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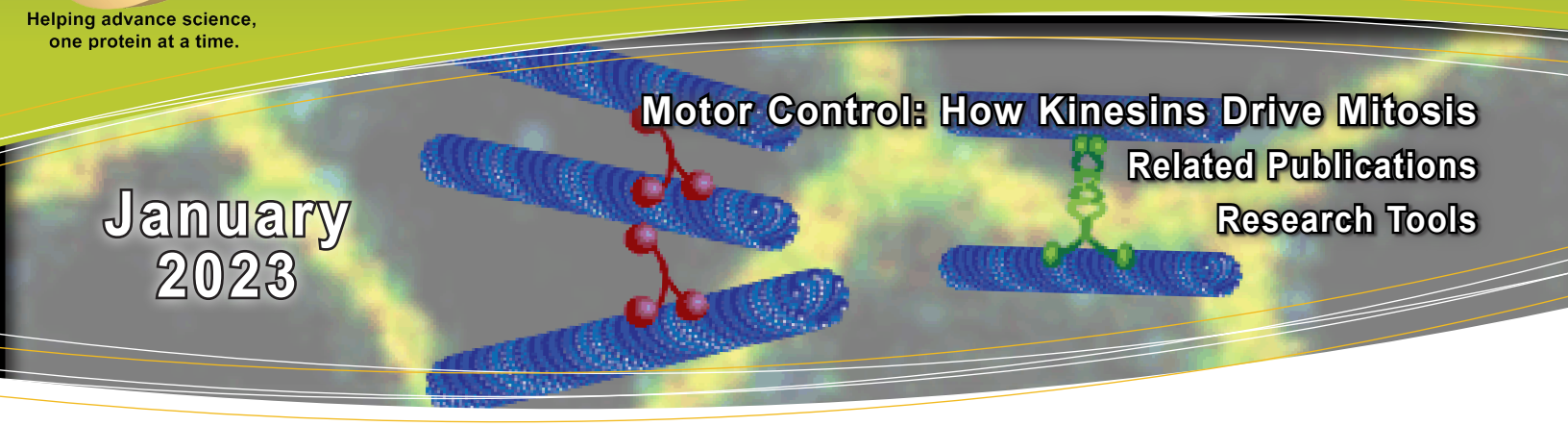

Motor Control: How Kinesins Drive Mitosis

One of the main components of the cytoskeleton is microtubules (MTs), which are comprised of heterodimers of α and ß-tubulin proteins. Tubulin can polymerize on both ends of MTs at different rates, where polymerization occurs more rapidly on the plus-end and is slower on the minus-end. The molecular motor families, kinesins and dyneins play a role in MT length by regulating the dynamic behavior of growth and shrinkage of MTs, known as dynamic instability[1]. These motors work along MTs to provide cellular functions involving organelle transport, vesicle transport, meiosis, and mitosis.

Kinesins were first discovered in 1985 when it was observed that a new class of ATPases could cycle on and off microtubules and induce organelle movement along the axons of squid and vertebrate brain[2,3]. Since then, 45 kinesin proteins have been identified in humans, with the Kinesins superfamily (KIF) organized into 14 recognized kinesin families[4]. Most kinesins have a plus-end directed motility, but motors in the kinesin-14 subfamily have a C-terminal motor and are minus-end directed. The general architecture of kinesins consists of three domains: 1) a catalytic motor or head domain that is well conserved within each kinesin family that can hydrolyze ATP and binds microtubules, 2) an adjacent neck domain that is involved in coiled-coil interactions and can organize the motor into higher-order oligomers, 3) On the opposite end of the protein is the tail domain, which can be highly divergent within a kinesin family, and is sometimes used to interact with cargo proteins, DNA, or regulatory domains[5-8]. Below, we will look at recent findings on how kinesins affect the formation of the mitotic spindle and the organization of the chromosomes through the various stages of mitosis[9].

Kinesins Play a Role in Mitosis

The process of mitosis occurs in somatic cells, where a copy of each chromosome of the parent cell is separated into two daughter cells during cell division. There are 5 phases of mitosis: prophase, prometaphase, metaphase, anaphase, and telophase.

