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Cell Division: Thinking Inside and Out

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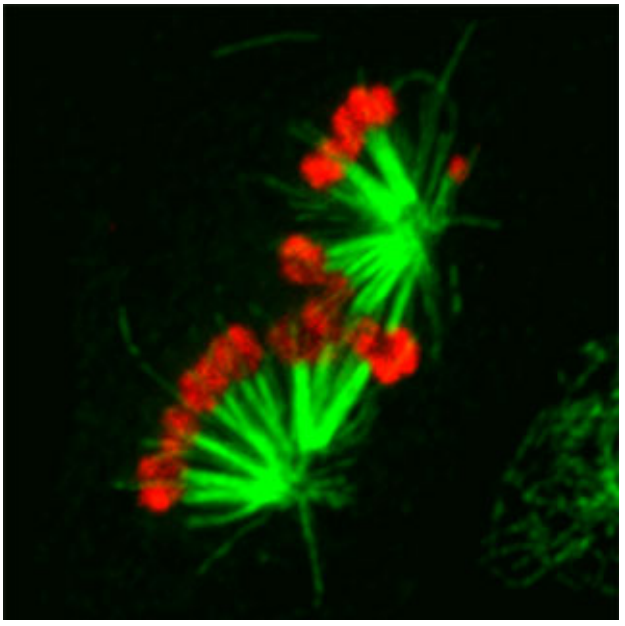
Caitlin Smith

Researchers are teasing apart the complex events governing mitosis. But can they find and measure the forces involved in this fundamental process? Caitlin Smith reports.

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When a cell contains too many or too few chromosomes, there's usually some consequence. For example, this issue is common in human cancers and tumor development. A cell gets its chromosomes during the M (mitosis) phase of its parent cell, when the neatly doubled and aligned pairs of chromosomes are elegantly separated to opposite poles of the dividing cell. When errors occur and the chromosomes are not evenly divided, an abnormal number of chromosomes—known as aneuploidy—results, possibly having some rather unhealthy consequences. Oddly, despite the importance of this process, we know little about the physical and mechanical forces that pull these DNA-protein structures apart during mitosis.

But now, researchers are developing new approaches to get a handle on the forces that pull apart chromosomes during mitosis and are gaining some rather interesting insights.



Spindles during mitosis. Source: T. Maresca.

A New Twist on Inside-out

One way to study the forces governing mitosis is by removing the cell membranes, leaving a cell-free mitotic system. The advantage of cell-free systems is that removing the cell membrane renders the cellular contents more accessible to mechanical manipulation of organelles. Until recently, many researchers used the extracted contents of amphibian eggs from *Xenopus* to prepare cell-free systems. Despite its merits, the *Xenopus* system also has disadvantages, such as the lack of genetic manipulation and a low rate of mitosis.

But Postdoctoral fellow Ivo Telley and colleagues in the lab of senior scientist Anne Ephrussi at the European Molecular Biology Laboratory (EMBL) have a solution: a cell-free system using extracts from *Drosophila* embryos, as described in a paper published in *Nature Protocols* (1). This innovation allows genetic manipulation of the mitotic machinery while taking advantage of a particular stage in early *Drosophila* embryonic development, the syncytial blastoderm stage. By this stage in early embryos, DNA-containing nuclei have been duplicated on a massive scale without corresponding cell divisions in the course of an hour, resulting in over a thousand nuclei (each about one micrometer wide) within an embryo (about half a millimeter wide) that is still a single cell (2). Telley and colleagues extracted the contents of one of these syncytial blastoderm embryos to create a cell-free system rife with mitotic nuclei.

Telley's interest in the biomechanical forces at work in cells led him to this project, in which chromosome segregation exemplifies a mechanical process that uses chemical energy to power mechanical work. Most research on the mitotic machinery has been conducted by live-cell imaging, but that technique doesn't provide direct information about mitotic forces. "The causes for the motion, the forces, are implied by assuming a particular model," says Telley. "This is rather unsatisfactory, as in mechanics a model couples effect (motion) with its cause (force). If the latter is missing, the model remains speculative."

Thus, a crucial feature of the cell-free *Drosophila* system is its potential to allow researchers to separate cause and effect. With the ability to examine mitotic forces directly, rather than indirectly, the system engineered by Telley and colleagues is on the brink of yielding new insights into mechanisms of

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mitotic force generation—especially with the application of genetic manipulations such as inducible gene expression, mutant generation, and fluorescent probe labeling. "In a next step, mechanical measurement tools have to be developed, allowing accurate registration of the forces that build the mitotic machinery and pull on the chromosomes," says Telley. "Only then will we have a complete picture of the chemomechanical energy transfer."

