In This Issue

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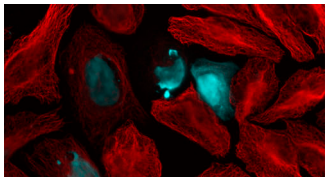
Polyglutamylation makes the cut



Cells expressing the polyglutamylase TTLL6 (cyan) lose their microtubules (red) because the filament-severing protein spastin is hyperactivated.

Lacroix et al. report that the addition of long glutamate side chains to tubulin stimulates microtubule disassembly by the microtubule-severing protein spastin.

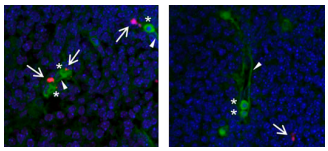
The C-terminal tails of tubulin subunits can be modified in different ways, which might alter the recruitment of molecular motors and other microtubule-binding proteins. A family of glutamylase enzymes generates glutamate side chains of varying lengths on tubulin tails. The purpose of this modification isn't clear, though it may stimulate microtubule severing: in the protozoan *Tetrahymena*, blocking tubulin glutamylation has a similar effect to deleting the microtubule-severing protein katanin.

Lacroix et al. transfected HeLa cells with different glutamylases and found that enzymes that added long glutamate side chains to tubulin stimulated microtubule disassembly. The addition of shorter chains by other family members had no effect. The microtubules were disassembled by the severing protein spastin, whose activity was boosted by the polyglutamylated tubulin tails—possibly because the negatively charged glutamate residues attract a positively charged region of spastin's active site. Microtubules assembled in vitro from polyglutamylated tubulin were quickly dismantled by spastin, while non-glutamylated microtubules were more stable.

Spastin's activity is reduced by mutations associated with spastic paraplegia; defects in tubulin polyglutamylation might therefore cause neurodegeneration as well. Senior author Carsten Janke thinks that the modification facilitates microtubule cutting by spastin and katanin, but that additional signals ensure filaments are only disassembled at the right moment. He now plans to investigate how polyglutamylation affects the activity of other microtubule-interacting proteins, such as molecular motors.

Lacroix, B., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201001024.

Deconstructing FAK function



Apoptotic cells (red) are found in blood vessels (green) lacking FAK (left), but are absent from vessels expressing a kinase-deficient mutant (right).

Focal adhesion kinase (FAK) has multiple functions in endothelial cells, but only some of them require the protein's kinase activity, Zhao et al. report.

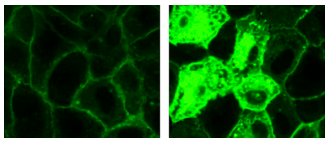
FAK promotes the migration and survival of many cell types, including endothelial cells. Mice lacking FAK in their vascular system die before birth due to defects in angiogenesis, but how FAK supports blood vessel development is unclear. In vitro experiments suggest that FAK sometimes acts as an adaptor for other kinases instead of phosphorylating target proteins itself. To investigate whether FAK's kinase activity is required for angiogenesis, Zhao et al. generated mice that only express a kinase-deficient version of the protein in their vasculature.

Although these mice still died before birth, they survived for a couple of days longer than animals without any FAK in their vascular system. Endothelial cells completely lacking FAK are prone to apoptosis, but kinase-dead FAK boosted cell survival by suppressing the cyclin-dependent kinase inhibitor p21. But other problems emerged as the embryos developed further: blood vessels were dilated and fewer in number. The researchers found that endothelial cell layers are more permeable in the absence of FAK due to mislocalization and reduced phosphorylation of the adhesion protein VE-cadherin. These defects weren't rescued by the kinase-deficient mutant.

FAK therefore has kinase-dependent and -independent functions in endothelial cells. The same may be true in cancer cells, says senior author Jun-Lin Guan, so drugs that only target FAK's kinase activity may not prevent the protein from promoting metastasis.

Zhao, X., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200912094.

Acid sphingomyelinase deals the seal



When ASM is inhibited (right), the holes produced by a bacterial toxin aren't resealed, allowing a lipophilic dye (green) to enter the cells.

Cells repair holes in their plasma membrane by secreting a lysosomal hydrolase to induce endocytosis, Tam et al. reveal.

If their outer surface is punctured by scraping or by pore-forming bacterial toxins, cells quickly fuse their lysosomes with the plasma membrane, which might provide extra lipids to patch the leak. But puncture repair also involves the removal of damaged plasma membrane by endocytosis. Tam et al. wondered whether the two processes were linked and found that blocking lysosome exocytosis in injured cells prevented endocytosis and plasma membrane repair.

How does lysosome secretion stimulate the internalization of damaged membrane? The endosomes generated during membrane repair look like the vesicles produced by cells exposed to the bacterial enzyme sphingomyelinase, which induces membrane budding by forming ceramide patches on the cell surface. The authors thought that secretion of the lysosomal version of this enzyme, acid sphingomyelinase (ASM), could have a similar effect and found that an ASM inhibitor blocked endocytosis and puncture repair. Cells lacking ASM—including fibroblasts derived from patients with Niemann-Pick disease type A—were also deficient in resealing their membranes after damage, but endocytosis and membrane repair were restored by adding ASM protein to the cells' medium.

Defective membrane repair may therefore contribute to the neurodegenerative pathology of Niemann-Pick type A, which was previously ascribed largely to the intracellular accumulation of sphingomyelin and cholesterol. First author Christina Tam and senior author Norma Andrews now want to determine whether other lysosomal hydrolases stimulate endocytosis and membrane resealing.

Tam, C., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.201003053.