

Fanning the flames of CIN

Comment on: Stolz A, et al. A phenotypic screen identifies microtubule plus end assembly regulators that can function in mitotic spindle orientation. *Cell Cycle* 2015; 14(6):827-37; PMID:25590964; <http://dx.doi.org/10.1080/15384101.2014.1000693>

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The fidelity of chromosome segregation during mitosis is intimately linked to the plus-end dynamics of kinetochore-microtubules to which they attach. Highly stable kinetochore-microtubules¹ or microtubules (MTs) that polymerize at increased rates² cause whole chromosomes to missegregate more frequently, a property referred to as chromosomal instability (CIN). Altered MT dynamics and CIN are both properties exhibited by cancer cells, and are particularly menacing because they increase tumor cell heterogeneity, enabling fast adaptation to chemotherapies. As restoration of proper MT dynamics suppresses CIN,^{1,2} a promising chemotherapeutic strategy may be to target proteins that promote abnormal MT dynamics. To this end, Stolz and colleagues report in this issue a new method to screen for factors that modulate the speed of MT polymerization.³

Systematic screening for factors that regulate MT dynamics, *eg.* by RNAi, is non-trivial because high resolution plus-end tracking over time is typically required. Small scale implementation of this strategy using *C. elegans*⁴ and human cultured cells⁵ have previously been reported, but additional approaches more amenable to high throughput screening would be beneficial. Stolz and colleagues report an unexpected but close relationship between MT polymerization rates and the percentage of cells with asymmetric monopolar spindles when Eg5 is inhibited by small molecule inhibitors (K5Is).³ The traditional image of an Eg5-inhibited monopole is a symmetric aster of MTs inside a shell of chromosomes.⁶ However, the authors show that

several chromosomally unstable colorectal cancer cell lines with heightened MT polymerization rates have a high incidence of asymmetric, fan-shaped monopolar spindles in the presence of K5Is. In addition, genetically or pharmacologically increasing MT polymerization rates can drive cells with normally symmetric monopoles to form asymmetric monopoles, while decreasing MT polymerization rate restores monopole symmetry. The authors leveraged this finding in a small RNAi screen and found that genes that impact monopole asymmetry also predictably change MT polymerization rates measured by tracking fluorescently labeled end-binding (EB) proteins. This demonstrates that monopole asymmetry can serve as a robust readout in large-scale screens for factors that influence MT polymerization rates, although hits should still be verified by EB tracking.

A strength of this assay is that it identifies not only factors which are likely to directly impact MT polymerization rates, but also kinases and other regulatory factors that are likely to impact MT dynamics indirectly. For example, the first factors tested are CHK2 and AURKA,^{2,3} kinases whose downstream targets affecting MT polymerization rates are not known. While this opens new avenues to study the upstream regulation of MT dynamics, it will also necessitate *in vitro* experiments to determine which molecules directly modulate MT polymerization, and which ones modulate polymerization by regulating other effectors. It will also be informative to show whether hits from a monopole asymmetry screen impact other MT dynamics parameters

besides polymerization rate. While these experiments may not be necessary for the authors' goal of understanding the genetics of CIN, they will help the fields of both mitosis and cytoskeletal dynamics better understand the mechanics at work in this system.

This paper leaves open several exciting questions. First, it remains unknown how increased MT polymerization rates drive monopolar spindles to be asymmetric. Second, because asymmetric monopoles are an intermediate step in a pathway of Eg5-independent spindle assembly,⁷ this work raises the possibility that cell lines with heightened MT polymerization rates may be better able to overcome Eg5 inhibitors. Third, the author's original question remains: what genetic lesions contribute to enhanced MT polymerization rates and CIN? Stolz and colleagues have laid the groundwork to answer this question and uncover new regulators of MT dynamics that will be of broad interest to the cytoskeleton community.

References

1. Bakhoum SF, et al. *Nat Cell Biol* 2009; 11:27-35; PMID:19060894; <http://dx.doi.org/10.1038/ncb1809>
2. Ertych N, et al. *Nat Cell Biol* 2014; 16:779-91; PMID:24976383; <http://dx.doi.org/10.1038/ncb2994>
3. Stolz A, et al. *Cell Cycle* 2015; PMID:25590964; <http://dx.doi.org/10.1080/15384101.2014.1000693>
4. Srayko M, et al. *Dev Cell* 2005; 9:223-36; PMID:16054029; <http://dx.doi.org/10.1016/j.devcel.2005.07.003>
5. Sironi L, et al. *Cytoskeleton* 2011; 68:266-78; PMID:21491614; <http://dx.doi.org/10.1002/cm.20510>
6. Mayer TU, et al. *Science* 1999; 286:971-4; PMID:10542155; <http://dx.doi.org/10.1126/science.286.5441.971>
7. Sturgill EG, Ohi. *Curr Biol* 2013; 23:1280-90; PMID:23791727; <http://dx.doi.org/10.1016/j.cub.2013.05.043>