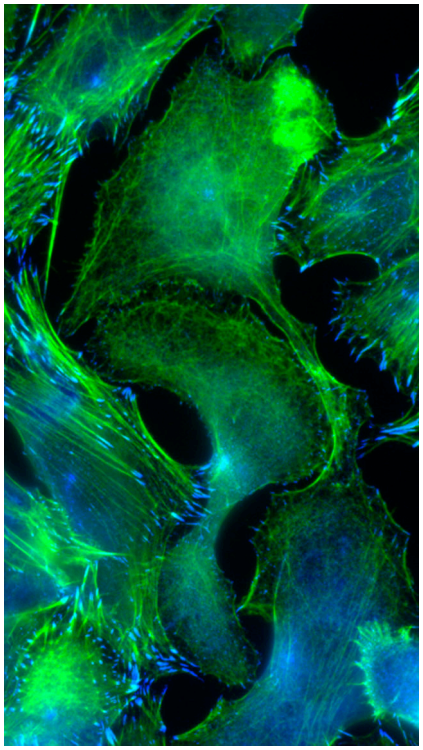
In This Issue



Cells low in ROCKI (center) lack stress fibers.

One ROCK is not like another

The mammalian rho kinases, ROCKI and ROCKII, control distinct cytoskeletal functions from distinct locations, report Yoneda et al. on page 443. ROCKI is required for formation of actin stress fibers and cell adhesion, whereas ROCKII is needed for phagocytosis.

The ROCK proteins are major effectors of the Rho signaling pathway, controlling cell adhesion, motility, and other actin-based functions. In kinase overexpression assays, ROCKI and ROCKII appear redundant, but several experiments, including mouse knock-outs, hinted that the proteins have unique duties despite their structural similarity.

While studying a signaling pathway that links integrins and cell surface proteoglycans to adhesion, Yoneda et al. saw that ROCKI but not ROCKII activity increased during adhesion. To learn how the proteins differ functionally, the team knocked down each ROCK gene independently using siRNA sequences that left the other gene unchanged. Cells lacking ROCKI formed few stress fibers or focal adhesions. ROCKII-depleted cells produced oversized stress fibers and adhesions, and showed problems with phagocytic uptake of fibronectin-coated beads.

The PH domain is the most divergent region of the two ROCKs, and GFP-PH from each protein localized to distinct subcellular locations. The ROCKI PH domain was evenly distributed at membranes, whereas ROCKII PH concentrated in ruffles at the cell surface. Subsequent analysis showed that only ROCKII PH binds to PIP_3 lipids and may, therefore, be regulated by PI3-kinase.

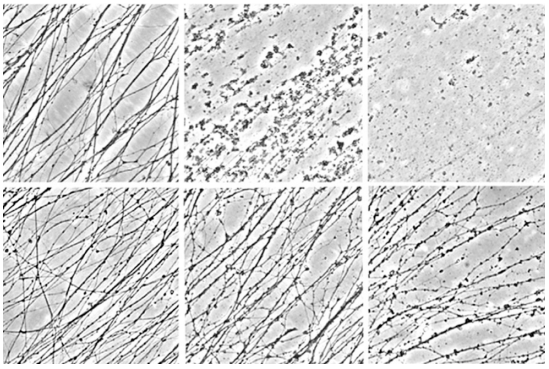
Although the ROCK proteins phosphorylate similar targets in vitro, Yoneda et al. think that subcellular targeting restricts the pair's functions. This is consistent with overexpression of either protein masking their differences. If the team is right, then what holds for real estate holds for the ROCKs: it's all about location, location, location. JCB

NAD to the rescue

NAD helps counteract energy leakage from severed axons, report Wang et al. on page 349.

Recently, Araki et al. (*Science*. 305:1010–1013) showed that NAD protects axons from degeneration after injury, apparently by activating SIRT1, a nuclear deacetylase involved in aging. Now, Wang et al. show that NAD prevents degeneration independent of SIRT1 and works locally to protect membrane integrity.

Axons degenerate when they are cut from their cell body. However, neurons from mice are protected from degeneration when they overexpress either Nmnat1, which synthesizes NAD, or the Wld^s fusion protein that contains Nmnat1.



Severed axons degenerate (top; left to right) but can be rescued by Nmat expression or NAD (bottom).

Wang et al. found that overexpression of Wld^s or Nmnat1 prevented a decrease of NAD, normally seen in axons after severing, and delayed degeneration. Moreover, addition of NAD to culture media delayed axonal degeneration, even when it was added to an axonal segment already isolated from its soma. This suggests that NAD acts locally rather than via transcription. Because NAD is essential for ATP synthesis, and a drop in ATP parallels the drop in NAD in severed axons, the team asked if exogenous pyruvate could delay degeneration. The energy-rich chemical did protect axons, indicating that NAD may be required to sustain ATP levels necessary to maintain the integrity of the cell boundary.

So what is the difference between this work and the earlier study? It could be that Wang et al. used higher concentrations of NAD and thereby bypassed a need for transcriptional activation of SIRT1. No difference after NAD addition in neurons from wild-type and SIRT1-deficient mice makes that less likely. Instead, Wang et al. point to the contrast between isolated axons in their study and surrounding glial cells in the Araki et al. study.

