A protein hidden in plain sight helps cells time their escape

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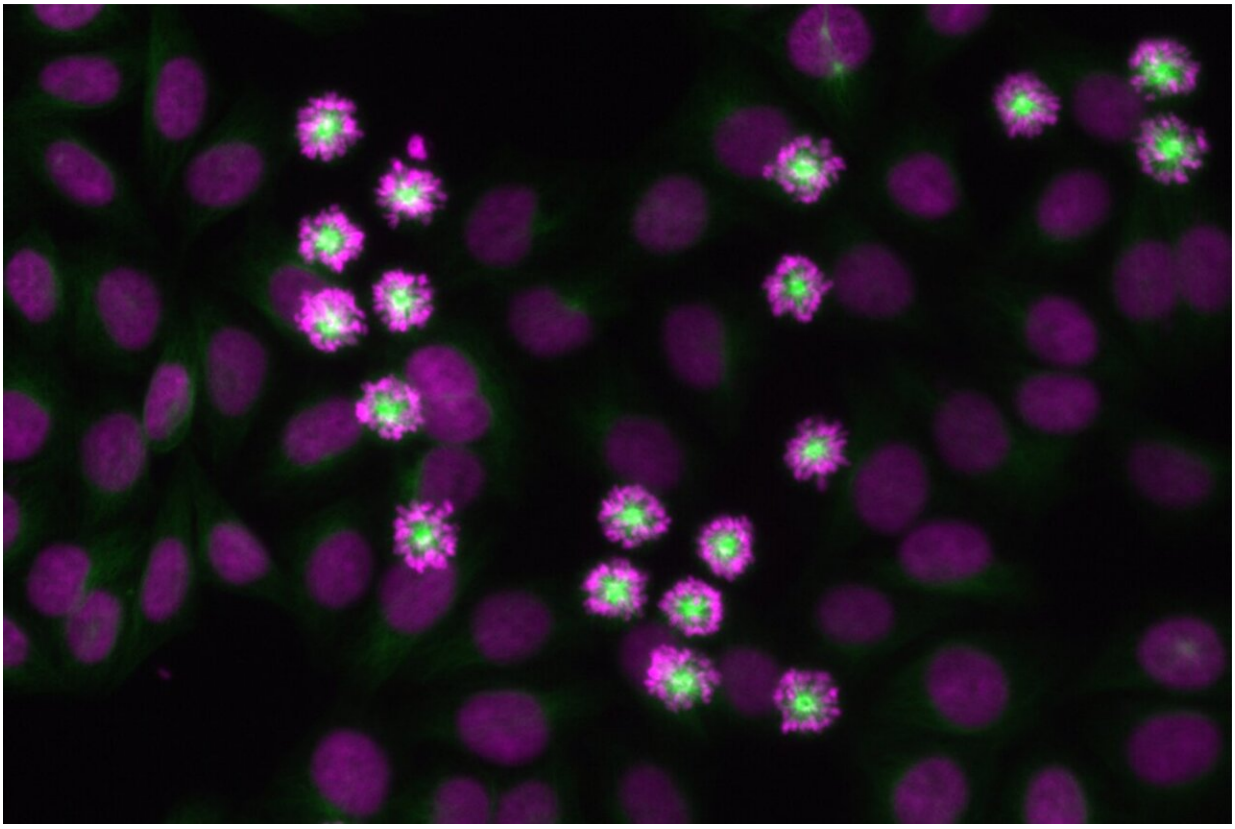

Cells in a state of arrest, or cell division that has been paused due to the detection of errors. Credit: Kuan Chung Su/ Whitehead Institute

When a cell is getting ready to divide, it needs to duplicate its DNA, which is divided among its chromosomes, and arrange the chromosomes

so that each new cell gets one complete set. If the chromosomes get sorted incorrectly, the resulting cells with the wrong number or set can become dysfunctional, or even cancerous.

Because the risks are so severe, cells have evolved strong controls to ensure that upon division, each of the daughter cells has the correct chromosomes. If a cell's machinery detects errors while the cell is preparing to divide, division is paused until those errors are corrected.

However, if division gets paused for too long, a state called being in arrest, the cell will eventually die. To escape this fate, every type of cell has a different timer for how long it will stay in arrest before escaping. When the timer runs out, cells exit the process of cell division without completing it, and resume life with double the normal number of chromosomes.

