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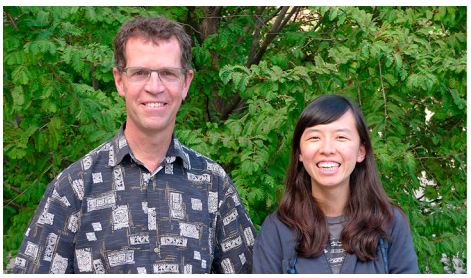
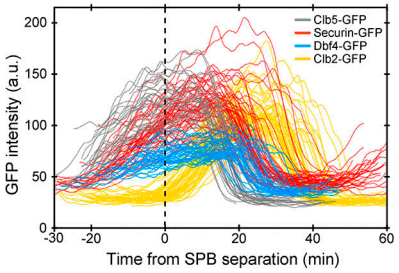
How mitosis keeps itself in order

Researchers describe how multiple mechanisms ensure that mitotic proteins are degraded in the correct sequence.

Focal Point





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David Morgan (left), Dan Lu (right), and colleagues (not pictured) describe how budding yeast ensure that proteins are degraded in the correct order during mitosis. By analyzing the disappearance of APC/C^{Cdc20} substrates in individual yeast cells (right), the researchers find that the S phase cyclin Clb5 (gray) is targeted for degradation six minutes earlier than the chromatid cohesion regulator securin (red). This difference is maintained by multiple mechanisms that either promote or inhibit the substrate proteins' direct or indirect association with the APC/C^{Cdc20}.

Cells progress through mitosis by switching protein activities on and off in a clearly defined order. The ubiquitin ligase APC/C deactivates mitotic proteins by targeting them for degradation by the proteasome. The APC/C is activated by two different subunits that recognize short sequence motifs, known as D and KEN boxes, in the target proteins. In early mitosis, once the spindle assembly checkpoint (SAC) has been satisfied, the APC/C partners with the activating subunit Cdc20 to promote the cell's entry into anaphase. The APC/C then pairs up with Cdh1 to degrade a different set of substrates and promote the cell's exit from mitosis. But even substrates targeted by the same activating subunit are degraded in a specific sequence. Lu et al. dissect the different ways that mitotic budding yeast achieve this orderly progression (1).

Protein degradation during yeast mitosis is typically studied using synchronized cell cultures and Western blotting. "The problem is that the cells rapidly lose synchrony, and you're only looking at the average behavior of the population," explains David Morgan from the University of California, San Francisco. "We wanted to get a more precise view." Morgan and colleagues, led by graduate student Dan Lu, therefore followed the disappearance of GFP-tagged APC/C^{Cdc20} substrates from individual yeast cells by fluorescence microscopy, an approach that allowed them to measure the timing, rate, and inherent variability of mitotic protein degradation (1).

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Lu et al.'s analysis revealed that the APC/C^{Cdc20} initially degrades the S phase cyclin Clb5 and then, six minutes later, targets a protein called securin for destruction, triggering the separation of sister chromatids and the yeast cell's entry into anaphase. "That six-minute difference would be very hard to see by any other method," Morgan says. Moreover, the precision of the single-cell analysis allowed the researchers to investigate the mechanisms underlying this six-minute gap.

Lu and colleagues first examined the role of the SAC, which inhibits the APC/C^{Cdc20} until the cell's chromosomes are correctly attached to the mitotic spindle. In human cells, the SAC prevents the destruction of securin but has little effect on the degradation of the mammalian S phase regulator, cyclin A. Lu et al. found that Clb5 was degraded a few minutes earlier in yeast lacking a functional SAC, indicating that the checkpoint usually inhibits Clb5 turnover. In contrast, disabling the SAC had no effect on the timing of securin degradation and anaphase onset. "That's the exact opposite of what happens in human cells," Morgan says. "So budding yeast use other mechanisms to ensure securin is degraded at the right time."

One mechanism delaying securin's degradation is the phosphorylation of two residues near the protein's D and KEN boxes by the cyclin-dependent kinase Cdk1, which inhibits securin's ubiquitination by the APC/C^{Cdc20} (2). Lu et al. found that mutating these two phosphorylation sites to non-phosphorylatable alanine brought securin's degradation forward by two minutes.

Clb5 was still degraded before non-phosphorylatable securin, however, so Lu et al. looked for mechanisms promoting the cyclin's early destruction. In human cells, cyclin A's degradation is accelerated by its association with Cdk1 and an accessory protein called Cks1, which together link cyclin A to the APC/C^{Cdc20} (3). Lu et al. found that the same mechanism applies to Clb5. Clb5 mutants unable to bind Cdk1 were degraded later than the wild-type protein, a phenotype that could be reversed by fusing Clb5 directly to Cks1.

Lu et al. then uncovered one more factor that makes Clb5 face its Waterloo. Bioinformatician Norman Davey found that Clb5 contains an ABBA motif, a short amino acid sequence that, like the D and KEN boxes, binds directly to the APC/C activators Cdc20 and Cdh1. Clb5 mutants lacking both the ABBA motif and the ability to bind Cdk1 were degraded at approximately the same time as securin.

It's not clear why yeast cells are so determined to degrade Clb5 before securin that they use multiple mechanisms to enhance Clb5's affinity for the APC/C^{Cdc20} and decrease securin's. Another mystery is how these differences in affinity result in Clb5 and securin being degraded at the same rate but at different times, instead of being degraded at the same time but at different rates. "That's a puzzle we're trying to work out now," Morgan says.

1. Lu, D., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201402041.
2. Holt, L.J., et al. 2008. *Nature*. 454:353–357.
3. Di Fiore, B., and J. Pines. 2010. *J. Cell Biol.* 190:501–509.