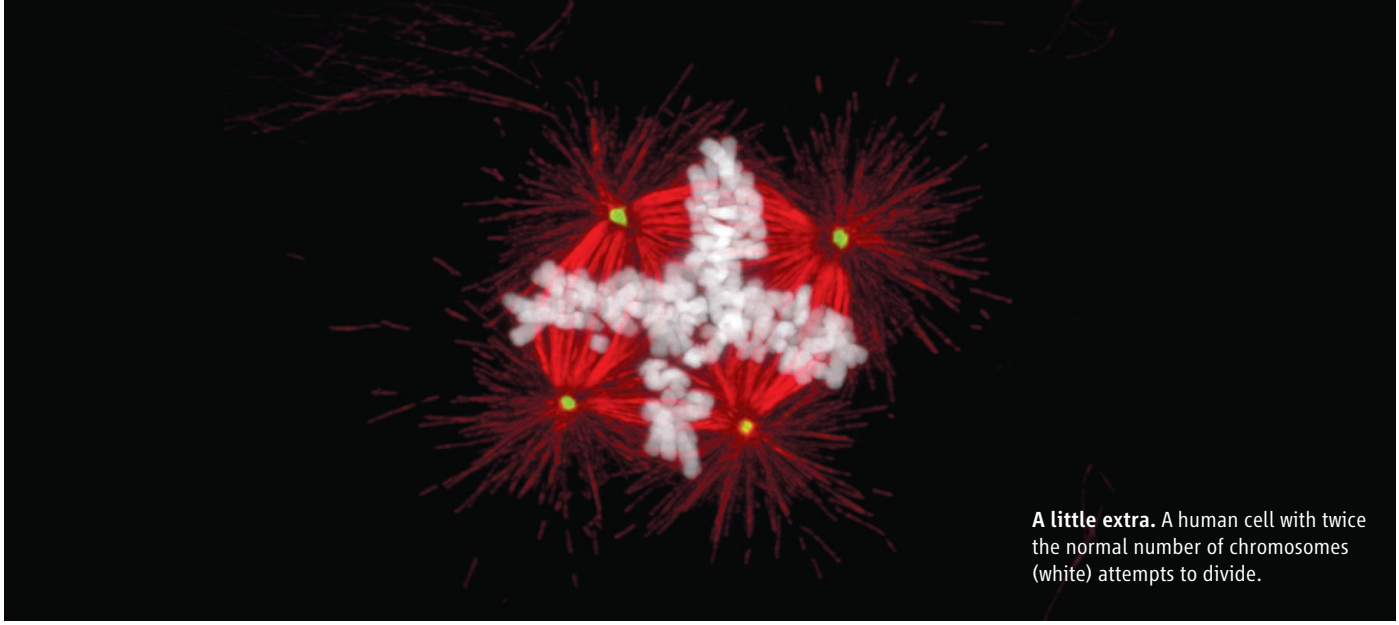

A little extra. A human cell with twice the normal number of chromosomes (white) attempts to divide.

CREDIT: NEIL J. GANEM/BOSTON UNIVERSITY

Strength in Numbers?

Some mammalian cells are loaded with extra sets of chromosomes, a state called polyploidy. What on Earth for?

A dividing cell generally follows a simple rule. After duplicating its DNA, the cell splits, yielding two daughter cells. That's why the movies of dividing mouse liver cells shot several years ago by Andrew Duncan, then a postdoc in Markus Grompe's group at the Oregon Health & Science University in Portland, flabbergasted his lab mates. "We saw a single cell giving rise to three and four daughter cells," says Duncan, who is now a tissue biologist at the University of Pittsburgh in Pennsylvania. And though chromosomes normally line up neatly across the middle of a cell before it divides, the chromosomes in many of the liver cells were arranged in unconventional formations, including multiple clusters.

The parental liver cells were forced to go through unusual maneuvers because they were polyploid, carrying extra sets of chromosomes. Polyploidy is rife among plants, insects, fish, and some other groups of organisms. But most human cells are diploid, outfitted with two sets of chromosomes that trace back to the set each provided by an egg and a sperm. Indeed, extra chromosomes usually spell trouble in mammalian cells. A few normal cells in people and other mammals, however, brim with extra genome copies—sometimes as many as a thousand. The contortions of the liver cells were surprising, but they had long been known to have a surfeit of chromosomes—as do cells in the heart and bone marrow.

For decades, researchers have speculated about whether polyploidy offers any advantages to mammalian cells, such as ramping up protein synthesis, but haven't been able to test their ideas. That has changed with the identification of several proteins that help regulate polyploidy. By cranking cells' allotment of chromosomes up or down, scientists recently have begun to explore the possible function of the odd cellular state. Do the extra chromosomes simply add bulk to cells that need it? Do they give cells reserve capacity that enables them to respond to stress and damage? "The real unanswered question is why any cell type is polyploid," says developmental geneticist Robert Duronio of the University of North Carolina (UNC), Chapel Hill. "We are poised to begin answering that question."