Thinking Inside the Box

In contrast, Tom Maresca, assistant professor at the University of Massachusetts, Amherst is studying the force that pulls on the chromosomes from within the cell. His lab investigates how forces generated by motor proteins and microtubule dynamics regulate the interaction between spindle microtubules and the kinetochores that assemble on each sister chromatid during cell division. “Chromosome biorientation generates tension across sister kinetochores that stabilizes kinetochore-microtubule attachments,” says Maresca. “Erroneous, nonbioriented attachments are commonplace but typically short lived due to the action of an error correction pathway that destabilizes attachments that do not support tension.”

One of the forces involved in mitosis is the polar ejection force (PEF), of which we know little save that it is thought to be generated by the protein chromokinesin, known as NOD in *Drosophila*. The Maresca lab studied the PEF and its proposed role in aligning chromosomes. The team created cell lines that expressed fluorescently labeled NOD with an inducible promoter and then quantified NOD levels by fluorescence imaging. As a result, the researchers found that cells overexpressing NOD showed more syntelic attachments —errors in which both sister kinetochores attach to the same, rather than opposing, spindle pole. Their quantitative fluorescence assay also showed a dose-dependent relationship between NOD fluorescence and syntelic attachments, suggesting that syntelic attachments were being stabilized by the PEF.

Though NOD had previously been classified as a nonmotile kinesin, the Maresca lab’s results suggest that NOD generates force in two ways—by tracking the plus ends of microtubules during polymerization and by plus end–directed motility. Further plans to investigate these ideas include isolating these mechanisms. “First off, we are trying to reconstitute plus end–directed motility and tip tracking by NOD in vitro,” says Maresca. “We are also generating separation of function mutants to tease apart the relative contributions of tip tracking and motility to force production and PEF-mediated stabilization of syntelic attachments.”

Maresca suggests that future studies may shed light on the magnitude of the force pulling on kinetochores. Analysis of the transmission of force through kinetochores, which are somewhat akin to focal adhesions, may benefit from the techniques used to study focal adhesions, such as combining high-resolution imaging with biosensors. Optical or magnetic traps have also been used to study kinetochores in vitro, but Maresca is uncertain that such methods would work in living cells. He thinks that their PEF assay can overcome some of the limitations of other biophysical approaches by taking advantage of the cellular machinery to introduce forces from internal rather than external sources.

In the end, having too many kinetochores, and hence too many chromosomes after mitosis, can be the difference between life and death for a cell. Although it seems logical that therapeutic drugs currently on the market to treat cancer might be work by disrupting the cell spindle assembly in cancer cells and, as a result, killing them, recent work suggests that effective chemotherapy drugs—such as taxol—

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Matt Moreno · San Diego, California

Interesting Idea, but still a coincidental observation, maybe after a zit forms the zit environment selects for specific strains. They really need to show a connection between the bad bacteria strains and acne. This is where a dermatologist and MIcrobiologist can team up.

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Mark A. Plough · Texas State Technical College

One bit of research leads to another. This could lead to other treatments but the Acne is so difficult for some at a difficult time of life.

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do not work that way at all.

“Regardless of how ongoing trials of mitosis-specific drugs turn out, I think that it cannot hurt to reevaluate the philosophical underpinnings of how novel antimitotic chemotherapies could work,” says Maresca. “Targeting unique yet commonly shared aspects of cancer cell division—for example, mitosis with multiple centrosomes—is a relatively new idea with a lot of promise.”

References

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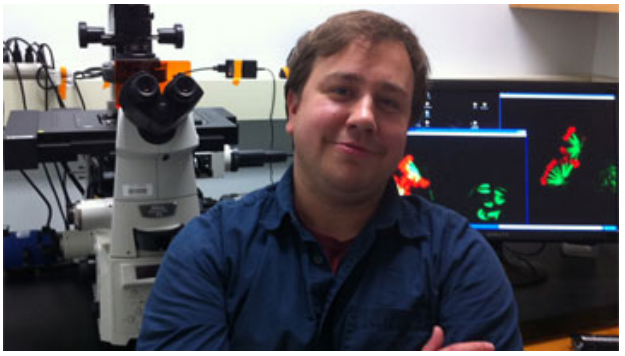
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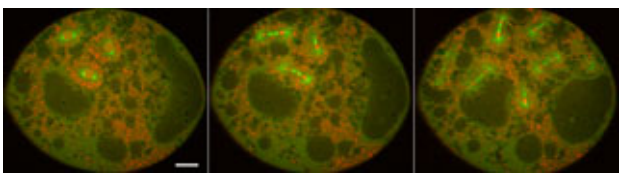
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Tom Maresca, assistant professor at the University of Massachusetts, Amherst, MA. Source: Thom Maresca



Nuclear divisions in a cell-free extract system. Microtubules (in the mitotic spindle) are green; DNA in the nuclei (and also lipid droplets in the cytoplasm) are red. The large dark spheres are either yolk from the embryo or oil . The series of images displays two consecutive divisions. One whole division cycle takes about nine minutes. Bar, 20 µm. Source: I. Telley.

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