Cutting edge science: Laser surgery illuminates viscoelasticity of merotelic kinetochores

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Increasing evidence in eukaryotic cells suggests that mechanical forces are essential for building a robust mitotic apparatus and correcting inappropriate chromosome attachments. In this issue, Cojoc et al. (2016. *J. Cell Biol.*, http://dx.doi.org/10.1083/jcb.201506011) use laser microsurgery in vivo to measure and study the viscoelastic properties of kinetochores.

Eukaryotic cells maintain a correct chromosome number by equally segregating their replicated chromosomes into two daughter cells at each division. When chromosome segregation is abnormal, aneuploid daughter cells are produced. Aneuploidy in germ cells is a well known cause of severe genetic diseases and is the leading cause of miscarriage in humans. To prevent aneuploidy, protein structures known as kinetochores (KTs) assemble at the chromosome centromeres and attach the centromeres to microtubules (MTs) from the two facing spindle poles, resulting in chromosome bi-orientation (Musacchio and Salmon, 2007). After capture, chromosomes align at the spindle center and form the metaphase plate as a result of forces generated by KT-bound mitotic motors and MT depolymerization. Once the chromosomes are correctly bi-oriented, KT motor forces act in opposition to chromosome cohesion forces, generating tension across sister chromatids, and the destruction of cohesion between sister chromatids triggers anaphase onset. At this stage, spindle elongation relies on both the sliding of interpolar MTs with antiparallel overlap and force generation by motor proteins acting at the spindle midzone (Pellman et al., 1995). The mechanisms of spindle assembly and error correction have been largely explored from a biochemical point of view, but the contribution of forces to spindle robustness has recently emerged from interdisciplinary studies combining cell biology, biophysics, and computational modeling (Mogilner and Craig, 2010). Such interdisciplinary approaches have helped address a fundamental question: How do the molecular components of the mitotic spindle interact to segregate the chromosomes both robustly and with fidelity?

Inappropriate chromosome attachments, such as merotelic attachments, in which one centromere is attached to both poles are actually very frequent during mitosis (Cimini et al., 2003). These can often be corrected by Aurora B kinase (Cimini et al., 2006), which detects tensionless attachments before anaphase onset. Merotely can be artificially induced in mammalian cells (Cimini et al., 2004) or genetically produced in fission yeast (Gregan et al., 2007; Courtheoux et al., 2009; Rumpf et al., 2010). In both models, merotelic attachment leads to intra-KT stretching, and these aberrant attachments are corrected in anaphase by the mechanical forces of spindle elongation (Cimini et al., 2004; Courtheoux et al., 2009). In fission yeast, the presence of merotelic chromosomes antagonizes spindle elongation, and the correction of merotely in anaphase prevents spindle collapse and cell death (Courtheoux et al., 2009). This correction is dependent on tension produced by spindle midzone forces and can be described with a simple force–balance model in which the merotelic KT is modeled with classical mechanical tools (spring and dashpot; Courtheoux et al., 2009; Gay et al., 2012). Thus, the contribution of tension and mechanical force to timely and accurate chromosome segregation has been increasingly appreciated.

In this issue, Cojoc et al. performed laser microsurgery of merotelic attachments to probe the mechanical properties of KTs in two model organisms, PtK1 rat kangaroo cells and fission yeast, to determine whether the mechanical properties of KTs were conserved throughout evolution (Fig. 1). Cojoc et al. (2016) first laser ablated MTs on one side of the merotelic KT and measured the change in KT length over time. They found that after MT severing, the once-stretched KT progressively shortened, with a relaxation shape characteristic of viscoelastic properties. Interestingly, the inner KT (defined by CENPA in PtK1 cells or Cnp1 in *Schizosaccharomyces pombe*) and the outer KT (defined by HEC1 in PtK1 cells or Ndc80 in *S. pombe*) both displayed viscoelastic responses but distinct relaxation kinetics. Upon MT severing, the inner KT relaxed more quickly than the outer KT, which Cojoc et al. (2016) suggest could be because of the elastic properties of the underlying chromatin. To further investigate these differences in the relaxation kinetics, Cojoc et al. (2016) then severed the MT bundles on both sides of the merotelic KT. These double ablations led to more similar relaxation kinetics for both the inner and outer KTs, suggestive that the slowing of outer KT relaxation in single ablation assays was a result of the unsevered MT bundle. Cojoc et al. (2016) also found that in PtK1 cells both the inner and outer KTs failed to relax completely to the length of unstretched KTs, even after double ablation. It is tempting to speculate that this residual stretch arises from nonelastic relaxation because of hyper-stretching of the KT structure. Alternatively, this