Online
sciencemag.org
Podcast interview with editor John Travis (http://scim.ag/pod_6172).

And even though the mystery of polyploidy's benefits remains unsolved, some researchers already hope to exploit the phenomenon. They are trying to turn polyploidy against certain cancers, compelling cells to cease their out-of-control division.

Risky excess

Polyploidy can seem like "a dangerous escapade," as Duronio and his colleagues put it in a 2009 paper. For cells that usually get along just fine with two sets of chromosomes, even one additional chromosome can be disastrous. An extra copy of chromosome 21 during development produces the disabilities of Down syndrome, for instance.

There's another potential drawback to polyploidy. "It can drive cancer," says David Pellman, a cell biologist and pediatric oncologist at the Dana-Farber Cancer Institute in Boston. He points to a 2013 *Nature Genetics* paper by Rameen Beroukhim, also of Dana-Farber, and colleagues that reported duplicated genomes in 37% of cancers. Polyploidy doesn't lead to cancer in every case, Pellman says, but it's a big enough risk that many cells go to great lengths to thwart it. p53, the watchful protein dubbed the guardian of the genome, often prompts cells with abnormal amounts of DNA to commit suicide or to curtail division. To become polyploid, therefore, cells have to disable it and other safeguards that protect against genome damage, notes biologist Gustavo Leone of Ohio State University, Columbus.

Researchers have gradually acquired a good grasp of the molecules and mechanisms that make cells polyploid, thanks mainly to their work on the cell cycle. A cell's life cycle includes milestones such as DNA duplication and division. An intricate network of proteins controls the cell's progress through the cycle, pushing it forward or holding it back. Under the right circumstances, researchers have found, some of these proteins steer cells toward polyploidy.

To tweak the chromosome content of cells, several research teams have recently genetically engineered mice to make more or less of these polyploidy promoters.

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For example, biochemist Katya Ravid of Boston University School of Medicine and colleagues enhanced polyploidy to test its role in megakaryocytes, hefty immune cells that dwell in the bone marrow and generate the platelets that help stanch bleeding. Megakaryocytes often harbor more than 100 copies of their genome, and researchers conjectured that the extra genes help the cells crank out platelets.

In 2010, Ravid's team engineered mice to manufacture excess amounts of a polyploidy-promoting protein. Although the alteration boosted the number of chromosome sets the cells contained, it didn't cause a corresponding rise in platelet numbers, the team revealed in *The Journal of Biological Chemistry*. Ravid suggests that polyploidy instead benefits megakaryocytes by boosting production of proteins that the cells need for structural support and sticking to their neighbors.

Bulking up

Biophysical engineer Dennis Discher of the University of Pennsylvania School of Medicine offers another explanation. He suspects that polyploidy helps a megakaryocyte in the same way a high-calorie diet helps a sumo wrestler—by increasing bulk. A membrane perforated by small pores separates the bone marrow from the bloodstream, and a megakaryocyte has to stay on the bone marrow side. Discher and his colleagues recently examined what size pores different types of bone marrow cells could slip through, and they found that megakaryocytes had trouble squeezing through even the largest openings, probably because of their chromosome-packed nuclei. "If you ask me why this cell is polyploid, I'd say it helps anchor the body of the cell in the marrow," says Discher, whose team reported its findings in the 19 November 2013 issue of the *Proceedings of the National Academy of Sciences*.

Researchers already have evidence from other species that extra heft is a benefit of polyploidy. In a 2012 study of fruit flies, Terry Orr-Weaver of the Massachusetts Institute of Technology and her colleague Yingdee Unhavaithaya found that when they reduced the levels of a polyploidy-stimulating protein in cells forming the blood-brain barrier in flies, the cells shrank and the barrier became leaky. The pair also showed that enlarging the undersized cells restored a tight seal. Boosting the size of existing cells might cause less disruption than producing more cells through division, which requires that a cell disengage from its neighbors, notes cell biologist Brian Calvi of Indiana University, Bloomington.

Yet for one mammalian cell type that takes polyploidy to the extreme, work by Leone's team downplays the size connection. Cells in the outer layer of embryos, known as trophoblast giant cells, are polyploidy champions—in mice they pack up to 1000 genome copies. The cells help the embryo implant in its mother's womb, and researchers have suggested that adding chromosomes allows the cells to quickly enlarge, enabling the embryo to infiltrate the uterine lining.

