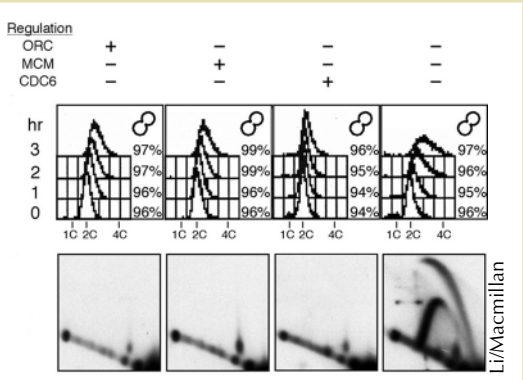
Research Roundup

Fail-safe replication

How does the cell ensure that its DNA is replicated once and only once in every cell cycle? Replication takes place at many sites and must be regulated separately at each one. That's a tough job even in budding yeast, with ~400 initiation sites, but a Herculean one in human cells, with as many as 100,000. Moreover, not all replication events are initiated at the same time.



Only yeast cells lacking all three regulation mechanisms (right) rereplicate their DNA.

Researchers in Joachim Li's lab (University of California, San Francisco, CA) are among those who have implicated three mechanisms in the prevention of reinitiation: phosphorylation of the origin recognition complex (ORC), downregulation of Cdc6 protein levels, and exclusion of the Mcm2–7 complex from the nucleus. Yet the authors were frustrated to find that when they disrupted any one of these mechanisms, the block to reinitiation remained intact.

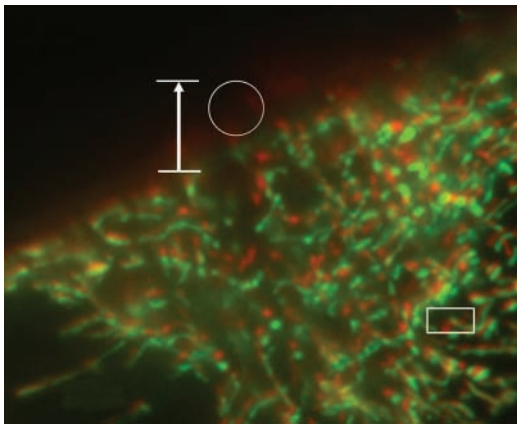
One possible explanation is that any one of the mechanisms is sufficient to maintain control of reinitiation. "So we had to start knocking out multiple mechanisms. We did that, and the bottom line is that when we perturbed all three of them, when we prevented the ORC from being phosphorylated, and forced the Mcm to stay in the nucleus, and we ectopically expressed Cdc6 protein, now we could see cells reinitiate DNA replication," Li reports. It makes perfect sense, he points out, that the cell would use multiple overlapping mechanisms as a fail-safe system, one that could all but guarantee that no segment of DNA would replicate more than once per cell cycle. ■

Reference: Nguyen, V.Q., et al. 2001. *Nature.* 411:1068–1073.

Tensegrity lives

For 20 years the notion that stability of cell shape is based on the rules of an architectural system known as tensegrity has been raising hackles in cell biology. Now tensegrity's foremost proponent, Donald Ingber of Harvard Medical School, Boston, MA, reports additional experimental evidence supporting the hypothesis.

Tensegrity (tensional integrity), exemplified by Buckminster Fuller's goedesic dome, is a tension-dependent building system, as opposed to a compression-dependent system. An example of the latter is a conventional house made of brick stacked on brick that gets its stability from gravity. By contrast, a tensegrity structure like Fuller's gets its stability from continuous tension that is transmitted over all of its elements and is balanced by a subset of elements that cannot be compressed. Ingber has long argued that cells are not, as conventionally pictured, elastic bags of viscous gel. Instead, cells create tension in their contractile microfilaments and transmit the tension all over the cell. The tension is resisted both by external attachments and by internal elements, such as microtubules, that Ingber likens to compression-resistant tent poles.



Mitochondrial positions before (green) or after (red) a micromanipulator (circle) was used to exert upwards tension on a surface-bound bead.

In their most recent paper, Ingber and colleagues deal with several criticisms of tensegrity via experimental results that are also consistent with the a priori predictions from a mathematical model of tensegrity previously devised by coauthor Dimitrije Stamenovic (Boston University, Boston, MA). For example, they allowed a cell containing GFP-tagged mitochondria to bind to coated beads that stick to integrin receptors across the cell surface. They then pulled the bead away from the cell, which caused the integrins to link up to the microfilament cytoskeleton and made the fluorescent mitochondria move at a distance.

Ingber argues that this means forces are transmitted across the integrin, over the microfilaments, and through structural interconnections to the microtubules. "So it confirms that if you physically connect to the right receptors that couple to the internal cytoskeleton, you get long-distance force transfer," he says.