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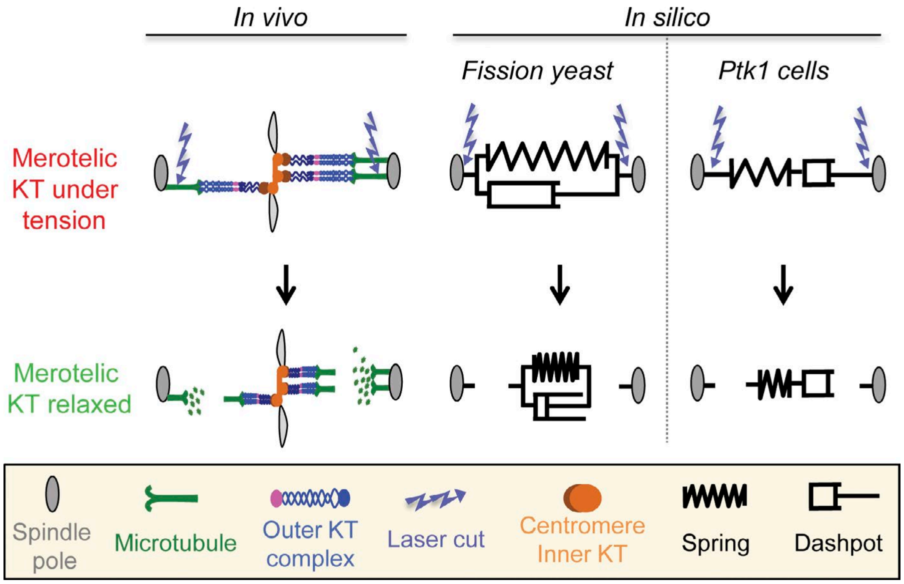

Figure 1. **The viscoelastic responses of merotelic KTs to laser surgery.** (left) Schematic description of a stretched merotelic KT in vivo. Both the inner and outer KT structures undergo stretching through the pulling forces produced by MTs. Laser ablation leads to the relaxation of the merotelic KT. (right) In silico, merotelic KT relaxation can be modeled through a combination of a spring and dashpot and reveals the viscoelastic properties of this structure. Note that in PtK1 cells, as opposed to fission yeast, the spring relaxes but the dashpot remains stretched (i.e., the KT does not regain its initial length).

nonelastic response could be explained by the presence of undetected MTs interacting laterally with the KT or with residual MT stubs. Indeed, as previously described, residual MTs that remain attached to the KT after MT severing can affect relaxation (Maiato et al., 2004; Elting et al., 2014; Sikirzhytski et al., 2014; Kajtez et al., 2016).

Cojoc et al. (2016) then turned to simple biophysical models to explain the viscoelastic and plastic properties of the KTs in the two cell types (Fig. 1). They considered two minimal models of viscoelastic material, which include a Hookean spring (characterized by a spring constant) and a linear dashpot (characterized by a drag coefficient) connected either in series or in parallel. The models can be distinguished by their differential response to equal force inputs. When the spring and dashpot were considered in parallel, both the relaxation kinetics and relaxed length of fission yeast KTs were best reproduced. In contrast, consideration of the spring and dashpot in series reproduced the residual length of PtK1 KTs, but not their relaxation kinetics. Cojoc et al. (2016) suggest that this discrepancy could be a result of the more complex structure of mammalian KTs compared with those in fission yeast. A model that recapitulates both viscoelastic and plastic properties of KTs could better reproduce the in vivo observations of PtK1 KT behavior.

Seminal work from Nicklas and Ward (1994) remains a clear example of how direct measurement of forces in live cells has informed our understanding of complex processes like error correction during chromosome segregation. Laser microsurgery has proven to be a valuable tool to dissect the mechanical properties of the spindle in live cells (Khodjakov et al., 1997; Maiato et al., 2004). The work by Cojoc et al. (2016) now establishes an experimental model to measure KT viscoelastic properties in vivo. This study raises the question: How does identification of these viscoelastic behaviors of KTs inform our understanding of mitosis? The authors report that merotelic relaxation after laser surgery is very similar to the "natural" correction supported by spindle elongation. Therefore, correction in anaphase is likely to occur spontaneously, following physical laws, as previously suggested in Gay et al. (2012). Consequently, the disruption of KT viscoelasticity may drive spindle collapse or aneuploidy. Proper KT viscoelastic properties could also be essential for satisfying the spindle assembly checkpoint because spindle checkpoint proteins are integrated within the substructure of the KT, placing them in a prime location to respond to mechanical inputs (Varma et al., 2013). Thus, through the use of laser microsurgery, Cojoc et al. (2016) have begun to decipher the contribution of mechanical properties in the mitotic spindle for the maintenance of genome stability.

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