Prophase begins when the duplicated centrosomes move to the opposite sides of the nucleus. Here, the MTs overlap and are orientated antiparallel to each other. The KIF11 motor (aka Eg5), a member of the kinesin-5 subfamily, can crosslink to the MTs and promotes MT sliding outwards to help push the centrosomes apart (Figure 1)[10,11]. Dynein also assists in moving centrosomes by tethering the MTs to the cell cortex and nuclear envelope.

During prometaphase, the goal is to create bipolar mitotic spindles, form interactions between the chromosome and MTs, and then promote chromosomal congression of the chromosomes to the spindle equator. The self-organization of the spindle requires KIF11, KIF15, and KIFC1 opposing motor actions to increase the separation of centrosomes and maintain spindle length[10]. Once the nuclear envelope breaks down, this allows the MT plus-ends of the spindle access to the macromolecular protein complex known as the kinetochore. Any plus-end MTs that attach to the kinetochore are known as kinetochore-microtubules (kMTs). The collection of all kMTs, typically 10-30 kMTs, within an individual kinetochore makes up a kinetochore-fiber (K-fiber)[12]. At the opposite end, the minus-end directed motors of KIFC1 and dynein anchor the minus-ends of MTs to the centrosome to produce focused bipolar spindle poles[13]. Congression of the chromosomes to the spindle equator is aided by dynein and the kinesin-7 subfamily KIF10 motor (aka CENP-E), which promotes lateral sliding of the kinetochore (Figure 1)[14]. Kinetochore-mediated pushing and pulling forces need to be generated at the kinetochore by the kinesin motors KIF10, KIF18A, KIF2B, KIF2C, and others for proper chromosomal alignment[15-18]. Next, forces must be generated to move the chromosomes from the spindle poles toward the spindle equator. These mechanical forces are known as polar ejection forces, but further studies are needed to understand how much these forces contribute to chromosome congression[19,20]. It is believed that the chromokinesins KIF22 (aka KID) of the kinesin-10 subfamily, and KIF4 of the kinesins-4 subfamily, interact with chromosomes during mitosis and are the motors associated with generating MT forces that force the chromosomes from the poles (Figure 1)[20,21]. Depletion of

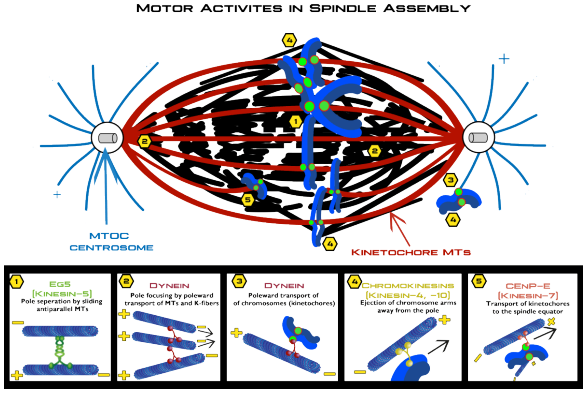

Figure Legend: Schematic representing bipolar spindle formation during mitosis. Microtubules and motor proteins play critical roles in kinetochore orientation and chromosome movement.

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Continued from Page 1

KIF22 via RNA interference in HeLa cells led to misaligned chromosomes and defects in spindle length, while KIF4-deficient cells resulted in disorganized chromosomes[22,23]. These motors play a vital role in chromosomal movement through prometaphase and metaphase.

During metaphase, the sister chromatids must continue to align along the spindle equator and form the metaphase plate. The coordination to synchronously align and separate the sister chromatids is a complex process requiring many factors. The kinesin-13 family members localize to minus-ends of the microtubules at the centrosome, provoking MT depolymerization and generating poleward pulling forces on the chromosomes[10,24]. KIF18A is also essential for proper metaphase plate alignment and timely anaphase onset[25]. Studies also point to KIF18A and KIF10's roles in maintaining attachment to kinetochores when MTs are depolymerizing[10,14,26].

The final stages of mitosis occur once the chromosomes of the mitotic spindle are correctly aligned to pass the spindle assembly checkpoint. The transition into anaphase leads to the loss of cohesion between sister chromatids, allowing them to be separated and moved to each spindle pole[27]. Less is known about kinesins and their function in anaphase, but depolymerization of the K-fiber is believed to be largely independent of motor proteins and more reliant on other MT-associated proteins and the intrinsic MT depolymerizing to complete cell division[28].

