

## A simple reentry into the cell cycle

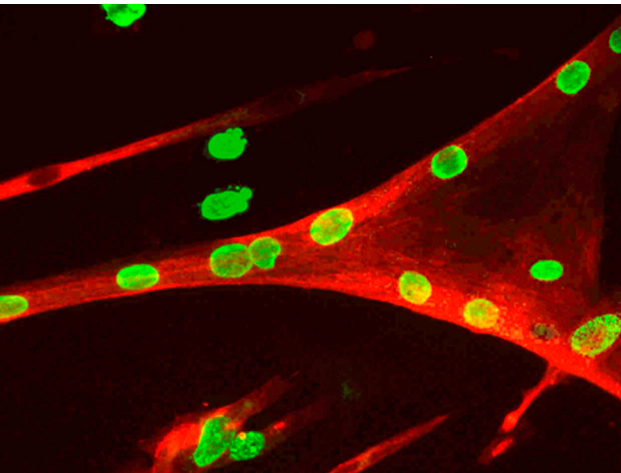
**I**t may be specialized, but a muscle cell is just a single step away from resuming division, as Pajalunga et al. reveal on page 807. The researchers show that removing one regulatory protein can push a range of nondividing cells back into the cell cycle.

Most cells in adults aren't cycling. Some have slipped into quiescence, a temporary lull triggered by scarcity of food or other necessities. Cells whose telomeres have worn down or that carry damaged DNA often retire by entering replicative senescence. And terminally differentiated cells are so specialized that they no longer divide. Although researchers can jump-start the cell cycle by, for example, adding growth factors, the recipe for reactivating cells in each state is different.

To their surprise, Pajalunga et al. found that a common factor, a protein called p21, controls whether all three types of cells reenter the cell cycle. p21 is a cyclin-dependent kinase inhibitor, or CKI, which counters division-triggering kinases.

Myotubes, or differentiated muscle cells, are stubborn, shrugging off growth factors, oncogenes, and other division promoters. But when the researchers used RNAi to quash p21, the cells awakened and underwent mitosis—though they usually died shortly afterward. “This is the first time anyone has reactivated terminally differentiated cells by removing something from them,” says team leader Marco Crescenzi. Eliminating p21 also restarted proliferation in quiescent and senescent cells, which continued to divide rather than dying.

The researchers wondered how the myotubes were able to respond without growth factors, which spur the assembly of the cyclin/cdk complexes essential for the cell cycle. Myotubes contain prefabricated complexes, the scientists determined, but p21 hooks onto and inactivates them. The results suggest that even differentiated cells are poised to reenter the cell cycle; they will start dividing if they don't get continuous inhibition from CKIs. The discovery could provide a boost to tissue engineering by allowing researchers to raise cells that are reluctant to grow in culture. **JCB**



These myotubes have reentered the cell cycle after p21 removal.

## How CENP-A reaches the centromere

**I**nstead of a unique DNA sequence, centromeres feature a distinctive histone known as CENP-A. Two papers in this issue help untangle when and how CENP-A gets into position.

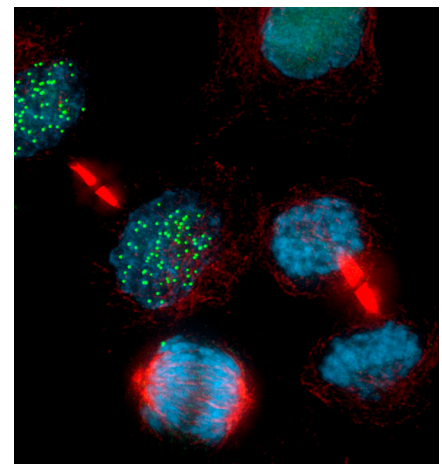
Centromeres serve as platforms for the kinetochores that are essential for divvying up chromosomes during mitosis. CENP-A's presence at centromeres, where it replaces the standard-issue H3 histone, poses several puzzles. On page 795 Jansen et al. answer the “when” question. The researchers applied a recently developed technique called SNAP tagging, which affixes a long-lasting label to a protein. The method allowed the team to pin down CENP-A's location and measure its turnover.

The logical time for cells to begin inserting fresh CENP-A into chromosomes is after S phase, to counter the dilution of the protein during DNA duplication. But what the researchers found was one of those surprises “that makes you jump to

the roof,” says lead author Lars Jansen. The addition doesn't begin until G1, after cells have gone through mitosis. Further experiments indicated that cells have to pass through mitosis before they can start incorporating new CENP-A. Why mitosis is essential for this process isn't certain. But the results reveal that chromosomes complete division with a reduced complement of CENP-A.

Maddox et al. tackle the “how” question on page 757. A previous screen of nematode genes had identified ~250 that are necessary for chromosome separation. By eliminating the corresponding proteins individually, the researchers pinpointed one that was crucial for kinetochore construction: KNL-2. Without it, CENP-A does not join the chromosome. And KNL-2 stays away if the histone is absent, suggesting that their interaction directs CENP-A to the correct location.

The researchers also found that the



Cells don't load new CENP-A (green) until they finish mitosis.

human version of KNL-2 homes in on the centromeres late in mitosis and early in G1, which jibes with the findings from Jansen et al. KNL-2 carries a DNA-binding Myb domain that might provide clues to another mystery about CENP-A: how the protein determines where on the chromosome to settle. **JCB**

## A sloppy checkpoint

If the G2 checkpoint were a building inspector, it would get fired for negligence. Deckbar et al. show on page 749 that the checkpoint, which is supposed to halt division after chromosome damage, allows cells to proceed into mitosis even if they harbor fractured DNA.

