Better 'mousetrap' discovered in fruit flies might stop human cancer-driving kinase in its tracks

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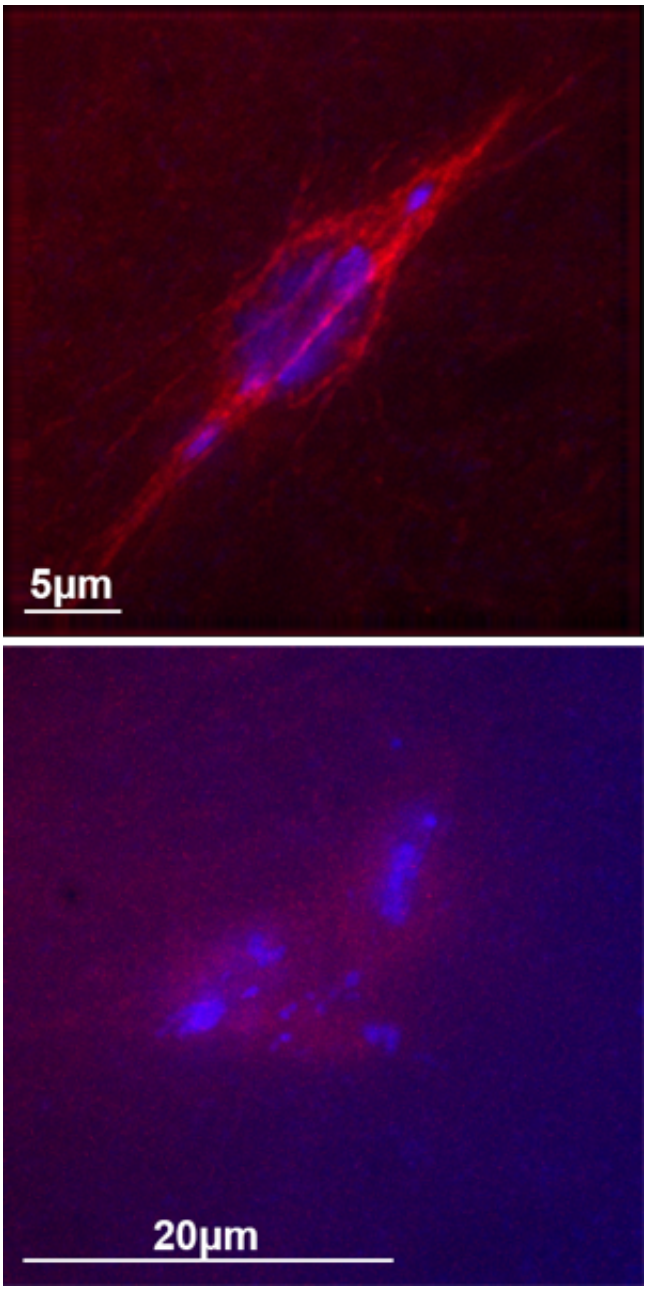

The top image shos: Fruit fly oocytes with fully functional Mtrm protein produce fully formed spindles. The bottom image shows: Without functional Mtrm protein, oocytes suffer catastrophic destruction of chromosomes and other structures required for cell division. DNA is shown in blue, the meiotic spindle in red. Credit: Image: Courtesy of Amanda Bonner, Stowers Institute for Medical Research

A seemingly obscure gene in the female fruit fly that is only active in cells that will become eggs has led researchers at the Stowers Institute for Medical Research to the discovery of a atypical protein that lures, traps, and inactivates the powerful Polo kinase, widely considered the master regulator of cell division. Its human homolog, Polo-like kinase-1 (Plk1), is misregulated in many types of cancer.

Stowers Investigator and senior author R. Scott Hawley, Ph.D., hopes that this highly selective kinase trap might give drug developers, who are working to inhibit Polo's crucial role in driving the multiplication of cancer cells, a new method to inactivate Polo without blocking other vital kinases in normal cells. "Our discovery will give people who do drug discovery a new way of thinking about inhibitors for Polo kinase," says Hawley. "At least that's my hope."

