



Nucleoli (raised, orange ovals) stand out in these colon cancer cells.

# Central command

More capable than once thought, the cellular structure known as the nucleolus may be a target for treating diseases

By Mitch Leslie

**H**it cancer cells where they're unique: the mutated genes and deranged molecular pathways that drive rampant growth. That's the strategy most researchers have adopted to kill cancer cells without massacring healthy ones, but it's backward, says cell biologist Ross Hannan of the Peter MacCallum Cancer Centre in East Melbourne, Australia. The way to take down tumor cells, he says, is to disturb the normal cellular "housekeeping processes," which are even more crucial to fast-growing cancer cells. And a focal point of those mundane processes is a dark spot in the cell nucleus called the nucleolus.

Hannan and his colleagues, who are running a clinical trial of an experimental

cancer drug targeting the nucleolus, are capitalizing on a new understanding of this obscure organelle. For more than 30 years, researchers thought that the nucleolus performed a vital but circumscribed role in the nucleus—manufacturing a specific type of RNA, dubbed rRNA, that assembles into ribosomes, the organelles that make proteins. But scientists have come to realize that, as molecular cell biologist Robert Tsai of the Texas A&M Health Science Center in Houston puts it, "the nucleolus is much more complex than rRNA synthesis."

Besides serving as a ribosome factory, the organelle also functions as a command center that monitors a cell's condition and orchestrates responses when it's under stress. By storing certain proteins and

doling them out when they are required, the nucleolus "endows cells with an accurate way to regulate distribution of proteins," says Michal Hetman, a molecular and cellular neurobiologist at the University of Louisville in Kentucky. Ultimately, the nucleolus helps determine whether cells reproduce and when they die.

All of that makes it a tempting target for treating diseases, starting with cancer. Researchers have known for more than 200 years that cancer cells often sport extra nucleoli, which balloon as they attempt to help the rapidly dividing cells meet their insatiable need for proteins. In recent years, hints have emerged that the nucleolus's activities also contribute to heart disease, neurological illnesses, and other diseases.



**DESCRIBED BY MICROSCOPISTS** in the 1830s officially, the nucleolus isn't enveloped by a membrane, unlike other cellular organelles. But nucleoli—even normal mammalian cells can have more than one—are distinct from the surrounding nuclear material. They are about twice as dense and, according to estimates by biophysicist Clifford Brangwynne of Princeton University and colleagues, roughly 100,000 times more viscous, like thick honey. In a 2011 paper in the *Proceedings of the National Academy of Sciences*, Brangwynne and his co-workers provided evidence that nucleoli in frog eggs are a distinct phase of matter. “They behave like liquid droplets,” he says, perhaps because of the unique combination of proteins—more than 4000 different kinds spend time there—and RNA molecules they harbor.

The recipe for these organelles resides in chromosome stretches called nucleolar organizing regions, or NORs, which contain numerous copies of genes encoding rRNAs. NORs prompt a nucleolus to form around them, as cell and molecular biologist Brian McStay of the National University of Ireland, Galway, demonstrated for the first time in vertebrate cells this year. His team crafted synthetic NORs that contain mouse ribosome genes and repeated DNA segments that bind to a nucleolar protein. When the researchers inserted these “neo-NORs” at different chromosome locations in cultured human cells, functional nucleoli sprang up around them, the team reported in the 1 February issue of *Genes & Development*.

The best-known function of the nucleolus came to light in the 1960s: making ribosomes. An enzyme called RNA polymerase I reads the ribosomal RNA genes in NORs and fashions rRNA molecules. After processing, these rRNAs combine in the nucleolus with proteins and other RNAs made elsewhere in the cell. The final assembly of ribosomes occurs in the cytoplasm, where they get to work making proteins.

These days, however, researchers refer to the “plurifunctional nucleolus.” Cell biologist Thoru Pederson of the University of Massachusetts Medical School in Worcester, who coined the term in the 1990s, says he chose “plurifunctional” over “multifunctional” because it would be correct even if the nucleolus had only two functions. “I was being cautious,” he says.

