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CELL CYCLE

Ubiquitin — a ticket through the checkpoint

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> “…an elegant mechanism by which the spindle checkpoint is inactivated.”

Successful chromosome separation requires the attachment of the bipolar mitotic spindle to each of the sister chromatids. To ensure reliable segregation and the equal division of chromosomes between daughter cells, a fail-safe mechanism — the spindle checkpoint — has evolved that surveys the spindle-attachment sites (kinetochores) on chromatids and arrests the cell cycle until the spindle is correctly attached. Two recent papers identify an elegant mechanism by which the spindle checkpoint is inactivated.

The separation of chromatids is promoted by the anaphase-promoting complex (APC), an E3 ubiquitin ligase that, when active, causes the degradation of proteins that arrest the cell cycle. The APC is assisted by two further proteins: CDC20 (cell division cycle-20), which recruits substrates to APC, and UBCH10, an E2 ubiquitin-conjugating enzyme that facilitates the transfer of ubiquitin to substrates. During the spindle checkpoint, the activity of the APC is inhibited by the spindle-checkpoint proteins MAD2 and BUBR1, which bind and inactivate CDC20. Upon correct spindle attachment, CDC20 is somehow released from the MAD2–BUBR1 complex and APC–CDC20–UBCH10 promotes the segregation of sister chromatids.

How is the CDC20–MAD2–BUBR1 complex regulated? Reddy *et al.* show that the checkpoint complex is regulated by ubiquitylation — a modification that often leads to protein degradation. In this instance, multi-ubiquitylation of CDC20 by APC–UBCH10 results in its dissociation from MAD2–BUBR1. It is therefore the APC itself that drives spindle-checkpoint inactivation.

However, without mechanisms to limit CDC20 ubiquitylation by the APC, the checkpoint would continually be inactivated. In a second study, Stegmeier *et al.* used a library of short hairpin RNAs in a spindle-checkpoint assay to identify the de-ubiquitylating enzyme USP44 as a spindle-checkpoint regulator. USP44 was shown to inhibit activation of the APC and could de-ubiquitylate CDC20 both *in vivo* and *in vitro*. The authors propose that USP44 antagonizes the APC–UBCH10-mediated ubiquitylation of CDC20 and keeps the spindle checkpoint active.

Upon correct kinetochore–spindle attachment, the balance between ubiquitylation and de-ubiquitylation activities must change so that ubiquitylation of CDC20 dominates. Stegmeier *et al.* made the intriguing observation that USP44 incubated with extract from spindle-checkpoint-arrested cells had enhanced activity; however, this was not seen in USP44 incubations with extract from cells arrested at another point in the cell cycle. The authors speculate that the checkpoint-arrested extract may contain a USP44 co-activator or an active kinase that upregulates the activity of USP44, which therefore keeps the checkpoint active.

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ORIGINAL RESEARCH PAPERS Reddy, S. K. *et al.* Ubiquitination by the anaphase-promoting complex drives spindle checkpoint inactivation. *Nature* **446**, 921–925 (2007) | Stegmeier, F. *et al.* Anaphase initiation is regulated by antagonistic ubiquitination and deubiquitination activities. *Nature* **446**, 876–881 (2007)
FURTHER READING Peters, J. M. The anaphase promoting complex/cyclosome: a machine designed to destroy. *Nature Rev. Mol. Cell Biol.* **7**, 644–656 (2006) | Musacchio, A. & Salmon, E. D. The spindle-assembly checkpoint in space and time. *Nature Rev. Mol. Cell Biol.* **8**, 379–393 (2007)

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