

SPOTLIGHT

Too close for comfort? Endomembranes promote missegregation by enclosing lost chromosomes

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Correct segregation of chromosomes during mitosis is essential to prevent aneuploidy. In this issue, Ferrandiz et al. (2022. *J. Cell Biol.* <https://doi.org/10.1083/jcb.202203021>) show that endomembranes can promote chromosome missegregation by “ensheathing” misaligned chromosomes, preventing their integration into the metaphase plate. Their findings point toward endomembranes as a potential risk factor for aneuploidy.

Mitosis is a carefully orchestrated process that ultimately results in faithful separation of DNA and other cellular material between the newly formed daughter cells. To ensure equal separation of DNA, chromosomes must align at the metaphase plate before being pulled apart, a process regulated by binding of kinetochores to the spindle microtubules. Errors in mitosis can cause chromosomes to misalign and, if not timely corrected by the mitotic spindle, this could result in chromosome missegregation. Chromosome missegregation leads to whole chromosome aneuploidy (i.e., the presence of an abnormal number of chromosomes within a cell) and is associated with the formation of micronuclei, both of which contribute to cancer progression (1, 2). There have been significant advances in our understanding of the mechanisms underlying chromosome missegregation, the majority of which involve defects in the mitotic spindle and spindle assembly checkpoint (1). Despite this, it remains largely unclear why some misaligned chromosomes are rescued by the mitotic spindle while others become missegregated.

Entry into mitosis is accompanied by an extensive reorganization of intracellular organelles (3). The nuclear envelope (NE) breaks down to enable the assembly of the mitotic spindle, while the ER and the Golgi apparatus disperse through the cytosol. The

remnants of these organelles, termed “endomembranes,” become displaced toward the cell periphery, thereby creating an “exclusion zone,” a membrane-free space for the mitotic spindle to operate (4). In this issue, Royle and colleagues ask what happens to misaligned chromosomes when they exit the exclusion zone.

To study this, Ferrandiz et al. (5) artificially induced chromosome misalignment through inhibiting centromere-associated protein E (CENP-E), a kinesin-like motor protein that is essential for correct chromosome alignment (6). In addition, they used a system in which the Y chromosome can be forced to detach from the spindle, resulting in misalignment. In both model systems, misaligned chromosomes that exit the exclusion zone become trapped in several layers of endomembranes, which the authors referred to as “ensheathing” of chromosomes (Fig. 1). They also noticed that cells with at least one ensheathed chromosome had a delay in mitotic progression, which likely results from activation of the spindle assembly checkpoint.

The authors then wondered about the fate of these ensheathed chromosomes. They observed that misaligned “free” chromosomes (those localized within the exclusion zone) were frequently rescued by the mitotic spindle and thus aligned at the metaphase plate before the cell proceeded to

anaphase. In contrast, ~66% of ensheathed chromosomes that arise following CENP-E inhibition failed to align, as spindle microtubules were unable to penetrate the layers of endomembranes and bind to kinetochores. Thus, when eventually cells proceeded with division, ensheathed chromosomes were missegregated and led to the formation of micronuclei with a ruptured NE (Fig. 1). However, CENP-E inhibition skews misaligned chromosomes toward the exclusion zone border, which could increase the likelihood of chromosomes becoming ensheathed. Furthermore, most free chromosomes were captured by spindle microtubules, and very few formed micronuclei. Therefore, in this context it is unclear whether NE rupture is a consequence of the ensheathing process.

Next, Ferrandiz et al. went on to demonstrate that ensheathing of misaligned chromosomes is causal for chromosome misalignment (5). To this end, they came up with a clever strategy to displace the entire mitotic ER to the plasma membrane using the rapamycin-inducible FKBP-FRB interaction system. By fusing an ER-resident protein (Sec61 β) to FKBP and a plasma membrane anchor to FRB, they managed to clear the cytosol of ER within 12–24 min following rapamycin treatment. Importantly, this intervention enabled the previously ensheathed chromosomes to align with the metaphase plate. It is thus tempting