Targeting Kinesins for Drug Therapy

Mitotic drugs have been of great interest as a cancer treatment. For example, Paclitaxel (Taxol) is an anti-mitotic drug that targets MTs to disrupt cell division, and is used as a cancer treatment but is known to have serious side effects as it can also affect normal dividing cells. Screening for potential kinesin inhibitors that target these motor proteins that may be dysregulated in cancer presents an alternative to tubulin-based drugs. Compound screening has identified leads that inhibit kinesins, including KIF11, which have made it to Phase II drug trials[29]. However, up to this point, most drug trials have been disappointing due to a lack of efficacy and potential side effects[30]. Nevertheless, other motors like KIF18A appear to be exciting new drug targets, as the viability of cancer cells with chromosomal instability (CIN) depends on KIF18As ability to maintain chromosomal alignment[31]. Targeting motors vital for chromosomal alignment in cancer cells with CIN may make them more vulnerable to anti-mitotic therapies while having minimal effects on normal cells[32].

Conclusion

This newsletter offers a glimpse into kinesins' complex mechanistic role in mitosis. Other proteins, microtubule-associated proteins, and types of MTs in the spindle also play significant roles in cell division. Further work is needed to understand how the motors coordinate with each other to generate the type of forces that orient the MTs and align the chromosomes for movement across the cell during the different stages of mitosis. Cytoskeleton Inc. has assisted many drug discovery efforts in this area[33,34]. With a better understanding, kinesins may present a promising therapeutic target for cancer treatment.

Motor Proteins

Product	Amount	Cat. #
Dynein motor protein	1 x 50 µg	CS-DN01-A
Kinesin heavy chain motor domain protein GST tagged: Homo sapiens recombinant	2 x 25 µg 1 x 1 mg	KR01-A KR01-XL
KIF3C kinesin motor domain protein GST tagged: Homo sapiens recombinant	2 x 25µg	KF01-A
KIFC3 kinesin motor domain protein GST tagged: Homo sapiens recombinant	2 x 25 µg	KC01-A
KIF7 Motor Domain Protein (H. Sapien)	1 x 100 µg	CS-KF51

References

1.Bringmann, H. et al. A Kinesin-like Motor Inhibits Microtubule Dynamic Instability. Science 303, 1519–1522 (2004).

2. Vale, R. D., Reese, T. S. & Sheetz, M. P. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell 42, 39–50 (1985).

3. Brady, S. T. A novel brain ATPase with properties expected for the fast axonal transport motor. Nature 317, 73–75 (1985).

4. Lawrence, C. J. et al. A standardized kinesin nomenclature. J Cell Biology 167, 19–22 (2004).

5. Wang, W., Cao, L., Wang, C., Gigant, B. & Knossow, M. Kinesin, 30 years later: Recent insights from structural studies: Kinesin, 30 Years Later: Structural Insights. Protein Sci 24, 1047–1056 (2015).

6. Cochran, J. Kinesin Motor Enzymology: Chemistry, Structure, and Physics of Nanoscale Molecular Machines. Biophysical Rev 7, 269–299 (2015).

7. Cason, S. E. & Holzbaur, E. L. F. Selective motor activation in organelle transport along axons. Nat Rev Mol Cell Bio 23, 699–714 (2022).

8. Miki, H., Okada, Y. & Hirokawa, N. Analysis of the kinesin superfamily: insights into structure and function. Trends Cell Biol 15, 467–476 (2005).

9. Dumont, S. & Mitchison, T. J. Force and Length in the Mitotic Spindle. Curr Biol 19, R749–R761 (2009).

10. Cross, R. A. & McAinsh, A. Prime movers: the mechanochemistry of mitotic kinesins. Nat Rev Mol Cell Bio 15, 257–271 (2014).

11. Tanenbaum, M. E. & Medema, R. H. Mechanisms of Centrosome Separation and Bipolar Spindle Assembly. Dev Cell 19, 797–806 (2010).

