Slip slidin' away of mitosis with CRL2^{ZYG11}

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The spindle assembly checkpoint arrests mitotic cells by preventing degradation of cyclin B1 by the anaphase-promoting complex/cyclosome, but some cells evade this checkpoint and slip out of mitosis. Balachandran et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201601083) show that the E3 ligase CRL2^{ZYG11} degrades cyclin B1, allowing mitotic slippage.

Mitotic cyclins were serendipitously discovered by Tim Hunt while studying mRNA translation in sea urchin oocytes in 1982. The oscillating cyclin, which disappeared every time cells divided, was subsequently shown to bind and activate Cdk1, a cyclin discovered as cdc2 in fission yeast by Paul Nurse and Cdc28 in budding yeast by Lee Hartwell a decade earlier. Tim Hunt, Paul Nurse, and Lee Hartwell shared the 2001 Nobel Prize for Physiology or Medicine for the discovery of this major cell cycle regulator, whose activity was identified as the long sought-after maturation-promoting factor discovered by Yoshio Masui and Clement Markert in 1971 (http://www.nature.com/celldivision/milestones/index.html). In the early nineties, it became clear that ubiquitin-mediated proteolysis was responsible for the oscillations of cyclin B. In 1995, a heroic effort led to the identification of the ubiquitin ligase complex by Avram Hershko, who called it cyclosome (Sudakin et al., 1995), and by Mark Kirschner, who called it the anaphase-promoting complex (King et al., 1995). Anaphase-promoting complex/cyclosome (APC/C) is apparently the largest ubiquitin ligase with a mass of 1.2 MD and 19 subunits. The APC/C has dozens of substrates and is activated in mitosis by Cdc20, throughout G1 and G0 by Cdh1, and in meiosis by Ama1 (Simpson-Lavy et al., 2010).

Entry into mitosis during prophase entails a dramatic cellular reorganization: depolymerization of the cytoskeleton microtubules and their polymerization into a mitotic spindle, breakdown of the nuclear lamina, chromosome condensation, and much more. Most of these changes are instigated by phosphorylation of hundreds of proteins by the cyclin B1–Cdk1 kinase (Holt et al., 2009). Subsequently, during prometaphase, chromosomes get attached to the spindle and get aligned on the metaphase plate in metaphase. The accurate bipolar attachment of all chromosomes to the spindle is of utmost importance for maintaining genomic stability. Any error in this process will lead to unbalanced segregation of the chromatids into the two daughter cells, resulting in cell death or to a viable aberrant cell that can cause trouble down the way. The mechanism that ensures that sister chromatids will not separate prematurely is called the spindle assembly checkpoint (SAC). This elaborate mechanism, which has puzzled researchers for many years, has recently been elucidated at the structural level (Alfieri et al., 2016). The SAC inhibits the APC/C until all chromatids are properly attached. Once they are attached, SAC inhibition of the APC/C is released, and securin and cyclin B1 ubiquitination is initiated. Securin degradation allows cleavage of cohesin (the complex that holds sister chromatids together), and sister chromatids are separated and segregated to opposite poles of the cell. Exit from mitosis into interphase is a reversal of entry into it—reassembly of the cytoskeleton and the nuclear lamina as well as chromosome decondensation. This reversal requires the inactivation of cyclin B1–Cdk1 kinase activity, brought about by APC/C-mediated degradation of cyclin B1.

Drugs that interfere with microtubule dynamics, either by preventing tubulin polymerization like nocodazole or preventing microtubule depolymerization like taxol, arrest cells in prometaphase by activating the SAC. Such drugs are commonly used for anticancer chemotherapy to target dividing tumor cells. Surprisingly, many cells treated with these drugs do eventually, after a prolonged mitotic arrest, often undergo what is called mitotic slippage and become interphase cells (Rieder and Maiato, 2004; Gascoigne and Taylor, 2008). Several mechanisms could explain slippage. The most obvious is that cyclin B1–Cdk1 gets inactivated as in yeast (Vernieri et al., 2013), but data showing that cyclin B1 is degraded in slipping cells by a proteasome-dependent mechanism go against this hypothesis (Brito and Rieder, 2006). Up until now, the APC/C was the only known ubiquitin ligase to mediate the degradation of cyclin B1 in mitosis. Some groups suggested a possible failure of the SAC mechanism after a prolonged arrest, leading to low levels of APC/C activity (Lee et al., 2010). In this issue, Balachandran et al. challenge this idea and show that slippage happens also when the APC/C is inactive. They further identify another ubiquitin ligase (CRL2^{ZYG11}) as the one targeting cyclin B1 in the cells slipping out of SAC arrest.

