Keeping order in anaphase

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Abstract

The critical components of chromosome segregation machinery are relatively well established, but how they orchestrate the relative order of events during mitosis is mostly unknown. Kamenz et al. (2015) now report in *Molecular Cell* quantitative data suggesting competing networks and adaptive thresholds in the control of mitotic exit by the Anaphase-promoting Complex.

Text

Proper chromosome segregation during mitosis depends on the tight coordination of multiple molecular and cellular events required to form two daughter cells. In anaphase, sister chromatids replicated in the preceding S phase separate into individual chromosomes. Once sister chromatids segregate, the mitotic spindle elongates and chromosomes continue moving towards the poles until they separate to form the two new daughter nuclei. Molecularly, anaphase onset is determined by the activity of an E3 ubiquitin ligase named after this function, the Anaphase-promoting Complex or Cyclosome (APC/C). By targeting critical cell cycle regulators with ubiquitin and inducing their proteasome-dependent degradation, the APC/C triggers a cascade of ordered events resulting in the formation of two new nuclei that will be later separated into individual cells after cytokinesis. The major regulatory players involved in this process have been conserved through evolution from yeast to human (Sullivan and Morgan, 2007). Yet, our view of this process lacks the quantitative perspective required to understand how these events are coordinated during mitotic exit to prevent genomic instability. New data reported by Kamenz et al. (2015) in *Molecular Cell* shed light on a level of control that permits variations in the levels of APC/C substrates without affecting the order of anaphase events.

The current view establishes that the APC/C drives the metaphase-to-anaphase transition and mitotic exit through the ubiquitination of two essential targets, securin and cyclin B (Thornton and Toczyski, 2003). These proteins control two separate pathways. Securin is an inhibitor of a protease, called separase, which cleaves the cohesin rings that hold sister chromatids together (Figure 1). Securin degradation and the subsequent cohesin

cleavage results in sister chromatid separation as a consequence of the pulling forces exerted from the spindle poles. Cyclin B, on the other hand, is the activating subunit of cyclin-dependent kinase 1 (Cdk1) and its APC/C-mediated degradation results in Cdk1 inactivation and reactivation of phosphatases, such as PP1, PP2A and Cdc14 (Grallert et al., 2015; Sullivan and Morgan, 2007). These changes contribute to several processes – generally referred to as "mitotic exit" – such as stabilization of the attachment of chromosomes to the spindle microtubules, spindle elongation and disassembly, and decondensation of chromosomes, among others. Both securin and cyclin B are degraded at the same time after APC/C activation. However, Kamenz et al. (2015) now show that variations in the ratio between these proteins in the fission yeast *Schizosaccharomyces pombe* do not alter the order of these events and the fact that they are simply targeted for degradation in parallel is insufficient to explain the robustness in the order of anaphase events. Variations in securin or cyclin B levels result in similar temporal changes in these two pathways, indicating an intriguing robustness in the relative timing. Using an elegant combination of yeast genetics, time-lapse live microscopy and computer simulation techniques, the authors demonstrate that this crosstalk is at least partially dependent on competition between securin and cyclin B for binding to the APC/C, whose concentration is a limiting factor in the system. Although this concept is not completely new (Marangos and Carroll, 2008), its implications for ordered anaphase progression and mitotic exit had not been analyzed in detail before. In the presence of altered ratio of securin/cyclin B protein levels, the competition model ensures that both proteins are ubiquitinated in an ordered manner determined by their relative levels and affinities towards the APC/C (Figure 1).

The competition model per se is also insufficient to explain the robustness in anaphase order. Kamenz and colleagues find that the threshold level of securin that needs to be reached for sister chromatid separation scales with the initial securin expression level, suggesting the presence of flexible thresholds. In an attempt to explain these results, the authors suggest that ubiquitination of securin without the subsequent degradation may be sufficient to impair securin activity, similarly to that already observed for cyclin B (Chesnel et al., 2006; Nishiyama et al., 2000). Thus, robustness arises from a combination of competition for the APC/C and the inability of ubiquitinated securin and cyclin B to bind separase or Cdk1 (Figure 1). Despite the conservation of mitotic exit regulation through evolution, securin is not essential in vertebrates, and separase can be directly inhibited by cyclin B-Cdk1 complexes (Gorr et al., 2005). However, this does not argue against the existence of flexible thresholds, and Kamenz et al. (2015 suggest that the scaling mechanism also has the capacity to ensure timing robustness against fluctuations in the cyclin B degradation kinetics.

How these adaptive thresholds respond to the different initial amounts of protein is not understood in detail. Although the combination of quantitative biology and computer modeling is very powerful, there are multiple factors that are not still ~~unclear~~ clear in the picture. For instance, we still do not understand the exact effect of ubiquitination in the inhibition of securin and cyclin B, or the putative role of deubiquitinases in the control of anaphase. Since the inhibition of the proteasome prevents anaphase onset, it is also unclear why ubiquitinated securin and cyclin B are unable to promote anaphase onset in these conditions. In addition, this study revives questions on the definition of “thresholds” in securin and cyclin B degradation. What is the nature of these thresholds? Is sister chromatid

separation or phosphatase reactivation determined by the relative amount of ubiquitinated versus non-ubiquitinated proteins? The resurrection of these very interesting questions will hopefully lead to a new quantitative view of the regulation of chromosome segregation.

Many of these questions about the specific quantitative aspects of securin and cyclin B degradation are not only critical for understanding cell division but might also shed light into the cellular alterations that accompany human disease. The mammalian securin gene (*PTTG1*) was originally characterized as an oncogene and overexpression of securin or cyclin B is commonly observed as part of the overexpression signature that defines chromosomally unstable tumors (Carter et al., 2006). The combination between competition for APC/C binding and thresholds that dynamically adjust to the degradation rate may help those cells survive to these defects in the chromosome segregation machinery. Identifying additional alterations that transform this state into a lethal phenotype thus would also have potentially relevant implications for cancer therapy.