Researchers have wondered what mechanisms determine how long a cell will remain in arrest and how they manage to escape it. The question is particularly important in the context of cancer cells, which can use early escapes from arrest to evolve—changing their sets of chromosomes—and resist common cancer drugs.

New research from Whitehead Institute Member Iain Cheeseman and postdoc Mary-Jane Tsang identifies a way in which cells set their timers for arrest. The key player is a previously undiscovered variant of a known protein, CDC20.

What Cheeseman and Tsang discovered, as published in *Nature* on April 26, is that cells produce both full-length and shortened, alternative versions of CDC20, and that the shifting ratio of these versions determines when cells will escape arrest.

Alternative proteins like these are very hard to find, because cells don't

make them in the way that researchers and common analytic tools typically look for, but researchers including Cheeseman are coming to appreciate their prevalence and importance to biology.

"By looking at data in a new way, we were able to discover this alternative protein that turns out to be central to a very important process in cells," Tsang says. "The protein has been there all along, but no one knew to look for it because the cell doesn't make it in the traditional way."

CDC20—the full-length protein, that is—has a well-known role in cell division. If no issues are detected at the checkpoint before chromosomes are pulled apart, then CDC20 binds to and activates a molecular complex called the anaphase-promoting complex (APC/C), which in turn initiates the end stages of cell division. If an issue is detected, then a mechanism called the spindle assembly checkpoint (SAC) inhibits CDC20, arresting cell division.

Tsang discovered that CDC20 plays another important role at this checkpoint, thanks to its previously undetected alternatives. As a protein, CDC20 is assembled according to a genetic sequence contained in messenger RNA. However, Tsang found that sometimes the machinery translating the CDC20 RNA into protein skips the normal starting point, and begins following the instructions from one of two unofficial starting points farther down the RNA sequence, which causes it to create alternative short versions of the molecule. These short versions vary from the full-length protein in one crucial way: they are not inhibited by the SAC. This means that the cell cannot stop them from activating the APC/C, even in the presence of errors that should arrest cell division.

This difference between versions of CDC20 enables cells to set a timer for arrest. Early in cell division, the APC/C is most likely to be bound by full-length CDC20, because cells produce more of the full-length protein

than the alternatives. This keeps the cells responsive to the signal to enter arrest.

As cells spend more time in arrest, they continue to produce all versions of CDC20, but they break down full-length CDC20 faster than the shorter versions. The ratio of full-length to short CDC20 shifts in favor of the short versions. Eventually, the ratio shifts enough that the APC/C is most likely to be bound by short CDC20, which means that the SAC can no longer inhibit it. At this point, the timer runs out: the cells activate the APC/C and escape arrest.

A cell's arrest timer is therefore determined by factors that affect its starting levels of full-length and short CDC20 and the speed at which it makes and breaks them down, such as what machinery the cell has active for translating RNA. These factors vary from cell type to cell type, so different cell types have different length timers. Tsang notes that this is likely not a complete picture of how cells set their timers—other molecules than CDC20 may be able to affect timer duration—but the shift in CDC20 ratio is a key regulator of the process.

Understanding how cells set their timers helps to explain why some cancer cells are better at resisting certain cancer drugs. Drugs that work by trapping cancer cells in arrest (to then be killed) are common treatments for breast, ovarian, and other cancers. The researchers found that different cancer cell lines had different ratios of full-length to short CDC20. This correlated with how long the cells would spend in arrest before escaping, and, correspondingly, to how effective arrest-causing drugs were against them.

Additionally, when Tsang added full-length CDC20 to cells that only had the short version, the cells became more sensitive to the drugs. These findings could be useful for predicting whether arrest-causing drugs will be effective for a given patient, and they also suggest a possible strategy

for sensitizing resistant cancer cells.

This work has shifted the Cheeseman lab's focus towards searching for other proteins hiding in plain sight. The mechanism that allows cells to make short variants of CDC20 could do the same for many other proteins, and other mechanisms may also create variant proteins.

"The differential turnover of these forms of CDC20 creates an elegant timer for arrest, and we never would have known that if Mary Jane hadn't looked at CDC20 in a way no one else had thought to before," says Cheeseman, who is also the Herman and Margaret Sokol Professor of Biology at the Massachusetts Institute of Technology. "This work demonstrates that there's a world of hidden biology out there waiting to be discovered."

More information: Mary-Jane Tsang et al, Alternative CDC20 translational isoforms tune mitotic arrest duration, *Nature* (2023). DOI: 10.1038/s41586-023-05943-7

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