12. Conway, W. et al. Self-organization of kinetochore-fibers in human mitotic spindles. Elife 11, e75458 (2022).

13. Merdes, A. & Cleveland, D. W. Pathways of Spindle Pole Formation: Different Mechanisms; Conserved Components. J Cell Biology 138, 953–956 (1997).

14. Gardner, M. K. CENP-E hangs on at dynamic microtubule ends. Nat Cell Biol 15, 1030–1032 (2013).

15. Mayr, M. I. et al. The Human Kinesin Kif18A Is a Motile Microtubule Depolymerase Essential for Chromosome Congression. Curr Biol 17, 488–498 (2007).

16. Stumpff, J., Dassow, G. von, Wagenbach, M., Asbury, C. & Wordeman, L. The Kinesin-8 Motor Kif18A Suppresses Kinetochore Movements to Control Mitotic Chromosome Alignment. Dev Cell 14, 252–262 (2008).

17. Zhu, C. et al. Functional Analysis of Human Microtubule-based Motor Proteins, the Kinesins and Dyneins, in Mitosis/Cytokinesis Using RNA Interference. Mol Biol Cell 16, 3187–3199 (2005).

18. Czechanski, A. et al. Kif18a is specifically required for mitotic progression during germ line development. Dev Biol 402, 253–262 (2015).

19. Brouhard, G. J. & Hunt, A. J. Microtubule movements on the arms of mitotic chromosomes: Polar ejection forces quantified in vitro. Proc National Acad Sci 102, 13903–13908 (2005).

20. Vukušić, K. & Tolić, I. M. Polar Chromosomes—Challenges of a Risky Path. Cells 11, 1531 (2022).

21. Almeida, A. C. & Maiato, H. Chromokinesins. Curr Biol 28, R1131–R1135 (2018).

22. Tokai-Nishizumi, N., Ohsugi, M., Suzuki, E. & Yamamoto, T. The Chromokinesin Kid Is Required for Maintenance of Proper Metaphase Spindle Size. Mol Biol Cell 16, 5455–5463 (2005).

23. Short, B. KIF4 helps mitotic chromosomes get in shape. J Cell Biol 199, 715–715 (2012).

24. Diez, S. Multi-talented MCAK: Microtubule depolymerizer with a strong grip. Nat Cell Biol 13, 738–740 (2011).

25. Orr, B. & Maiato, H. No chromosome left behind: The importance of metaphase alignment for mitotic fidelity. J Cell Biol 218, 1086–1088 (2019).

26. McIntosh, J. R., Molodtsov, M. I. & Ataullakhanov, F. I. Biophysics of mitosis. Q Rev Biophys 45, 147–207 (2012).

27. Pandey, H., Popov, M., Goldstein-Levitin, A. & Gheber, L. Mechanisms by Which Kinesin-5 Motors Perform Their Multiple Intracellular Functions. Int J Mol Sci 22, 6420 (2021).

28. Vukušić, K., Buđa, R. & Tolić, I. M. Force-generating mechanisms of anaphase in human cells. J Cell Sci 132, jcs231985 (2019).

29. Garcia-Saez, I. & Skoufias, D. A. Eg5 targeting agents: From new anti-mitotic based inhibitor discovery to cancer therapy and resistance. Biochem Pharmacol 184, 114364 (2021).

30. Yan, V. C. et al. Why Great Mitotic Inhibitors Make Poor Cancer Drugs. Trends Cancer 6, 924–941 (2020).

31. Tamayo, N. A. et al. Targeting the Mitotic Kinesin KIF18A in Chromosomally Unstable Cancers: Hit Optimization Toward an In Vivo Chemical Probe. J Med Chem 65, 4972–4990 (2022).

32. Marquis, C. et al. Chromosomally unstable tumor cells specifically require KIF18A for proliferation. Nat Commun 12, 1213 (2021).

33. Perdomo, D. et al. TbKINX1B: a novel BILBO1 partner and an essential protein in bloodstream form Trypanosoma brucei. Parasite 29, 14 (2022).

34. Fukai, R. et al. Design, synthesis, and evaluation of a novel prodrug, a S-trityl- l -cysteine derivative targeting kinesin spindle protein. Eur J Med Chem 215, 113288 (2021).

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