Figures

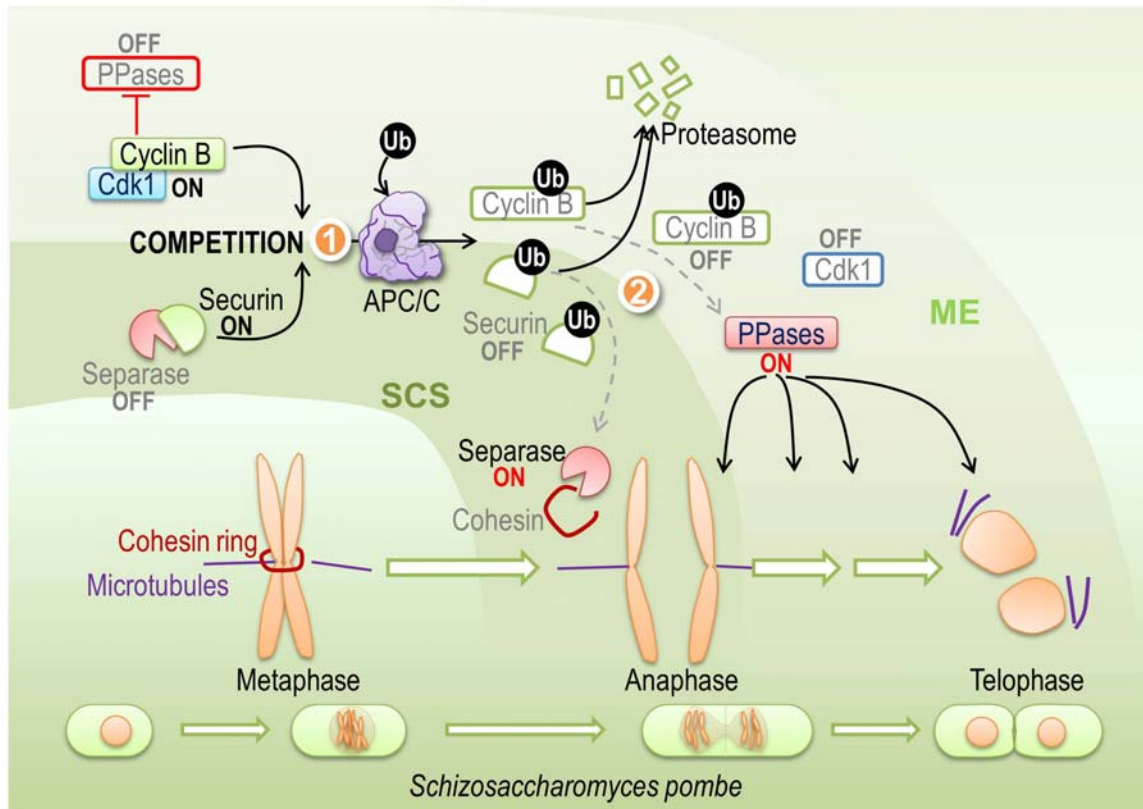

Figure 1. Coordination between sister chromatid separation (SCS) and mitotic exit (ME) events in fission yeast.

Anaphase onset is inhibited by securin, which prevents separase function, and cyclin B-Cdk1, a heterodimeric kinase complex that phosphorylates a large number of mitotic substrates and inhibits counteracting phosphatases (PPases). Upon complete bipolar attachment of chromosomes to microtubules, the Anaphase-promoting Complex (APC/C)

targets both securin and cyclin B with ubiquitin (Ub) for proteasome-dependent degradation. The parallel ubiquitination of these two factors coordinate downstream events using at least two different mechanisms. Both securin and cyclin B compete for APC/C binding (1), which buffers the relative ubiquitination of each of these components depending on their relative protein levels. APC/C activity then results in the presence of ubiquitinated, non-degraded forms (2) of securin and cyclin B, which are incapable of performing their inhibitory functions in anaphase. These forms reduce the dependence of the proteasome and permit downstream anaphase events are determined by flexible, rather than fixed, thresholds for securin and cyclin B degradation.

References

Carter, S.L., Eklund, A.C., Kohane, I.S., Harris, L.N., and Szallasi, Z. (2006). Nature genetics *38*, 1043-1048.

Chesnel, F., Bazile, F., Pascal, A., and Kubiak, J.Z. (2006). Cell cycle *5*, 1687-1698.

Gorr, I.H., Boos, D., and Stemmann, O. (2005). Mol Cell *19*, 135-141.

Grallert, A., Boke, E., Hagting, A., Hodgson, B., Connolly, Y., Griffiths, J.R., Smith, D.L., Pines, J., and Hagan, I.M. (2015). Nature *517*, 94-98.

Kamenz, J., Mihaljev, T., Kubis, A., Legewie, S., and Hauf, S. (2015). Mol Cell *60*, 446-459.

Marangos, P., and Carroll, J. (2008). Nature cell biology *10*, 445-451.

Nishiyama, A., Tachibana, K., Igarashi, Y., Yasuda, H., Tanahashi, N., Tanaka, K., Ohsumi, K., and Kishimoto, T. (2000). Genes & development *14*, 2344-2357.

Sullivan, M., and Morgan, D.O. (2007). Nature reviews Molecular cell biology *8*, 894-903.

Thornton, B.R., and Toczyski, D.P. (2003). Nature cell biology *5*, 1090-1094.