He didn't need to worry. The nucleolus has turned out to be a busy cellular housekeeper. It helps manage the cell cycle, the series of events that culminates in cell division. It stimulates DNA repair and organizes a molecular complex necessary for sorting proteins in the cytoplasm. Pederson and

colleagues recently identified a hint of yet another function. “We've discovered a small number of microRNAs in the nucleolus,” he says. These diminutive RNAs usually operate in the cytoplasm, fine-tuning protein production. What microRNAs are doing in the nucleolus “is a development we need to pin down,” Pederson says.

The most important job for the nucleolus might be reining in p53, the protein known as the guardian of the genome because it protects against cancer. p53 is so potent—it can trigger cellular suicide—that cells continually make and then destroy the protein, thus keeping its levels low. But myriad stimuli, ranging from DNA damage to heat, can slow the destruction and allow

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Ross Hannan, Peter MacCallum Cancer Centre

the protein to accumulate. In 2003, a finding by biochemist Carlos Rubbi, now at the University of Liverpool in the United Kingdom, and his colleague Jo Milner suggested that the nucleolus is a key sensor for such stresses. The pair showed that even if cells carried injured DNA, they didn't begin to amass p53 unless their nucleolus was zapped with radiation. Moreover, Rubbi and Milner revealed that breaking up the nucleolus could liberate p53.

Nucleoli “are very sensitive to physical and chemical conditions,” Rubbi says. When all cellular readings are normal, the nucleolus keeps p53 under wraps. If the nucleolus is injured or the cell is under duress, it stops performing this function and unleashes p53, which can kill the cell.

**THAT LIFE-AND-DEATH POWER** makes the nucleolus a bull's-eye for cancer therapy. Hannan and colleagues are now testing a drug called CX-5461, which was designed to thwart RNA polymerase I, the enzyme that makes rRNAs. When rRNA synthesis slows drastically, the nucleolus allows p53 levels to rise. Two years ago, Hannan and colleagues demonstrated that administering CX-5461 to mice cranked up p53 to lethal levels in cancer cells and preferentially killed them, presumably because they are more sensitive than are normal cells to reductions in rRNA synthesis. “We've shown that a house-keeping process that's in every cell can be a good target,” Hannan says. The current trial of CX-5461 is largely to evaluate its safety in people, but the researchers will also moni-

tor if it slows or stops the blood cancers the subjects suffer from.

The compound might just be the start. Cancer biologist Marikki Laiho of the Johns Hopkins University School of Medicine in Baltimore, Maryland, and colleagues have found another compound that targets RNA polymerase I in the nucleolus, called BMH-21. Earlier this year, they revealed that it kills cancer cells in culture and curbs tumor growth in mice, and they are now looking for a biotech or pharmaceutical company that will help develop it further.

Researchers are also starting to uncover evidence for a nucleolar role in other diseases. In 2008, molecular biologist Mark Sussman of San Diego State University in California and colleagues stumbled on a link to heart disease while studying the heart's ability to renew itself after injury. When Sussman's team probed cells from damaged hearts for nucleostemin—a nucleolus protein thought to be made mainly by stem cells—they found a surprising pattern. “It's expressed in adult heart muscle cells, and they are generally thought not to be cells that renew,” he says.

To tease out the protein's role in the organ, the researchers disrupted blood flow to the hearts of mice to simulate a heart attack. Bloated, deformed nucleoli appeared in some cells in the damaged areas of the animals' hearts, the team revealed in the *Proceedings of the National Academy of Sciences* in 2011. That's a signal the organelles are under stress. In response, the nucleoli released nucleostemin and another protein called nucleophosmin. Sussman thinks the proteins may be the nucleolus's way of turning back the clock, reactivating a mechanism found in stem cells that would protect the organelle.

In other diseases, a stressed nucleolus may hasten damage rather than heal it. Dopamine-producing neurons die off in Parkinson's disease (PD), and in 2011 researchers reported injured nucleoli in samples of these cells from the brains of PD patients. They also showed that inducing nucleolar stress in mice with a PD-like illness leads to the death of these neurons. Findings such as these suggest that compounds to soothe the nucleolus might stem neurodegenerative diseases—although such drugs are still far off.

These days, says Texas A&M Health Science Center's Tsai, “more and more people are thinking about the nucleolus,” as they realize how many cellular processes it touches. The spotlight on this small dark spot will likely illuminate more surprises, Hannan says. “We've hardly scratched the surface of what the nucleolus has evolved to do in mammalian cells.” ■