The real test of NAD's protective abilities will come when Wang et al. start to look at neurons in the brains of animals. Such cells are postmitotic and can't synthesize NAD from tryptophan. If they start to lose NAD for some reason, such as axonal injury, they may become more susceptible to degeneration—and adding it back may be protective. JCB

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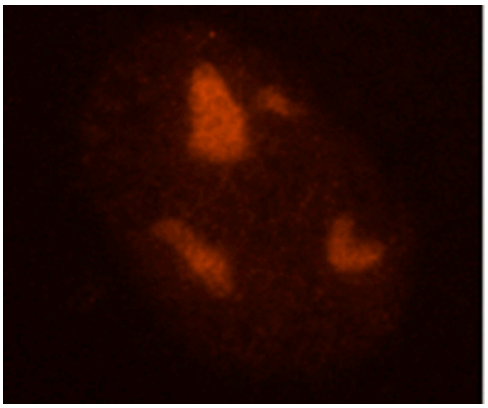


Nucleolar WDR12 (orange) helps coordinate ribosome biogenesis and the cell cycle.

Ribosome cycling

Ribosome biogenesis consumes up to 80% of the energy of the cell. To prevent wastage of all of that energy, coordination with the cell cycle is vital. Both ribosome biogenesis and cell proliferation go awry when a the integrity of a three-protein complex (Nop7p-Erb1p-Ytm1p) in yeast is disrupted. Now on page 367, Hölzel et al. show that a homologous complex comprised of Pes1-Bop1-WDR12 exists in mammals and, like its yeast counterpart, relates cell cycle progression to ribosomal RNA processing.

Like Pes1 and Bop1, which have been characterized previously, Hölzel et al. find that WDR12 is up-regulated in response to c-myc expression and cell proliferation. Pes1, Bop1, and WDR12 copurified in immunoprecipitation experiments.

A link between ribosome biogenesis and the cell cycle may be provided by p53. This protein accumulates when the rRNA-encoding nucleolus is disrupted. Here, the researchers found that a WDR12 variant (lacking the most NH_2-terminal of its seven WD repeats) stabilized p53, localized to the nucleolus, and inhibited rRNA processing, causing the accumulation of the 32S precursor. Cells expressing this mutant WDR12 failed to proliferate normally.

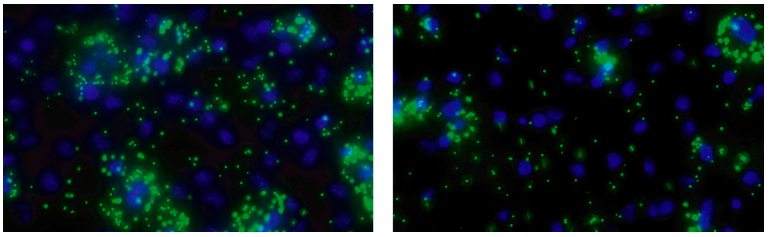
p53 stabilization by the WDR12 mutation was not dependent on the tumor suppressor p19ARF, which is a known to disrupt the ubiquitination of p53. The actual mechanism of p53 stabilization is unclear. The Pes1-Bop1-WDR12 complex might actively regulate p53 turnover, or p53 stabilization might be a secondary effect of mutations in Bop1 or WDR12. Such mutations disrupt ribosome assembly so they probably lead to an accumulation of free ribosomal proteins, such as L5, L11, and L23. These proteins bind an inhibitor of p53, and may thus allow p53 to delay the cell cycle. JCB

Eating Staph

The scavenger receptor CD36 is required for binding and internalization of *Staphylococcus aureus*, report Stuart et al. (page 477). After recognizing lipoteichoic acid on the bacterial surface, CD36 teams up with toll-like receptors, TLR2/6, to trigger expression of pro-inflammatory cytokines.

Researchers already knew that CD36 recognized apoptotic cells in both mammals and flies. In a large-scale RNAi screen in *Drosophila*, Stuart et al. saw evidence that CD36 was necessary for *S. aureus* internalization; they then looked for a similar function in mammals. CD36 was necessary for *S. aureus* engulfment, but not *E. coli* uptake, and mutations in the cytoplasmic tail of CD36 disrupted bacterial phagocytosis. Although some cytokine expression was detected in the absence of CD36, cotransfection of CD36 and TLR2/6 demonstrated a synergistic effect in the presence of *S. aureus*. Mice lacking CD36 were unable to clear *S. aureus* infection.

Stuart et al. show that CD36-mediated internalization of *S. aureus* boosts downstream TLR-induced responses, and the team hypothesizes that the TLRs' affinity for lipoteichoic acid may increase upon bacterial uptake into the phagosome. Other scavenger receptors are known to interact with bacteria and with TLRs and, like CD36, may modulate TLR signaling, which would otherwise behave as a simple on–off switch. JCB



Macrophages lacking CD36 (right) take up less *S. aureus* (green).

Two-step adhesion

p21-activated kinases (PAKs) control actin dynamics and cell migration. On page 465, Leisner et al. report that CIB1, a widely distributed calcium binding protein, activates PAK1 immediately after cells bind fibronectin, whereas more familiar PAK activators such as small GTPases take over after adhesion is established.

CIB1 was identified in platelets where it binds an integrin involved in platelet aggregation. Further study showed that it was expressed in many cells types, leaving open the question of its function.

Leisner et al. found that CIB1 binds to and activates PAK1 in a variety of cell types. The increase in PAK activation when cells contacted fibronectin was CIB1 dependent, whereas only the later activity of PAK required small GTPases including Rac and Cdc42. Overexpression of CIB1 reduced the number of focal adhesions in cells, increased membrane ruffling, and decreased migration on fibronectin. Depletion of CIB1 reduced PAK activity and increased migration on fibronectin.

These data suggest that in addition to regulating platelet aggregation, CIB1 regulates cell migration and actin dynamics in other cell types. Leisner et al. hypothesize that CIB1 works immediately after cells make contact with the extracellular matrix, before the small GTPases bind PAK1. Just how CIB1 slows migration on fibronectin isn't yet clear, however, especially because prior data showed that PAK activity stimulates migration on collagen. JCB