The G2 checkpoint interrupts the cell cycle to allow repair of double-stranded DNA breaks (DSBs). But the checkpoint isn't perfect, as the researchers found when they irradiated normal human fibroblasts and Artemis cells, which have a normal checkpoint but sluggish DSB repair. Each of the cells that went on into mitosis had one to two chromosome breaks, which is 10 times the background value.

Not every DSB shows up as a complete chromosome break. So to gauge the number of DSBs that are slipping past the checkpoint, the researchers counted the number of repair foci, where enzymes have begun—but not finished—mending DNA. Fibroblasts left G2 with ~20 foci. Other methods gave similar values for unfixed DSBs, indicating that the checkpoint has a threshold of ~10 to 20 breaks.

Only one DSB is needed to activate this checkpoint in yeast, according to previous studies. Mammalian cells might be sloppier because they carry much more DNA. Whether unrepaired breaks cause trouble is unclear. Many of them might get fixed at the subsequent G1 checkpoint. **JCB**

## Die another way

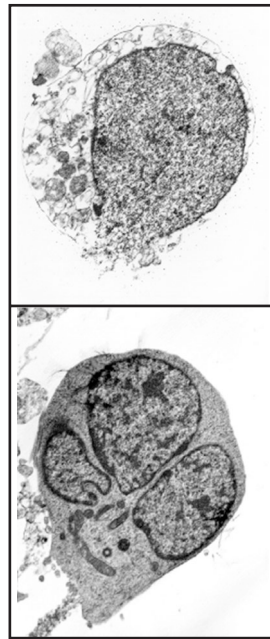
On page 853 Ueda et al. describe a protein that switches cells between two death mechanisms, preventing necrosis while promoting apoptosis. The molecule might provide a new way to save brain cells after a stroke.

A stroke delivers a double whammy. First, cells near the clot begin to perish from necrosis, which is triggered by ATP scarcity. Later, more distant cells start dying through apoptosis. Although anti-apoptosis compounds can stem some damage, their benefits are modest, possibly because the necrosis is more devastating. Molecules to halt necrosis have proven elusive.

Ueda et al. found one such molecule in cultures of rat cortical neurons. The protein ProT $\alpha$  curbs necrosis in cultures that lack serum or have been oxygen deprived. But it also boosts their apoptotic death rates. Adding growth factors that derail apoptosis protects most of the cells.

The group showed that ProT $\alpha$  works by keeping cells well-nourished. During necrosis, some of the GLUT transporters that usher glucose into the cell exit the plasma membrane. But ProT $\alpha$  prevents this relocation.

Ueda et al. conclude that ProT $\alpha$  flips cells from an uncontrollable form of cell death, necrosis, to a more controllable one. Other factors can derail cells from apoptosis. A treatment that mixes ProT $\alpha$  with some of these compounds might spare neurons after a stroke. **JCB**



The characteristic signs of necrosis (top) are missing in a neuron dosed with ProT $\alpha$  (bottom).

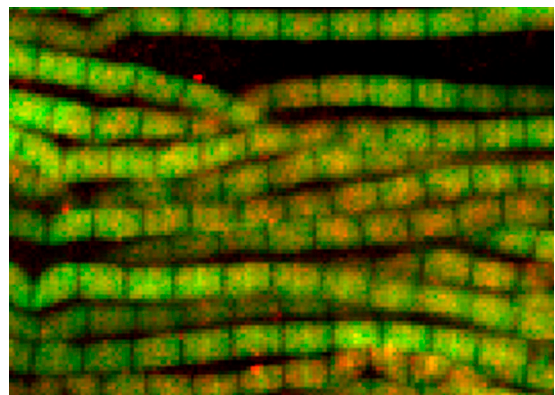
## Muscle first in neurodegenerative disease?

Researchers hunting for the molecular malfunction behind the paralyzing disease spinal muscular atrophy (SMA) might have been looking on the wrong side of the neuromuscular junction, as Rajendra et al. show on page 831. The work suggests that reduced levels of SMA's signature protein, SMN, disrupt the junction from the muscle side.

SMA is the second most prevalent fatal recessive illness after cystic fibrosis. Although its severity varies, the disease often kills patients before age two as motoneurons deteriorate and muscles wither. How reduced amounts of SMN trigger symptoms isn't clear. Because the disease involves neural breakdown, researchers have assumed that the loss of SMN mainly hits motoneurons, not muscles.

To model the disease, Rajendra et al. identified fruit flies that produce too little of the protein. Feeble flight muscles grounded the mutants. Consistent with previous mouse models, the neurons that innervate these weakened muscles grew and branched abnormally. In addition, the insects' flight muscles lacked actin, a sign that innervation had failed.

Localization experiments suggested SMN is crucial specifically in muscles. The researchers determined that, in normal flies, SMN accumulates in muscle sarcomeres and gloms onto  $\alpha$ -actinin, which interlinks actin filaments. Although the results do not rule out the "neuron first" hypothesis, they suggest that muscle dysfunction helps spur nerve deterioration. **JCB**



SMN (red) congregates at the actin-rich I-bands (green) in muscle fibers.