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Current Biology

Cells Escape an Operational Mitotic Checkpoint through a Stochastic Process

Highlights

- Yeast cells under nocodazole arrest adapt in the presence of an active SAC
- Adaptation dynamics can be described as a stochastic process
- All cells in the population share the same propensity to adapt
- Increasing Cdc20 levels favors adaptation but does not impair the SAC

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In Brief

Bonaiuti et al. show that cells adapt to an operational mitotic checkpoint and that adaptation dynamics can be described as a stochastic process where all cells share the same propensity to adapt. Increasing Cdc20 levels makes cells more prone to adapt, although they still maintain the ability to mount a mitotic arrest.

Bonaiuti et al., 2018, Current Biology *28*, 1–10
January 8, 2018
https://doi.org/10.1016/j.cub.2017.11.031

CellPress

Please cite this article in press as: Bonaiuti et al., Cells Escape an Operational Mitotic Checkpoint through a Stochastic Process, Current Biology (2017), https://doi.org/10.1016/j.cub.2017.11.031

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Cells Escape an Operational Mitotic Checkpoint through a Stochastic Process

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https://doi.org/10.1016/j.cub.2017.11.031

SUMMARY

Improperly attached chromosomes activate the mitotic checkpoint that arrests cell division before anaphase. Cells can maintain an arrest for several hours but eventually will resume proliferation, a process we refer to as adaptation. Whether adapting cells bypass an active block or whether the block has to be removed to resume proliferation is not clear. Likewise, it is not known whether all cells of a genetically homogeneous population are equally capable to adapt. Here, we show that the mitotic checkpoint is operational when yeast cells adapt and that each cell has the same propensity to adapt. Our results are consistent with a model of the mitotic checkpoint where adaptation is driven by random fluctuations of APC/C^{Cdc20}, the molecular species inhibited by the checkpoint. Our data provide a quantitative framework for understanding how cells overcome a constant stimulus that halts cell cycle progression.

INTRODUCTION

The mitotic checkpoint, also known as the spindle assembly checkpoint (SAC), arrests cells before anaphase when conditions for successful chromosome segregation are not met [1]. Activation of the SAC leads to inhibition of the anaphase promoting complex or cyclosome (APC/C), an E3 ubiquitin ligase whose activity is essential for entry into anaphase.

APC/C is active when bound to its coactivator Cdc20. Its inhibition takes place via a series of association/dissociation reactions that result in the sequestration of Cdc20 into a core complex, which also includes the checkpoint components Mad2, Bub3, and Mad3 (the "mitotic checkpoint complex core" or MCC core) [2]. The MCC core can bind APC/C, and recently it has been shown that MCC bound to APC includes in fact 2 molecules of Cdc20 [2–4]: one taking part in the MCC core and the second required for activating APC/C, as mentioned above. APC/C bound to MCC is unable to ubiquitinate its substrates, among them Clb2–the most important mitotic cyclin in budding yeast—and securin—an inhibitor of sister chromatids separation. This prevents their degradation by the proteasome and progression into anaphase.

Most of the essential checkpoint components (e.g., Mad1, Mad2, Bub3, Mad3/BubR1) are stable, and their concentrations have been reported to be constant throughout a cell cycle. By contrast, Cdc20, the target of the SAC, is actively transcribed and translated during an arrest [5–7], when it accumulates in the nucleus. As Cdc20 production increases during an arrest, so does Cdc20 degradation, which is believed to require the binding of Cdc20 (primarily in the MCC) to APC/C [7, 8].

Cells treated with agents that impair spindle assembly, such as nocodazole, can maintain an arrest for several hours before undergoing the metaphase-to-anaphase transition and leaving mitosis—an event known as adaptation [9]. Whether cells adapt with an active checkpoint is controversial. Originally, the process was described in mammals as "slippage," since cells enter anaphase slipping through an active checkpoint [10]. In molecular terms, it was proposed that mitotic cyclins were slowly degraded despite APC/C inhibition, eventually reaching insufficient levels to sustain mitosis [10]. Mad2 localization at the kinetochores, however, may require Cdk1 activity [11, 12], supporting an alternative scenario: the slow degradation of mitotic cyclins switches off the checkpoint, which in turn allows full APC/C activation and exit from mitosis. Obviously, these are two mutually exclusive options: in one case, adaptation takes place in the presence of an active checkpoint; in the second, it requires checkpoint silencing. A clear-cut analysis to assess the state of the SAC at the time of APC/C activation during adaptation has not been performed yet in any experimental system.

Understanding adaptation also requires addressing the observed large variability in adaptation times. In yeast, the earliest adapters enter anaphase after an arrest that lasts only