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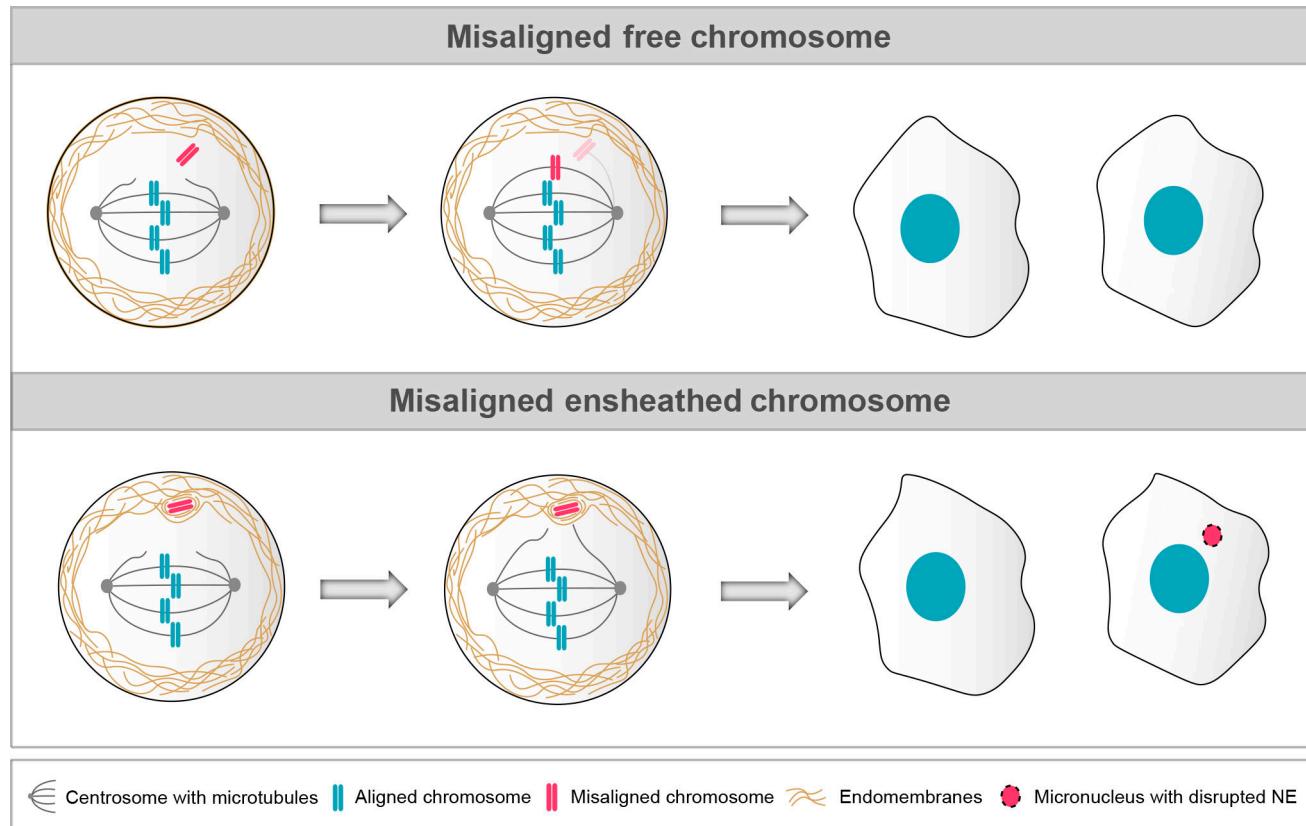


Figure 1. Schematic model depicting the fate of misaligned free and ensheathed chromosomes. Free misaligned chromosomes (those that remain within the exclusion zone; top panel) are frequently rescued by microtubules that pull them back in line with the metaphase plate. Once the correct position of the chromosome is restored, the cell proceeds through division, giving rise to two diploid daughter cells. Ensheathed misaligned chromosomes that are beyond the exclusion zone become surrounded by layers of endomembranes (bottom panel). The mitotic spindle is unable to rescue ensheathed chromosomes, as microtubules fail to penetrate their surrounding endomembranes. Eventually, the cell proceeds with division, resulting in aneuploidy and the formation of a micronucleus with a disrupted NE.

to speculate that this rescue involves newly formed interactions between microtubules of the mitotic spindle and the misaligned chromosomes. Collectively, the data show that endomembranes promote chromosome missegregation and micronucleus formation and could thus constitute a significant risk factor for aneuploidy.

Interestingly, a fraction of the cells with misaligned, ensheathed chromosomes eventually went through a normal mitosis. This suggests that these trapped chromosomes can be rescued during the later stages of cell division. While it remains elusive how this rescue occurs, it is possible that ER reorganization as cells progress to anaphase and telophase could make ensheathed chromosomes more accessible, thereby enabling their interaction with microtubules of the mitotic spindle that pull them back into place.

Micronuclei arising from ensheathed chromosomes frequently had a disrupted NE, which correlates with invasion of the

ER. The authors propose that NE disruption could arise from ER membranes physically interfering with NE reformation. However, it is also possible that ER invasion is a consequence of the defective NE, as previously suggested (7). Furthermore, prior work demonstrated that micronuclei arising from lagging chromosomes located within the exclusion zone, and thus unlikely to be ensheathed, also commonly display ruptured NE, which results from defects in the assembly of the NE and nuclear pore complex (8–10). Thus, additional work is needed to assess the impact of endomembranes on the integrity of the micronuclear envelope to fully understand what is driving NE disruption.

Finally, Ferrandiz et al. observed a higher fraction of ensheathed chromosomes in cancer cells compared to non-transformed cells (5). This raises the possibility that cancer cells could be more prone to chromosome missegregation as a consequence of ensheathing. There are several potential

explanations that could underlie this difference. First, cancer cells may simply have a higher amount of endomembranes, which would leave less space for the formation of an exclusion zone within the confinement of the cytosol. Second, cancer cells could have a different organization of the ER (e.g., sheet-to-tubule ratio; 11) that may impact the ability of chromosomes to physically invade the endomembrane compartment. Third, the formation of an exclusion zone at the onset of mitosis requires forces generated by microtubules that pull the endomembranes toward the cell periphery (3). Potentially, this microtubule-dependent reorganization of the endomembrane compartment could be disrupted in cancer cells, resulting in the formation of a more compact exclusion zone.

Overall, this work provides novel and surprising insights in the mechanisms underlying chromosome missegregation. These findings provide a plausible explanation for the outstanding question of why some misaligned

chromosomes are rescued while others become missegregated. Future work will likely establish to what extent endomembranes contribute to chromosome missegregation in the context of cancers *in vivo*, and whether this could be exploited for therapeutic interventions.

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