

The APC/C gets Trim'd down for apoptosis

Huang et al. describe how the anaphase-promoting complex (APC/C) limits cell death and how this inhibition is relieved following DNA damage.

Bcl-2 family proteins such as Bax stimulate apoptosis by promoting the release of cytochrome *c* from mitochondria. A protein called modulator of apoptosis-1 (MOAP-1) helps activate Bax, but this protein is usually degraded too rapidly for it to induce apoptosis. In response to DNA damage, however, an E3 ubiquitin ligase called Trim39 enhances cell death by stabilizing MOAP-1, though how the ligase prevents MOAP-1 turnover is unclear.

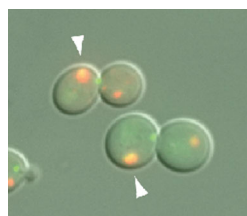
Huang et al. realized that Trim39 is homologous to a *Xenopus* E3 enzyme called Xnf7, which inhibits the APC/C, a multisubunit ubiquitin ligase that promotes mitotic exit. Trim39 also inhibited the APC/C, so the researchers wondered whether

MOAP-1 might be an APC/C substrate in the absence of an apoptotic signal. MOAP-1 was ubiquitinated by the APC/C, causing the pro-apoptotic protein to be degraded in G1 phase cell extracts. MOAP-1 ubiquitination and turnover was inhibited by wild-type Trim39 but not by a mutant version of the protein that lacked ligase activity.

MOAP-1 bound to the APC/C-activating protein Cdh1. Knocking down Cdh1 by RNAi increased MOAP-1 levels and facilitated Bax activation, enhancing cells' susceptibility to death in response to DNA damage. Huang et al. now want to investigate how DNA damage activates Trim39 and to determine how the E3 ligase then inhibits the APC/C to stabilize MOAP-1 and promote apoptosis.

Huang, N.-J., et al. 2012. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201111141>.

Prion puts yeast cells under arrest



The SPB component Spc42 (green) is sequestered into cytoplasmic deposits of misfolded Rnq1 (red).

Overexpression of a yeast prion protein prevents cells from undergoing mitosis by sequestering a key spindle pole body component, Treusch and Lindquist report.

The prion Rnq1 can adopt a self-perpetuating, amyloid conformation called $[RNQ^+]$. This conformation enables toxicity in cells overexpressing the protein. Misfolded Rnq1 molecules assemble into large, insoluble amyloid fibers, but these structures actually protect cells by removing from the cytoplasm smaller, soluble $[RNQ^+]$ oligomers that are thought to cause cell death. How these smaller, amorphous aggregates harm cells is unclear, however.

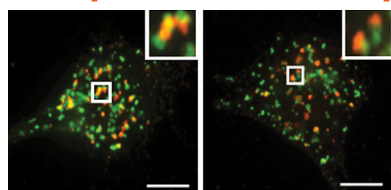
Treusch and Lindquist found that yeast overexpressing Rnq1 in the presence of $[RNQ^+]$ arrested in mitosis. The cells

failed to duplicate their spindle pole bodies (SPBs, the yeast equivalent of centrosomes) and therefore formed monopolar spindles that activated the spindle assembly checkpoint. The researchers analyzed several SPB proteins and found that Spc42, a core component required for SPB duplication, was sequestered into cytoplasmic $[RNQ^+]$ aggregates. Modestly increasing Spc42 levels was sufficient to restore normal growth to Rnq1-overexpressing yeast.

Though Rnq1 isn't normally involved in SPB duplication, the results demonstrate that misfolded proteins can cause toxicity through specific interactions with other proteins, rather than through general inhibition of the cell's protein folding and degradation machinery. Similarly specific interactions could underlie the toxicity associated with protein aggregates in human diseases like Huntington's and amyotrophic lateral sclerosis.

Treusch, S., and S. Lindquist. 2012. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201108146>.

Ubiquitin isn't ubiquitous in receptor trafficking



Unlike wild-type PAR1 (left, red), a PAR1 mutant unable to bind ALIX (right) isn't transported to lysosomes (green).

Dores et al. reveal how a G protein-coupled receptor (GPCR) can be sorted to multi-vesicular bodies (MVBs) and lysosomes without being ubiquitinated.

PAR1 is a GPCR for the protease thrombin. Like other GPCRs, activated PAR1 is downregulated by its internalization and transport to lysosomes for degradation. Unlike other receptors, however, PAR1 doesn't have to be ubiquitinated to move to lysosomes, and ubiquitin-binding components of the ESCRT complexes—which sort GPCRs and other cargo into intraluminal vesicles (ILVs) within MVBs en route to lysosomes—are not required for PAR1 trafficking.

Dores et al. found that, nevertheless, PAR1 passed through

ILVs on its way to lysosomes and that a PAR1 mutant that couldn't be ubiquitinated took an identical route. The sorting of PAR1 into ILVs required CHMP4, a core component of the ESCRT-III complex, which releases vesicles into the interior of MVBs. PAR1 sorting also depended on ALIX, a CHMP4-interacting protein that bound to a conserved motif in PAR1, thereby linking the receptor to the ESCRT-III sorting machinery.

ALIX links viral proteins to the ESCRT-III complex during viral budding, but the protein hasn't been implicated in mammalian receptor trafficking until now. Several other GPCRs have the same ALIX-binding motif as PAR1. The interaction with ALIX allows PAR1 to bypass the usual requirements for ubiquitination and ubiquitin-binding ESCRT subunits. Yet PAR1 is still ubiquitinated in response to thrombin. Senior author JoAnn Trejo now wants to investigate the function of this modification and to understand why PAR1 uses a sorting mechanism different from that used by most other GPCRs.

Dores, M.R., et al. 2012. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201110031>.