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CELL CYCLE

Destruct and arrest

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The spindle-assembly checkpoint (SAC) blocks chromosome segregation until all sister chromatids are properly attached to the mitotic spindle. It does so by inactivating CDC20 (also known as Slp1 and Fizzy) — an essential activator of the ubiquitin-ligase anaphase-promoting complex or cyclosome (APC/C), which promotes the degradation of proteins that arrest the cell cycle. Two studies now provide insights into how the SAC inhibits CDC20 and reveal a new role for CDC20 in APC/C-mediated protein degradation.

Using nocodazole (a microtubule-depolymerizing agent) to activate the SAC in live-imaging studies of human cells, Nilsson *et al.* observed that a fluorescent CDC20 fusion protein was degraded in response to SAC. Degradation of CDC20 started at the time of nuclear envelope breakdown, which coincides with the time when APC/C recognizes its early mitotic substrates. This implies that CDC20 might be a target of APC/C. Indeed, the use of small interfering RNAs (siRNAs) against APC/C or the SAC protein MAD2 blocked CDC20 degradation, which suggests that CDC20 is degraded by APC/C in response to the SAC.

Size-exclusion chromatography and quantitative immunoblotting experiments in nocodazole-treated cells revealed that CDC20 primarily interacts with the SAC component BUBR1 and not with MAD2, as previously thought.

A mutant version of CDC20 that could not be ubiquitylated could bind to the SAC protein complex and be released from it when the SAC was chemically inhibited. However, it was not degraded by APC/C and was unable to maintain the SAC-induced mitotic arrest. These findings contradict a previous model that suggests that ubiquitylation of CDC20 is needed to release it from the SAC complex and to activate APC/C. By contrast, Nilsson *et al.* show that ubiquitylation of CDC20 causes its degradation, which is required to maintain the SAC-associated mitotic arrest.

CDC20-family proteins recognize APC/C substrates through a carboxy-terminal WD40 repeat domain and recruit them to APC/C for degradation. Kimata *et al.* found that Nek2A, an APC/C substrate that interacts directly with APC/C independently of Cdc20, was not degraded in *Xenopus laevis* egg extracts that were depleted of Cdc20. This suggests that Cdc20 has an additional role besides substrate recruitment. By adding mutated versions of Cdc20 back to the egg extracts, the authors found that the amino-terminal C-box domain of Cdc20 facilitates Nek2A degradation. By contrast, the WD40-repeat domain was required for the degradation of 'canonical' substrates that are recruited to APC/C by Cdc20.

The ability of purified APC/C from Cdc20-depleted egg extracts to ubiquitylate Nek2A *in vitro* was restored by adding the C-box domain of Cdc20 to the reaction. By contrast, the ubiquitylation of canonical substrates required the WD40 repeat domain. However, canonical substrates that were directly fused to the C-box domain of Cdc20 were efficiently ubiquitylated by APC/C, thereby revealing a new role for Cdc20 in promoting substrate ubiquitylation by APC/C.

These studies represent important steps in understanding how the activity of APC/C is regulated during the cell cycle.

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PHOTODISC

ORIGINAL RESEARCH PAPERS Nilsson, J. *et al.* The APC/C maintains the spindle assembly checkpoint by targeting Cdc20 for destruction. *Nature Cell Biol.* **10**, 1411–1420 (2008) | Kimata, Y. *et al.* A role for the Fizzy/Cdc20 family of proteins in activation of the APC/C distinct from substrate recruitment. *Mol. Cell* **32**, 576–583 (2008)
FURTHER READING Peters, J.-M. The anaphase promoting complex/cyclosome: a machine designed to destroy. *Nature Rev. Mol. Cell Biol.* **7**, 644–656 (2006)