In a paper published in this week's online edition of the *Proceedings of the National Academy of Science* (*PNAS*), the Stowers researchers reveal in detail how Matrimony (Mtrm) stops the Polo kinase in its tracks in egg cells in the fruit fly *Drosophila melanogaster*. Hawley calls the most likely method by which Mtrm might bind and repress Polo the "mousetrap model." The Matrimony protein, which is expressed only in developing oocytes or egg cells, offers the Polo kinase "cheese" at its N-terminal end in the form of three phosphorylated amino acids that resemble Polo's favored canonical binding sites: phospho-serine or phospho-threonine residues.

Hawley explains, "The way we think of it, the N-terminal region of Matrimony serves as bait. To Polo, it looks like a canonical binding site with three such residues, saying 'Come look at me. I've got a phosphate and I'm a serine. Or I'm a threonine and I've got a phosphate,' because that's what Polo wants." As soon as Polo takes the bait, the C-terminal end of Matrimony wraps around Polo and represses its function. If the N-terminal phosphates are the cheese in the mousetrap, the C-terminus

would be the lever. "It springs, and Polo is trapped and repressed," he says.

"Polo is at the top of the regulatory hierarchy in almost all dividing cells," Hawley continues. "It phosphorylates targets that either phosphorylate or dephosphorylate other targets in every regulatory pathway in cell division. The fact that egg cells need to shut down Polo function to divide is a fascinating exception to this rule."

Hawley discovered the Matrimony gene in 2003. Over time, the Hawley lab learned that Mtrm was a critical player in the cell divisions that occur as an egg is being made. Using fly genetics, the researchers knocked out one and then both copies of the Mtrm gene in female flies. With one functioning Mtrm gene, the oocytes could make it through the two rounds of meiosis absolutely required for haploid reproduction, albeit with a high risk of chromosome defects. With both copies of Mtrm disabled, the oocyte suffered catastrophic destruction of chromosomes and other structures required for cell division. Yet, Mtrm also turned out to be a rare example in *Drosophila* of a protein that can stably bind (and turn off) Polo kinase.

Mtrm seemed to be facilitating meiotic cell division by shutting down Polo. But how did the Mtrm protein manage to slow Polo and stop its action? Answering that question took seven years. According to Hawley, it required important collaborations with the Stowers Institute's core facility in proteomics to characterize the Mtrm::Polo interaction and with the Stowers imaging facility to use an advanced imaging technology to follow the interaction of the two proteins in living oocytes. The project was initially started by S. Kendall Smith, an M.D.-Ph.D. student from the University of Kansas Medical School. After Smith graduated, Amanda Bonner, a research technician, assumed full responsibility for guiding the project and bringing it to its completion.

The project's success helped Bonner transition from her position as a technician in Hawley's lab to a graduate student in the first class of the new Stowers graduate school. The experimental results speak for themselves, she says. "The important thing was finding a small protein that can inhibit Polo. It provides some real therapeutic possibilities because Polo is misregulated in so many types of cancer. To find something small and specific to Polo that doesn't interact with anything else is pretty exciting."

For a basic researcher like Hawley, making a discovery that might have direct therapeutic impact is doubly exciting. "We are a *Drosophila* genetics lab, but there are lots of people out there in drug discovery working on Polo. I'm hoping that someone like that will read this and my other papers and think, 'I wonder if I can use this as a means of turning down Polo kinase'." Making a basic discovery about cancer is thrilling in another way for Hawley. "I have been funded by the American Cancer Society for almost 26 years, and I've been an American Cancer Society Research Professor for the last nine years. During that time, I think my contributions to chromosome biology have added to basic research that helps us understand how tumor cells divide. Now, I've actually done something that has a practical application."

Provided by Stowers Institute for Medical Research