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A little Cdc20 goes a long way

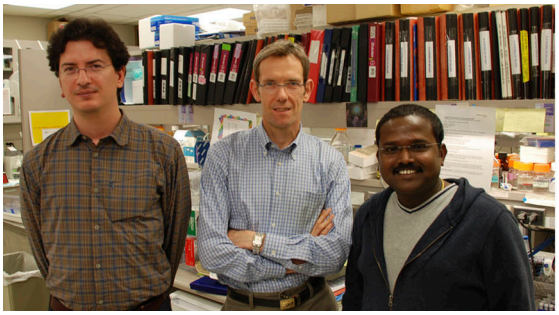
Hypomorphic mice reveal new details of Cdc20 and cyclin B1's mitotic activities.

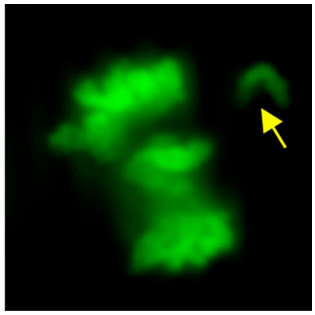
Cdc20 drives mitotic cells from metaphase into anaphase. Once metaphase chromosomes are aligned at the center of the mitotic spindle, Cdc20 activates the E3 ubiquitin ligase APC/C to trigger the degradation of cyclin B1 and securin, allowing sister chromatids to separate and move toward opposite poles (1). Cdc20 is so essential for mitotic progression that mouse embryos lacking the protein fail to pass the two-cell stage (2). "It's easy to assume that you'll be in big trouble if you don't have near-normal levels of important proteins like Cdc20," says Jan van Deursen, from the Mayo Clinic in Rochester, Minnesota. "But what happens if there's enough Cdc20 for cells to get through mitosis but not enough for them to do it perfectly?" To find out, van Deursen and colleagues generated a series of mice expressing progressively lower amounts of Cdc20 (3).

Malureanu et al. made animals expressing as little as 15–20% of normal Cdc20 levels. Yet the mice seemed completely healthy. "We were very surprised, and also disappointed," admits van Deursen. A closer look at isolated cells, however, revealed that aneuploidy—the presence of abnormal chromosome numbers—was increased in Cdc20 hypomorphic mice. "We looked at dividing cells and saw misaligned chromosomes, reflecting problems with kinetochore–microtubule attachments," van Deursen explains. Cdc20 hasn't been implicated in linking chromosomes to the spindle before, though a small portion of the protein localizes to kinetochores in wild-type cells. This localization was reduced in Cdc20-depleted cells, suggesting that Cdc20 could have a direct role in capturing microtubules to accurately segregate sister chromatids.

Alternatively, lower Cdc20 levels could indirectly affect chromosome alignment by reducing APC/C's ability to target cyclin B1 and other mitotic proteins for destruction, resulting in the misregulation of kinetochore–microtubule attachments.

Focal Point





(L-R) Liviu Malureanu, Jan van Deursen, Karthik Jeganathan, and colleagues (not shown) uncover new details of Cdc20's mitotic function by generating mice expressing greatly reduced amounts of the protein, which activates the APC/C ubiquitin ligase to drive mitotic cells into anaphase. The mice are overtly normal, but have higher levels of aneuploidy, probably due to defects in chromosome alignment (far right), indicating an additional function for Cdc20 in kinetochore–microtubule attachment. The approach also revealed that the mitotic regulator cyclin B1 is actively synthesized during metaphase, at the same time that it is targeted for destruction by Cdc20 and the APC/C.

Indeed, rather than gradually declining during metaphase, cyclin B1 levels increased as Cdc20 hypomorphic cells went through mitosis. Malureanu et al. found that cyclin B1 mRNA was translated during metaphase. Anaphase onset was delayed in Cdc20 hypomorphic cells, but blocking cyclin B1's mitotic synthesis reduced the protein's level and restored the timing of the metaphase-to-anaphase transition. Cyclin B1 was also synthesized in wild-type mitotic cells, though this is usually masked by the protein's turnover at the hands of Cdc20 and the APC/C.

> "Our findings open up a more dynamic view of cyclin B in mitosis."

Why would metaphase cells simultaneously synthesize and degrade cyclin B1? Perhaps, says van Deursen, because the APC/C isn't as selective as commonly thought. The complex appears to target cyclin A early in mitosis before turning its attention to cyclin B1, though how this order is maintained is unknown. Instead, van Deursen suggests, both cyclins may be targeted from the outset, but degraded cyclin B1 is initially replaced by the synthesis of new protein during metaphase.

"Our findings open up a more dynamic view of cyclin B in mitosis," van Deursen says. Rather than remaining stable until the last kinetochore is attached and metaphase is complete, cyclin B1 may be continuously degraded by Cdc20 and APC/C, which grow increasingly active as each chromosome correctly aligns. Cyclin B1 synthesis would maintain the protein's steady-state levels until all chromosomes were attached and ready for segregation.

"Our genetic approach gave us insights into Cdc20 and cyclin B1 function that weren't apparent from other studies," says van Deursen. "We could find subtle phenotypes that you wouldn't see in a mixed population of siRNA-treated cells." Remaining questions include why, despite increased aneuploidy, Cdc20 mice are no more tumor prone than wild-type animals, even after treatment with carcinogens. One possibility is that the tumor-promoting effects of aneuploidy are balanced by an increased susceptibility to apoptosis when anaphase is delayed (4). Van Deursen also wants to look at Cdc20's role in meiosis and to extend the hypomorphic approach to another APC/C activator called Cdh1.

1. Yu, H. 2007. *Mol. Cell.* 27:3–16.
2. Li, M., et al. 2007. *Mol. Cell. Biol.* 27:3481–3488.
3. Malureanu, L., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201003090.
4. Huang, H.C., et al. 2009. *Cancer Cell.* 16:347–358.