

In This Issue

Fewer phosphates but fatter axons

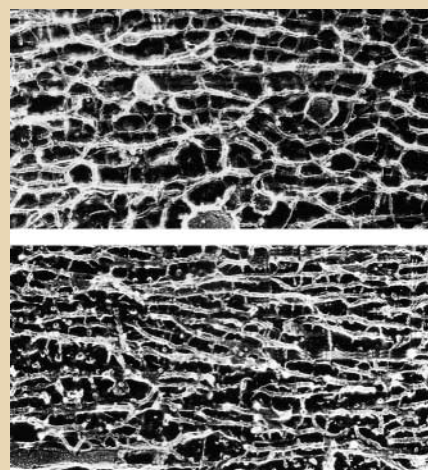
Axons are fattened by an unexpected neurofilament (NF) subunit, according to Garcia et al. (page 1011) and Rao et al. (page 1021), who find that more phosphorylation sites are not necessarily better when it comes to driving axon expansion.

The radial growth-inducing subunit is one of three that make up NFs. The COOH-terminal tails of two of these subunits, NF-H and NF-M, extend perpendicular to the main NF axis and thus bridge NFs with adjacent NFs, actin filaments, or microtubules. These COOH-terminal tails are phosphorylated in response to myelination, which also initiates a tenfold expansion in volume that is critical for fast conduction of action potentials. As NF-H has 51 phosphorylation sites in its COOH-terminal tail, and NF-M has just 7, NF-H was assumed to be the key phosphorylation target in axonal expansion, with some researchers suggesting that

repulsion between all these negatively charged groups was driving expansion. But both groups find that NF-M, but not NF-H, is the driving force.

The articles report on mice lacking the phosphorylatable NF tails. All of the mice had normal NF levels, but axons only expanded in mice that contained phosphorylated NF-M bridges. Axon filaments in the NF-M mutants were thus more closely packed. NF-M phosphorylation may be required for the binding of interlinking proteins, such as plakins, which then force expansion by pushing apart the filaments. NF-H tail deletions did not have the same effects—axons grew to normal size despite lacking the long NF-H cross-bridges—so the function of NF-H phosphorylation is still a mystery.

In the smaller axons of the NF-M mutants, action potentials were conducted at two-thirds the normal rate, although the mice developed and acted



Axonal neurofilaments are closely packed in mice lacking the phosphorylated NF-M tail (bottom).

normally. Larger animals, however, whose nerves must transmit impulses greater distances, display neurological disorders when conduction velocities are impaired. Perhaps NF-M phosphorylation is impaired in human disorders such as Charcot-Marie-Tooth disease, in which reduced myelination slows nervous impulses. ■

The APC–tumor connection

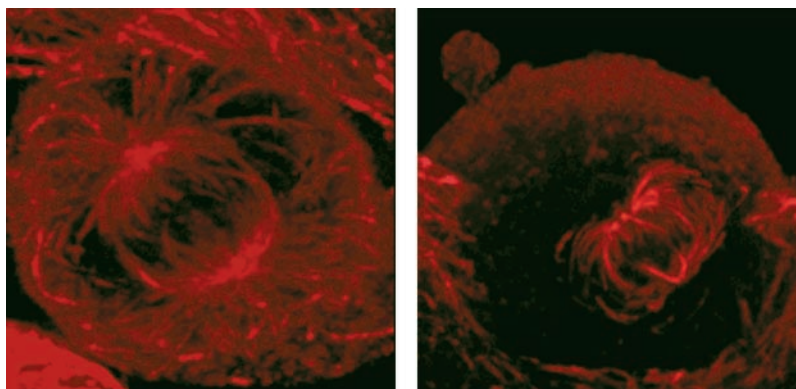
Chromosomal instability—the less than faithful mitotic segregation of chromosomes—is a hallmark of several cancers, particularly colorectal tumors. Also common in these tumors is a mutation that truncates the adenomatous polyposis coli (APC) protein. On page 949, Green and Kaplan show that this is more than coincidence—APC truncations lead to defective mitotic spindles resembling those of colorectal tumor cells.

The faulty spindles are a result of defective capture of microtubule plus ends. The authors expressed the truncated APC protein in noncancerous cell lines that also had normal APC and found that, in these cells, spindle microtubules could no longer grab hold of the kinetochores. This caused chromosome misalignment and segregation defects. Astral microtubules were lost, presumably because they were not stabilized by interactions with the cell cortex. Without complete asters, spindles were free floating and often mispositioned.

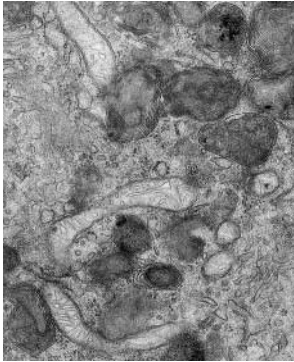
Similar spindle problems were seen in human colorectal tumor cell lines that have high rates of chromosome instability. Patients are more susceptible to colorectal cancer even when they have only one APC truncation allele. As

the truncated product dominantly interfered with plus-end attachments, Kaplan suggests that these individuals might have mitotic abnormalities in intestinal epithelial tissues. He plans to examine spindle morphology in a heterozygous APC mouse model.

