The separation of sister chromatids at anaphase cannot commence until separase cleaves the cohesin subunit Scc1. Separase is regulated by an inhibitor, securin, that seems to have contradictory characteristics—it prevents Scc1 cleavage by separase, yet at the same time it is required for separase function and correct chromatid separation. Now, Nadine Hornig, Frank Uhlmann (London Research Institute, London), and colleagues explain how securin primes separase for a rapid burst of activity in mitosis.

To promote correct separase function, securin first ensures the nuclear localization of separase. Securin levels normally drop during anaphase and do not increase again until S phase. When the group expressed securin in G1, they found it increased the amount of separase found in the nucleus. But just bringing separase to the nucleus was not enough; securin also acted as a chaperone for separase found in the nucleus. But just bringing separase to the right place and right time is not enough. Securin also prevented separase binding to Scc1. Degradation of securin at anaphase thus suddenly unleashes a high concentration of superactive separase at the right place and right time.

The group found that interaction of separase NH2-terminal and protease domains is crucial for the protease activity. Securin interacted with both domains, disrupting separase self-contact. Securin also prevented separase binding to Scc1. Degradation of securin at anaphase thus suddenly unleashes a high concentration of superactive separase at the right place and right time.

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While securin promotes separase activity, it also has two tricks to ensure that the protease does not cleave cohesin until the time is right. “Securin uses a foolproof twofold mechanism,” says Uhlmann. “It prevents separase from activating itself and at the same time prevents it from binding to its target substrate.”

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Reference: Hornig, N., et al. 2002. *Curr. Biol.* 10.1016/S096998222008151.

**Ubiquitin receptor found**

Patricia Bilodeau, Robert Piper, and colleagues (University of Iowa, Iowa City, IA) have identified an endosomal receptor complex that pushes proteins into the lumen of endosomes destined for the vacuole. This internalization step represents another possible site of regulation for controlling receptor activity.

Receptors and other plasma membrane proteins bound for destruction are tagged with one or more ubiquitin (Ub) molecules before they are internalized in vesicles that fuse with early endosomes. To be fully degraded when the endosomes fuse with the vacuole, the tagged proteins must bud off a second time to form a multivesiculated body (MVB)—a structure that has vesicles inside the lumen of the endosome. Proteins that escape this step never reach the inside of the vacuole and may still be recycled back to the cell surface. It turns out that Ub is a potent sorting signal for the incorporation of proteins into the lumenal vesicles of the MVB.

With the new results, the authors have identified the protein Hse1p, which, coupled with Vps27p, mediates the lumenal internalization decision in yeast. A complex of Hse1p and Vps27p localized to endosomal compartments and bound Ub through several Ub interaction motifs (UIMs). Mutation of HSE1 resulted in a class E phenotype, which is shared by a set of vacuolar protein sorting mutants that are defective in recycling Golgi proteins, forming MVBs, and sorting ubiquitinated proteins into the vacuole. Mutation of the Hse1p and Vps27p UIMs alone abolished sorting of Ub-tagged proteins into the vacuole, but did not cause the other class E phenotypes.

Separation of these phenotypes indicates that the Vps27p–Hse1p complex is involved in several aspects of endosomal function, one of which is to sort tagged proteins into the lumen of the endosome by binding Ub and dragging the cargo inside for destruction. Though it is not yet known how the complex mediates internalization, regulation of its activity could provide another means for controlling receptor levels. “For the most part, attention has been focused on how internalization of receptors is controlled at the plasma membrane,” says Piper. “But this is not enough. Internalization happens all the time, yet there must also be a mechanism to decide whether the receptors are recycled or degraded.”

Reference: Bilodeau, P., et al. 2002. *Nat. Cell Biol.* 10.1038/ncb815.