Leone and his colleagues deleted genes for polyploidy-promoting proteins from trophoblast giant cells in mice, anticipating that embryos would die because implantation would suffer. "We were expecting that polyploidy is really significant," Leone says. Although the giant trophoblast cells were smaller than normal and carried fewer chromosomes, the mouse embryos lived and grew up into seemingly healthy adults, the researchers reported in *Nature Cell Biology* in 2012.

Deep reserves

For the heart and the liver, two hard-working organs that also teem with polyploid cells, researchers are exploring a different explanation for polyploidy: The extra chromosomes boost performance under trying conditions and increase overall resilience. Indeed, "polyploidy may be an important stress response or adaptation" for many cell types, says cell biologist Donald Fox of Duke University Medical Center in Durham, North Carolina.

Support for that notion comes from a study of the mouse heart, in which almost all the cells sport four sets of chromosomes. In 2010, stem cell biologist Thomas Braun of the Max Planck Institute for Heart and Lung Research in Bad Nauheim, Germany, and colleagues examined genetically altered mice whose muscle cells—including those in the heart—were missing a gene that spurs polyploidy. Although the gene's absence didn't make all the animals' heart cells diploid, it did reduce the number of chromosome sets they contained by about one-third.

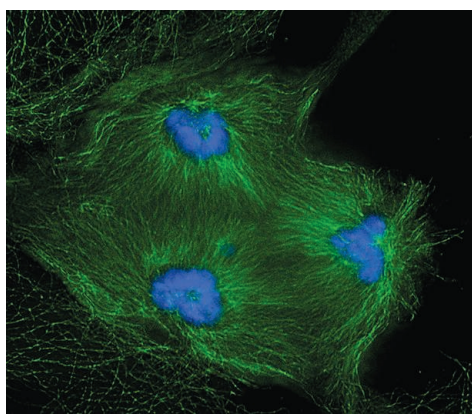
"At baseline conditions, they are pretty normal," Braun says of the mice. However, deficiencies appeared when the rodents had to cope with setbacks such as a heart attack. The hearts of animals with reduced polyploidy pumped less blood after an induced heart attack than did the hearts of control animals, the group reported in *Circulation Research*. How polyploidy enables the heart to rebound remains unclear, Braun says.

The work by Duncan and his colleagues on liver cells also backs the stress-response hypothesis. Unlike most mammalian organs, the liver has a remarkable ability to regenerate after injury. The liver is also well stocked with polyploid cells: In humans, about 50% of the liver cells called hepatocytes carry extra sets of chromosomes.

Duncan's team had originally explored

POLYPLOID CELL TYPES IN MAMMALS

CELL TYPE	LOCATION	FUNCTION	NUMBER OF GENOME COPIES
Megakaryocyte	Bone marrow	Producing blood clotting platelets	Up to 128
Hepatocyte	Liver	Detoxification, metabolism	Typically 4 to 16
Trophoblast giant cell	Embryo	Promote implantation	Up to 1000
Cardiomyocyte	Heart	Contraction	Typically 4



Bonus DNA. The polyploid cells in mammalian bodies differ in their location, function, and number of chromosome sets (table). In a liver cell (*right*), the multiple chromosome copies (blue) have sorted into three clusters in preparation for cell division.

CREDIT: (RIGHT) ANDREW W. DUNCAN AND MARKUS GROMPE

whether the polyploid cells within the liver are "terminally differentiated," meaning that they had become mature cells that don't divide and help replenish the organ. So the team transplanted polyploid hepatocyte cells into mice whose livers had been partially removed. "To our great surprise, they regenerated the liver perfectly," Duncan says.

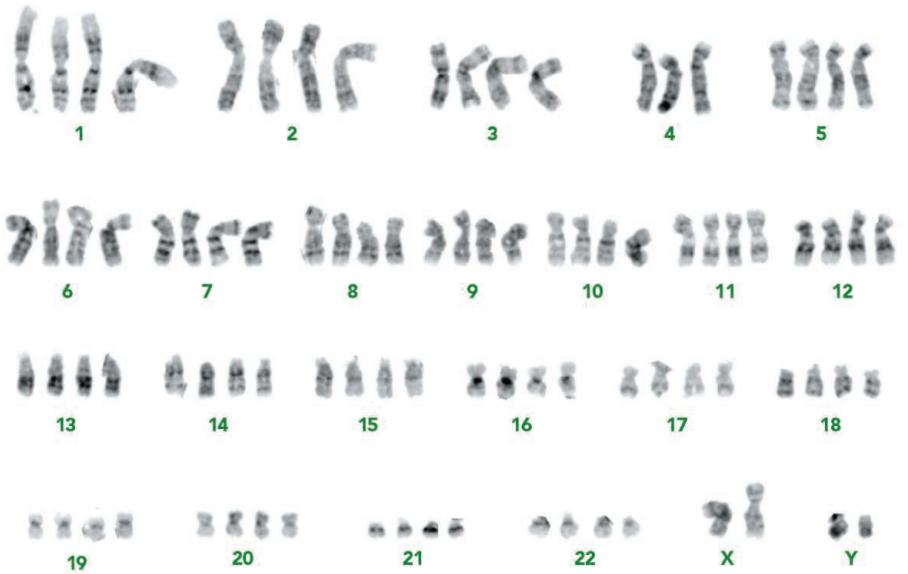
That's when Duncan put the liver cells under the microscope, turned on the camera, and noticed their unorthodox division style. The researchers discovered something else unusual about the cells. As the team revealed in *Nature* in 2010, when many of the polyploid cells divided, they spawned diploid daughter cells. But often these diploid daughters hadn't quite returned to normal—many of them had gained or lost an individual chromosome, a condition called aneuploidy that is generally considered ominous. "Most cancer folks will tell you that aneuploidy is synonymous with cancer," Duncan says.