It is not yet clear how APC makes microtubule plus ends sticky. APC associates with the plus-end binding protein EB1, whose loss phenocopies the APC mutant, so possibly this interaction is necessary for microtubule search and capture. ■



Spindles (red) are disrupted by a truncated APC protein (right).



Mitochondria are intact in neurons dying from GDNF deprivation.

Neurons die one way or another

On page 987, Yu et al. identify a novel cell death pathway that bypasses mitochondria. The results indicate that various strategies exist to kill off neurons.

A lot of the killing of sympathetic neurons occurs in the first week after birth. During that paring period, ~50% of sympathetic neurons die due to a lack of nerve growth factor (NGF). Neurons deprived of NGF in vitro die through the classical cell death pathway, which includes the release of cytochrome *c* from mitochondria and the resulting activation of caspases. Other factors can also promote neuronal survival, but it now seems that the method used to kill cells after withdrawal of these factors is not the same.

Yu et al. find that depriving sympathetic neurons in vitro of GDNF kills cells without mitochondrial involvement. Different caspases were activated than in NGF-dependent neurons, cytochrome *c* was not released, and mitochondrial structure was maintained in GDNF-deprived neurons.

Initiation of this pathway probably involves the GDNF receptor Ret, which may activate caspase-2 when GDNF is absent. Whether certain neurons depend solely on GDNF for survival in vivo remains to be seen. Perhaps the mitochondria-independent pathway is only a back-up in case the main system fails. But if death pathways are factor- and cell type-specific, some neuronal death might be blocked selectively by interfering with one but not another death pathway. ■

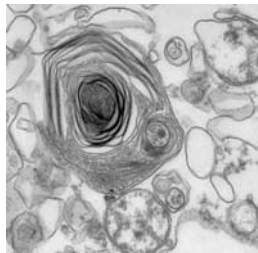
Fusion fuels killing

On page 1123, Hernandez et al. demonstrate that a human bacterial pathogen makes host defense cells self-destruct by engulfing their own organelles.

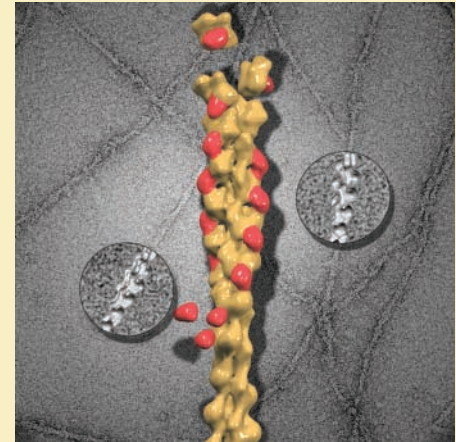
This engulfment, known as autophagy, is commonly used by eukaryotic cells to remove damaged organelles by enveloping them in membranes (probably derived from the ER) that later fuse with lysosomes. High levels of autophagy is also a form of programmed cell death—a process that the bacterium *Salmonella* is now shown to hijack.

The authors show that the *Salmonella* protein SipB is all the bacteria need to get autophagy rolling. SipB is a translocase that sends pathogenic effector proteins into host cells. The authors show that SipB also functions within the infected cell, where it finds its way to mitochondria. There, SipB's demonstrated fusion activity may explain the appearance of damaged and bloated mitochondria, which then become a target for the autophagic apparatus. The end results, visualized by the group, were multimembranous structures that resemble autophagic vesicles, contain mitochondrial and ER proteins, and occasionally surround entire mitochondria.

Salmonella can also kill macrophages through a faster necrosis-like pathway that, unlike autophagy, depends on caspase-1 activity. The two killing methods may result in different immunological responses from the host, but as yet it is not even clear whether macrophage death is a benefit for the host (by halting bacterial replication) or the pathogen (by impairing defense responses). ■



Macrophages infected by *Salmonella* accumulate vesicles that resemble autophagosomes.



Where AC proteins (red) bind, the structure of an actin filament (yellow) resembles its depolymerizing end.

Tearing down actin

Proteins that take apart actin filaments are hardly creative, according to Galkin et al. (page 1057), who show that destabilizing proteins induce structural states that are normally seen at the depolymerizing end of actin.

That end—the so-called pointed end—normally depolymerizes in the cell even as actin monomers are added at the other “barbed” end. This treadmilling is disturbed by ADF/cofilin (AC) proteins, which tear down actin filaments near the leading edge of migrating cells. The authors used electron microscopy and three-dimensional reconstructions to investigate how AC proteins promote instability and dismantle filaments.

AC-bound filaments looked like the pointed ends of naked actin filaments. Both were missing a contact between subdomain (SD) 2 of one actin monomer and SD1 of the monomer just above it in the filament. In contrast, strong SD1–SD2 interactions were found throughout the rest of the naked actin filaments. These contacts must be weakened for actin strands to adopt a tilted conformation that favors destabilization. AC proteins thus induce this naturally unstable state wherever they bind and cause either depolymerization (if subunits fall off the ends) or severing (if a filament segment is broken off). ■