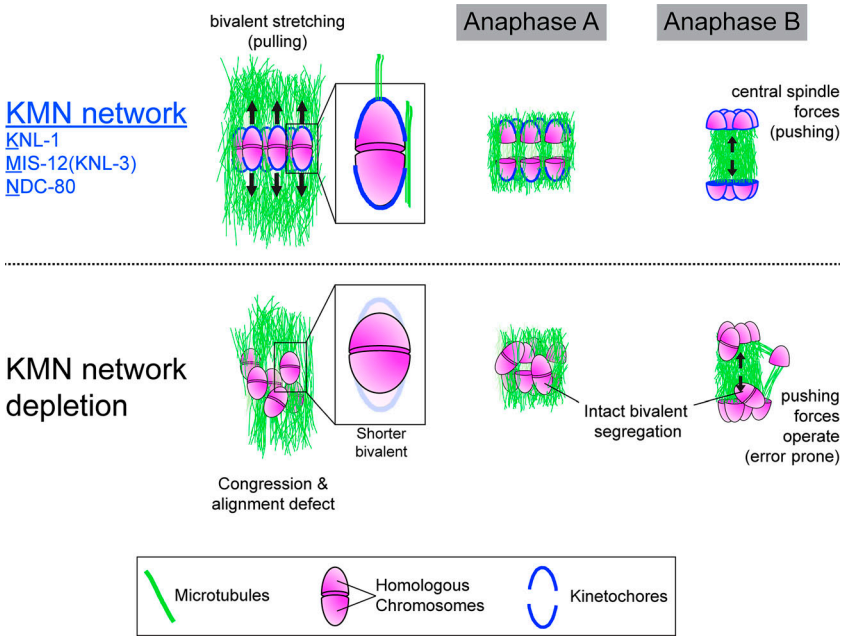

Figure 1. **Some of the key findings in Danlasky et al.** Kinetochore proteins surround the outer surface of the chromosomes, resulting in a characteristic cup shape. As anaphase progresses, chromosomes come into close contact to the spindle poles (anaphase A). Chromosome stretching is provided by KNL-1, MIS-12 (KNL-3), and NDC-80 (KMN)–dependent forces. Once the spindle starts elongating (anaphase B), central spindle microtubules provide the pushing forces for chromosome segregation. At this stage, kinetochore proteins also occupy the inward face of separating chromosomes. Upon KMN network depletion, bivalents flatten, and chromosome congression and alignment are defective. Anaphase A chromosome movement is almost absent, which leads to error-prone anaphase B.

Danlasky et. al found that this stretching of the chromosomes did not occur when KNL-1,3 were depleted, indicating that the kinetochore is required for this process (Fig. 1). Together with the observation that kinetochore proteins appear to extend toward the spindle poles, this finding suggested that pulling forces resulting from the interaction between the kinetochore and spindle microtubules are occurring during metaphase/pre-anaphase (Fig. 1).

The kinetochore is required for anaphase A. In *C. elegans* female meiosis, anaphase A occurs when homologous chromosomes begin to separate during spindle shortening, and anaphase B when the chromosomes separate alongside the spindle poles (10). Danlasky et al. observed that KNL-1,3 depletion drastically reduced the velocity of anaphase A, as chromosomes only separated when spindle poles began to move apart. This indicated that pulling forces caused by the interaction between the kinetochore and spindle microtubules are also important for the initial separation of homologous chromosomes in anaphase A.

The kinetochore is required for proper separation of homologous chromosomes. In KNL-1,3 depletion strains, 60% of bivalents failed to separate before segregation began, resulting in intact bivalents being pulled to the same spindle pole (Fig. 1). This failure of homologous chromosomes to separate was not thought to be a result of KNL-1,3 depletion interfering with the cleavage of cohesin that holds the two homologous chromosomes together because (a) separase and AIR-2^{AuroraB}, both of which are required for cohesin cleavage, localized normally during metaphase and anaphase, and (b) bivalents separated by metaphase II. This leaves the possibility open that the failure of bivalents to separate was due to the disrupted pulling forces thought to be important in bivalent stretching and anaphase A.

Altogether, these data strongly indicate that the kinetochore is required not only for chromosome congression and alignment but also for the early stages of homologue separation. Anaphase B occurred successfully in the absence of KNL-1,3 but was more error prone, likely as a result of the earlier congression and anaphase A defects. While it is clear that chromosome masses do segregate in the absence of the kinetochore, this segregation is highly erroneous as a result of defects during the earlier stages of segregation in anaphase A (Fig. 1).

The findings of Danlasky et al. raise testable hypotheses that could significantly enhance our understanding of acentrosomal chromosome segregation. Further investigation of the proposed pulling forces required during metaphase and early anaphase will be of great interest. Additionally, a more detailed analysis of the dynamic localization of separase and Securin, as well as assessing successful cohesin cleavage when KNL-1,3 are depleted, would back up the assertion that the failure of homologous chromosomes to separate was not due to the kinetochore impacting cohesin cleavage. It has previously been shown that the CLASP orthologue CLS-2 in *C. elegans* localizes to the kinetochore surrounding the bivalent chromosomes during metaphase before relocalizing to the central spindle during anaphase (7, 8, 13). It will be interesting to examine whether this key microtubule-stabilizing protein contributes to anaphase A pulling forces alongside its essential role in microtubule polymerization between chromosomes in anaphase B (8).

While the regulation of proper chromosome segregation during acentrosomal meiosis in *C. elegans* is not yet fully understood, Danlasky et al.'s results represent a significant step forward in this endeavor by showing that the kinetochore is in fact required for the early stages of chromosome segregation.

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References

1. Monda, J.K., and I.M. Cheeseman. 2018. *J. Cell Sci.* https://doi.org/10.1242/jcs.214577
2. Musacchio, A., and A. Desai. 2017. *Biology.* https://doi.org/10.3390/biology6010005
3. Asbury, C.L. 2017. *Biology.* https://doi.org/10.3390/biology6010015
4. Scholey, J.M., et al. 2016. *Biology.* https://doi.org/10.3390/biology5040051
5. Dumont, J., and A. Desai. 2012. *Trends Cell Biol.* https://doi.org/10.1016/j.tcb.2012.02.007
6. Severson, A.F., et al. 2016. *Curr. Top. Dev. Biol.* https://doi.org/10.1016/bs.ctdb.2015.11.031
7. Dumont, J., et al. 2010. *Nat. Cell Biol.* https://doi.org/10.1038/ncb2093
8. Laband, K., et al. 2017. *Nat. Commun.* https://doi.org/10.1038/s41467-017-01539-8
9. Muscat, C.C., et al. 2015. *eLife.* https://doi.org/10.7554/eLife.06462
10. McNally, K.P., et al. 2016. *Mol. Biol. Cell.* https://doi.org/10.1091/mbc.e16-05-0331
11. Redemann, S., et al. 2018. *Curr. Biol.* https://doi.org/10.1016/j.cub.2018.07.012
12. Danlasky, B.M., et al. 2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.202005179
13. Pelisch, F., et al. 2019. *J. Cell Sci.* https://doi.org/10.1242/jcs.232330