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SPOTLIGHT

Function through absence: Active RNA exclusion from chromosomes leads to proper cell division

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The dynamics and functional roles of chromatin-bound RNA during cell division are largely unexplored. In this issue, Sharp et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201910148) found that a mitosis-specific signal evicts RNA-bound SAF-A from chromosomes, and its absence leads to proper chromosome segregation.

The structure and organization of the eukaryotic genome have long puzzled many scientists. Since the early images of interphase chromatin (1) and mitotic chromosomes (2) by electron microscopy were published, questions of how this structure affects DNA-based processes such as transcription, replication, and repair have remained unresolved. Over decades of work, we have learned that DNA is wrapped around histone proteins to form the nucleosome, the building block of chromatin, and that nucleosomes are organized in neat arrays at the start of genes (3). The development of chromatin conformation capture assay (4) and its subsequent adaptations to high-throughput sequencing have further led to discoveries that chromatin is organized into loops, which are then grouped to form topologically associated domains (TADs), such that DNA regions within TADs interact more frequently than those outside of TADs (5). We have also learned that chromosomes occupy distinct territories inside the nucleus, and that actively transcribed regions are more centrally located, whereas silenced regions are generally concentrated in the periphery (5).

Despite all the focus on interphase chromatin, little is known about a crucial stage in the cell cycle. Cells massively reorganize the chromosomes every time they divide. Virtually all of the interphase loops and TADs are disassembled, and instead, a more or less linear condensation is observed (6). Though their precise structure, and even composition, remains a mystery, each chromosome inherently adopts the characteristic X-shape during mitosis. This macroscale structure is conserved throughout evolution and is important for accurate segregation of each copy of the genome to daughter cells. What contributes to this mitotic structure? How do structural factors during interphase influence the change to mitotic chromosomes?

Recent studies are shedding light on the role of RNAs and RNA-binding proteins in establishing the structure and organization of chromatin. Perhaps the most famous noncoding RNA known to affect gene regulation and chromatin structure is the XIST RNA. In XX cells, the *Xist* gene is transcribed from only one of the X chromosomes. The resulting XIST RNA coats the X chromosome from which it is transcribed, leading to massive condensation and inactivation of most genes within the chromosome (7). Though negatively charged RNA can directly bind to positively charged chromatin, this interaction is often mediated by RNA-binding proteins that act as a tether between DNA and RNA (8). One example of these proteins is the scaffold attachment factor A (SAF-A). Originally identified as a structural protein, SAF-A has a DNA-binding domain on one end and an RNA-binding region on the other. Recent studies have shown that RNA-bound SAF-A regulates chromatin organization in interphase cells (9). What role does it play in organizing mitotic chromosomes? In this issue, Sharp et al. (10) dove into the dynamics of SAF-A and its associated RNAs during mitosis. The authors identified the key players that lead to eviction of SAF-A from mitotic chromosomes, isolated the precise mechanism through which this process occurs, and demonstrated a clear phenotype linking the RNA eviction via SAF-A to proper segregation of mitotic chromosomes. In short, the authors found something surprising: in this case, active eviction rather than active binding is important for proper function.

Using both immunofluorescence and coimmunoprecipitation assays to establish the dynamic localization of SAF-A through the cell cycle, the authors showed that SAF-A changes from chromatin bound during interphase to excluded from chromosomes during mitosis. Importantly, RNA bound to SAF-A during interphase maintained binding to SAF-A through the cell cycle, leading to eviction of RNA from mitotic chromosomes. Given that SAF-A binds to ∼13% of all expressed RNA, the exclusion of SAF-A from mitotic chromosomes leads to substantial rearrangement of RNA localization during mitosis.

The timing of SAF-A exclusion at the prophase–prometaphase junction hints at a cell cycle–specific signal that is triggering

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the change in SAF-A localization. Sharp et al. checked the most likely candidates: mitosis-specific kinases such as Aurora-A, PLK1, and Aurora-B. Using specific inhibitors and RNAi knockdown for each kinase, the authors observed that Aurora-B kinase inhibition leads to retention of SAF-A, and hence RNA, on mitotic chromosomes, suggesting that Aurora-B triggers the exclusion of SAF-A early in mitosis. To test whether Aurora-B works directly via SAF-A to actively exclude RNA from mitotic chromosomes, the authors used acute, drug-mediated depletion of SAF-A along with Aurora-B inhibition and found that RNA was no longer retained on chromosomes, suggesting that Aurora-B is epistatic to SAF-A.

The interaction between SAF-A and Aurora-B defines the pathway for global eviction of RNA from chromosomes during early mitosis. Diving deeper, Sharp et al. used structural modeling, mass spectrometry, and in vitro reconstitution assays to identify precisely the serine residues within SAF-A that are the targets of Aurora-B phosphorylation during mitosis. They ultimately identified two serine residues within the SAF-A DNA binding domain that, when phosphorylated, are responsible for the release of SAF-A from mitotic chromosomes.

The researchers next identified the functional significance of this exclusion. They induced the expression of either the wild-type or nonphosphorylatable SAF-A in cells that are depleted of endogenous SAF-A and followed mitotic progression through live-cell imaging. Mutant SAF-A expression led to massive delays in early stages of mitosis that ultimately presented as aberrant anaphase structures, including increased rate of lagging chromosomes and anaphase chromosome bridge formation. Taken together, the authors concluded that global eviction of RNA via phosphorylation of SAF-A by the Aurora-B kinase during early mitosis is necessary for proper segregation of chromosomes.

In summary, Sharp et al. (10) combined a diverse set of techniques to dissect the mechanism of SAF-A:RNA dynamics during mitosis and found something surprising. As biologists, we normally associate phenotype with the presence of activity. In this case, the opposite is true: the exclusion of SAF-A bound RNA is related to the phenotype. Why does retention of SAF-A–bound RNA lead to chromosome segregation defects? Addressing this question presents a tantalizing future direction of research.

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References

1. Huxley, H.E., and G. Zubay. 1961. *J. Biophys. Biochem. Cytol.* https://doi.org/10.1083/jcb.11.2.273
2. Barnicot, N.A., and H.E. Huxley. 1965. *Q. J. Microsc. Sci.*
3. Andrews, A.J., and K. Luger. 2011. *Annu. Rev. Biophys.* https://doi.org/10.1146/annurev-biophys-042910-155329
4. Dekker, J., et al. 2002. *Science.* https://doi.org/10.1126/science.1067799
5. Dekker, J., et al. 2013. *Nat. Rev. Genet.* https://doi.org/10.1038/nrg3454
6. Gibcus, J.H., et al. 2018. *Science.* https://doi.org/10.1126/science.aao6135
7. Brockdorff, N., et al. 2020. *Genes Dev.* https://doi.org/10.1101/gad.337196.120
8. Hasegawa, Y., and S. Nakagawa. 2011. *RNA Biol.* https://doi.org/10.4161/rna.8.5.16367
9. Nozawa, R.-S., et al. 2017. *Cell.* https://doi.org/10.1016/j.cell.2017.05.029
10. Sharp, J.A., et al. 2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201910148