CRL2^{ZYG11} is a member of the huge modular cullin–RING (CRL) family of ubiquitin ligases (Cai and Yang, 2016). Substrate recognition is achieved via specific recognition subunits, like ZYG11 in the case of CRL2^{ZYG11}. CRL2^{ZYG11} has previously been shown to play a role in B-type cyclin degradation during *Caenorhabditis elegans* meiosis (Liu et al., 2004; Sonneville and Gönczy, 2004). In their current work, Balachandran et al. (2016) performed a genome-wide screen for suppressors of a temperature-sensitive mutant of CRL2^{ZYG11} in *C. elegans*. Through whole genome sequencing, they identified a strain with a homozygous mutation in the mitotic cyclin *cyb-2.1*. Cyb2.1

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is closely related to cyclin B1 in humans, and the authors observed that silencing of either cyb-2.1 or cyb-1, the cyclin B1 homologue, suppressed the lethality associated with mutant $CRL2^{ZYG11}$, suggesting that an overall decrease in mitotic cyclin B activity suppressed the *zyg-11* mutant phenotype. CUL-2 and ZYG-11 are required for CYB-1 degradation in meiosis, and Balachandran et al. (2016) investigated whether $CRL2^{ZYG-11}$ directly targets CYB-1 for degradation or facilitates the action of other E3 ligases, such as the APC/C, in mitosis. Through overexpression in HEK 293T cells, they established that $CRL2^{ZYG-11}$ influences the levels of CYB-1 in a proteasome-dependent manner and that $CRL2^{ZYG-11}$ and CYB-1 directly interact in vitro and in *C. elegans*. Further, the $CRL2^{ZYG-11}$ complex ubiquitylated cyclin B1 in vitro, and analysis of siRNA-mediated silencing of $CRL2^{ZYG-11}$ in human cells showed that $CRL2^{ZYG-11}$ only influenced the levels of cyclin B1 when the APC/C was pharmacologically inhibited. In particular, the pool of cyclin B1 localized to chromosomes was preferentially affected by knockdown of $CRL2^{ZYG-11}$. Interestingly, using time-lapse imaging in cancer cells, siRNA-mediated silencing, and pharmacological treatments, Balachandran et al. (2016) determined that $CRL2^{ZYG-11}$ is required for cyclin B1 degradation and mitotic progression when the APC/C is inhibited, but is otherwise dispensable. Further, silencing of $CRL2^{ZYG-11}$ in SAC-inhibited, mitosis-arrested cells strongly reduced the rate of mitotic slippage, whereas pharmacological inhibition of the APC/C in these cells did not lead to a significant decrease in the rate of slippage.

Like every interesting study, this one solves one outstanding problem—the mechanism of mitotic slippage, which is of great medical significance—and raises several new ones, of more fundamental biological significance. The most intriguing question raised by Balachandran et al. (2016) is the functional rationale of $CRL2^{ZYG11}$ degrading cyclin B1 in mitosis. Many proteins are targeted for degradation by more than a single ubiquitin ligase. However, it is rare that these ligases act in parallel at the same time and display specificity to a cell cycle transition or stress signal. For example, cyclin B1 is targeted by BRCA1 upon DNA damage to prevent damaged cells from entering mitosis before repair (Shabbeer et al., 2013). In contrast, $CRL2^{ZYG11}$ seems to be active in parallel to the APC/C in mitosis and to override the highly robust SAC. Given the importance of mitotic arrest and the elaborate mechanism that enforces it, it seems peculiar that cells express a bypass mechanism to short-circuit it. It is valid to claim that the observed effect is caused by nonphysiological conditions. However, given that $CRL2^{ZYG11}$ targeting of cyclin B1 is evolutionary conserved in nematodes, perhaps there is an alternative explanation. One could think of a drug-induced SAC arrest as sitting in one's car and simultaneously engaging the breaks and the accelerator in full power for as long as it takes for something to happen (please do not do this at home). Unless there is a built-in safety switch that turns off the engine, the motor will eventually burn or the breaks will give in and the car will start moving. Maybe $CRL2^{ZYG11}$ is such a safety switch turning off cyclin B1, which is the engine that drives the cell into mitosis. To appreciate such an option, we have to reconsider the term mitotic slippage that is incorrectly defined as bypass of mitosis and entry of G1. I would like to propose that cells do not bypass mitosis, but rather slip back into G2, like in the song by Paul Simon: "You know the nearer your destination / The more you're slip slidin' away." The slipped cells have not separated their chromosomes and remain 4N, they have not undergone cytokinesis, and they are likely to have an inactive APC/C. The only difference between these cells and proper G2 cells is that they have no cyclin B1. In fact, this difference is rather minor, because even though cyclin B1 is present in G2, it remains inactive until prophase. If we view mitotic slippage in this manner, degradation of cyclin B1 by $CRL2^{ZYG11}$ in response to an unsatisfied SAC makes sense. Such degradation is analogous to that of cyclin B1 by BRCA1 in response to an unsatisfied DNA damage checkpoint. Moreover, cyclin B1 inactivation in response to an unsatisfied SAC is functionally conserved in yeast, which use a different mechanism to achieve the same effect.

Mitotic slippage was in part viewed as a breakdown of the checkpoint mechanism in response to artificial and prolonged drug abuse. The discovery of the $CRL2^{ZYG11}$ pathway of cyclin B1 degradation by Balachandran et al. (2016) suggests for the first time that slippage is a genuine cellular defense mechanism.

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