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SPOTLIGHT

# Small-number effects limit chromosome segregation synchrony

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**Feedback loops have been described as universal, recurring motifs that regulate major cell cycle transitions. In this issue, Williams et al. (https://doi.org/10.1083/jcb.202602088) show, however, that chromosome segregation synchrony in fission yeast is feedback-independent, and instead, temporal precision of anaphase is dictated by molecular noise arising from small-number stochastic effects.**

The eukaryotic cell cycle abounds with negative, positive, and double-negative feedback loops that render cell cycle transitions sharp, switch-like, and unidirectional (1). At anaphase onset, sister chromatids separate abruptly and synchronously, upon cleavage of the cohesin protein complex, a ringlike structure that entraps the two sister chromatids (2). This proteolytic cleavage is executed by a molecular scissor called separase (3, 4). The sharp and abrupt nature of this process led to the hypothesis that the anaphase switch is indeed sharpened by a positive feedback loop (5). It has been proposed that budding yeast separase accelerates the degradation of its own inhibitor securin by indirectly promoting securin dephosphorylation (separase activates the phosphatase Cdc14, which dephosphorylates securin). Dephosphorylated securin is readily recognized by the anaphase-promoting complex (APC/C; a large, multi-subunit E3 ubiquitin ligase), which triggers securin degradation through the ubiquitin–proteasome system. In contrast to the described yeast data, dephosphorylation of securin in human cells leads to a stabilization rather the destruction of securin (6). Therefore, it has remained unclear whether a separase-mediated positive feedback loop is a conserved and essential mechanism that drives chromosome segregation synchrony or whether other and perhaps simpler mechanisms are at play.

To resolve this ambiguity, Williams and colleagues employed live-cell imaging with high temporal resolution (sampling every 3.5–7 s) in combination with rigorous computational modeling (7). In a first experiment, the authors tracked sequence-specific centromere markers across the three *Schizosaccharomyces pombe* chromosomes, showing that centromeres split within a narrow time window (but not perfectly synchronous). It also showed that chromosome separation contains a stochastic component, meaning chromosomes do not separate in a strict temporal order. To investigate what controls this temporal window, the authors used a temperature-sensitive separase mutant and followed sister chromatid separation at a semi-permissive temperature. Importantly, impaired separase activity resulted in a significantly higher chromosome asynchrony and slower centromere movement toward the spindle poles. This slower pole movement indicates that either separase activity must be sustained to remove the remaining cohesin at the chromosome arms or, more speculatively (and perhaps more excitingly), separase activity after anaphase onset might be necessary to cleave yet unknown substrates. It is well known that separase can cleave substrates other than the kleisin subunits of mitotic (SCC1 also known as RAD21) or meiotic (REC8) cohesin (8). However, overexpressing separase did not significantly improve segregation synchrony. This finding implies that additional molecular mechanisms limit anaphase synchrony. The authors therefore next investigated the involvement of spindle forces in this process. They perturbed microtubule dynamics by weakening microtubule forces using the drug MBC or by stabilizing microtubules through the depletion of the kinesin-8 (*klp5*). While anaphase synchrony worsened slightly using MBC, anaphase synchrony was nearly unchanged in *klp5Δ* cells. These results thus suggest that separase activity plays a central role in anaphase synchrony, while microtubule forces only seem to play a secondary role.

As mentioned above, a prior study in budding yeast suggested that active separase drives a positive feedback loop by promoting the dephosphorylation of its inhibitor securin, thereby accelerating securin's degradation. To test whether this positive feedback also exists in *S. pombe*, Williams et al. conditionally expressed a nondegradable version of cyclin B at endogenous levels, resulting in perpetually high CDK activity. While some cells expressing nondegradable cyclin B were able to undergo nuclear division and exit mitosis, another fraction of cells was incapable of dividing the nucleus. In both cases, regardless of nuclear divis, the synchrony of sister chromatid separation was comparable to that of wild-type cells. Next, the authors utilized a nondegradable securin mutant that should decelerate the degradation of wild-type securin according to the positive

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feedback model. However, using a nondegradable securin mutant to block separase activity had no impact on the degradation rate of wild-type securin. This finding also argues against a positive feedback loop in *S. pombe*. Finally, the authors tested the possibility that a positive feedback loop could act downstream of securin degradation, e.g., through separase autoactivation. Using computational models, they simulated securin degradation and separase release, both with and without separase autoactivation. In both cases, decreased APC/C activity resulted in slower securin degradation. However, without positive feedback, the increase of active separase in this model is gradual. With a positive feedback loop, though, the increase in separase cleavage activity is sharp and occurs later. The computational model was then experimentally tested by slowing down securin degradation through a described APC/C mutant (9) or proteasome inhibition with Velcade (bortezomib). Both approaches resulted in asynchronous chromosome separation, which is inconsistent with separase autoactivation.

If positive feedback does not sharpen the anaphase switch, then what limits its precision? The study's most profound implication stems from fitting a stochastic model of cohesin cleavage to the experimental data. This simple stochastic model is based on the following assumptions and parameters: (1) a period ($\tau$) during which separase activity steadily rises to its maximum cleavage rate ($k_{max}$). (2) Separase cleaves cohesin complexes with no preference for either of the three chromosomes. (3) Sister chromatid separation occurs when the initial number of chromosomal cohesin complexes (N) is reduced to a critical threshold value (n). If the rupture forces on a cohesin complex are strong enough, the cohesin complexes will dissociate from the bound DNA independently of separase cleavage. This explains why (n) is not zero. This basic stochastic model successfully replicated the patterns of sister chromatid separation synchrony under all experimental conditions. The authors then modified the parameters in their computational model and found that the parameters (n) and ($k_{max}$) have the strongest impact on anaphase synchrony. Additionally, the number (n) was at the lowest possible value allowed within the parameter range. In other words, the model is based on the hypothesis that only a few cohesin complexes remain at the centromeres before sister chromatid separation occurs. Because the timing of cleaving the last few remaining cohesin complexes at the centromere is stochastic, this also reduces the likelihood of perfect synchrony of sister separation. This model also explains why reduced separase activity strongly impacts anaphase synchrony. Low separase activity increases the time between successful cohesin cleavage events, promoting asynchrony.

In this remarkable study, the authors convincingly demonstrate that in fission yeast, chromosome segregation synchrony is achieved independently of separase-mediated positive feedback. They also disprove the assumption that complex cellular events always require complex regulatory circuitry. Rather, the temporal precision of anaphase synchrony is dictated by a fundamental, physical constraint: molecular noise resulting from the small-number effects of stochastic events (e.g., the cleavage of only a few remaining cohesin rings). Many exciting evolutionary questions remain: for instance, did higher eukaryotes evolve additional regulatory layers, such as separase inhibition by the cyclin B–CDK complex (10), to specifically suppress molecular noise and enforce tighter synchrony?

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