But some researchers have proposed that aneuploidy can create useful genetic diversity in a tissue or organ, allowing cells to add a copy of a beneficial gene or throw out a copy of a detrimental one. And when Duncan and colleagues studied an example of liver regeneration in mice, they found that sites where regrowth occurred were rich in aneuploid cells. They have discovered that aneuploid cells are abundant in human livers, too.

Duncan now hypothesizes that polyploidy in the liver is a roundabout way to produce aneuploid cells that have regenerative properties. His team is now working to confirm that these cells spur regeneration in people suffering from hepatitis B, in which a virus devastates the liver. Some patients die unless they get a liver transplant, but others survive as sections of the organ regenerate. The researchers are collecting tissue samples to determine if areas of the liver that regrow are high in aneuploid cells.

But the idea that polyploidy helps tissues regenerate remains a hypothesis, as findings from Leone's group and that of Alain de Bruin, a pathologist and veterinarian at Utrecht University in the Netherlands, emphasize. They genetically engineered mice so the animals' livers lack two polyploidy-promoting proteins. "We can generate a mouse whose liver is almost entirely [diploid] cells," De Bruin says. Both teams expected that the animals would suffer ill effects. Instead, the mice were vigorous, each group reported in *Nature Cell Biology* in 2012, and their livers were no less able to regrow after injury. The mice De Bruin and colleagues studied, for example, could restore their livers after surgical removal of two-thirds of the organ. "This polyploidization does not have an effect on regeneration or on proliferation rate," De Bruin says.

The work from both teams also undermines another older polyploidy hypothesis. The liver, De Bruin notes, "is all the time exposed to toxins." Hepatocytes work hard to detoxify all those noxious substances, and some researchers had speculated that their extra genetic material could boost the output of proteins crucial to this. Yet the mice whose livers had reduced polyploidy had no problems breaking down toxins, De Bruin's group found.



Double down. The chromosome copies from a polyploid liver cell arranged by size, showing that the cell carries four copies of almost every one.

> "The real unanswered question is why any cell type is polyploid. We are poised to begin answering that question."
>
> —Robert Duronio, University of North Carolina, Chapel Hill

Exploiting polyploidy

Even as they wrestle with mystery of polyploidy, researchers wonder whether they can put what they've learned to use. Leukemia biologist John Crispino of Northwestern University's Feinberg School of Medicine in Chicago, Illinois, and his colleagues have trained their sights on a type of acute myeloid leukemia, triggered by megakaryocytes, that kills most adults who develop it. Mature megakaryocytes don't divide, but in this form of cancer, the cells remain immature, don't become polyploid, and replicate prodigiously, causing the leukemia. Crispino and colleagues propose that forcing the cells to become polyploid and mature might treat the cancer.

The team revealed in *Cell* in 2012 that it had identified more than 200 compounds that, in lab dishes, spur polyploidy in human megakaryocytes. One of these molecules, alisertib, is already under-going clinical trials for several other types of cancer—though not because of its ability to stimulate polyploidy. Crispino's group is now trying to organize an initial safety trial of the drug in people with acute myeloid leukemia.

Although polyploidy research has recorded some progress in recent years, the field still hasn't nailed down the benefits polyploidy provides to different mammal cell types. To move forward, Leone says, researchers should take a cue from plant biologists, who have tested polyploidy's advantages in specific environmental conditions, showing that it boosts tolerance for salinity (*Science*, 9 August 2013, p. 658). Scientists could perform similar studies on liver cells, for example, by gauging whether polyploidy helps them deal with different diets. Delving further into polyploidy's cellular roles will probably produce some surprises, UNC Chapel Hill's Duronio predicts. "There are going to be many uses for polyploidy, and we are just scratching the surface."

–MITCH LESLIE