Consistent with the idea of an interconnected cytoskeleton, Anne-Marie Yvon and her colleagues in Patricia Wadsworth's lab (University of Massachusetts, Amherst, MA) show that actomyosin generates tension that moves microtubules in cells, whereas cytoplasmic dynein/dynactin complexes resist that tension. ■

References: Wang, N., et al. 2001. *Proc. Natl. Acad. Sci. USA.* 98: 7765–7770.
Yvon, A., et al. 2001. *Proc. Natl. Acad. Sci. USA.* 10.1073/pnas.141224198.
http://www.pnas.org/cgi/doi/10.1073/pnas.141224198

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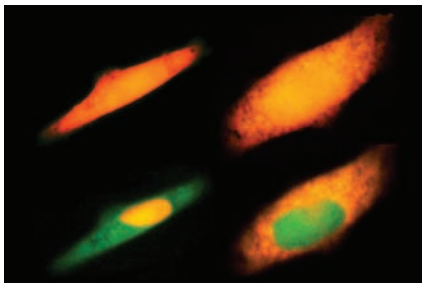
Christmas in July

First there was green fluorescent protein (GFP) from jellyfish, then came red fluorescent protein (DsRed) from coral. Now researchers from the University of California at Irvine show that DsRed may become equally indispensable for tracking cells, organelles, and fusion proteins by turning them... green?

Ian Parker says he and his colleagues made the discovery quite by accident while exploring the use of DsRed for multiphoton confocal imaging. Brief exposure to a femtosecond-pulsed laser beam turned DsRed's fluorescence from red to green when viewed with a conventional one-photon microscope. The excitation, they explain, selectively bleaches the mature, red-emitting form of DsRed, which enhances emission from the immature green form.

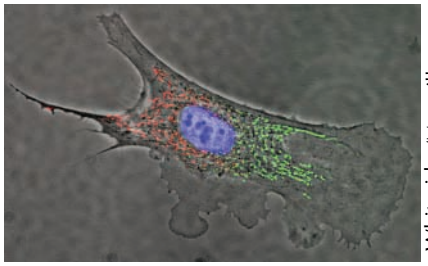


Parker/Macmillan

DsRed-expressing cells before (upper) and after (lower) "greening" of the cytoplasm (left) or nucleus (right).

The color change persists for hours or even days, and appears to do the cells no damage. Parker likens the technique to using a highlighter pen to mark important bits of text. The color change, he says, provides a powerful tool for picking out individual cells, fusion proteins, or even small regions of cells, by bleaching them green and leaving red everywhere else. Thus, the technique is suitable for studying cell lineages, organelle dynamics, and protein trafficking, as well as for selective retrieval of cells from a population.

Another method for labeling part of a cell has been described by Shuichi Takayama and colleagues (Harvard University, Cambridge, MA). They developed a chip that uses multiple laminar streams in a microfluidic channel; it can deliver membrane-permeable molecules to selected bits of a cell without causing major disruptions.



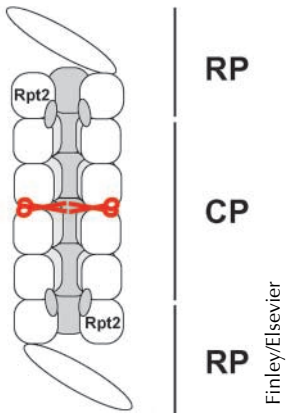
Whitesides/Macmillan

Selective labeling of mitochondrial subpopulations using PARTCELL.

The researchers are calling the method "partial treatment of cells using laminar flows," or PARTCELL, and have used it to study mitochondrial movement and changes in cytoskeletal structure. One example: they streamed different solutions containing fluorescent tags over opposite poles of a live bovine capillary endothelial cell. This enabled them to selectively label mitochondrial subpopulations in different parts of the cell and watch the two kinds of mitochondria moving around and mixing together. ■

References: Marchant, J.S., et al. 2001. *Nat. Biotech.* 19:645–649.
Takayama, S., et al. 2001. *Nature.* 411:1016.

Keeping the lid on destruction



Finley/Elsevier

Rpt2 (top and bottom) controls access to enzymes in the proteasome core.

Proteins slated for destruction enter the proteasome core particle (CP) through a channel that usually remains closed for safety's sake. It opens when a CP associates with the proteasome regulatory particle (RP), which recognizes the substrates and ushers them into the channel. What persuades the channel to open? A ring of six ATPases straddles the channel, and those enzymes have been thought to figure in this process. Now, Alwin Köhler and his colleagues in the laboratories of Daniel Finley and Alfred Goldberg (Harvard Medical School, Boston, MA) report, surprisingly, that just one of the six ATPases, Rpt2, is the key that unlocks the channel. In addition, the products of protein degradation exit the proteasome the same way that they came in, pointing to some possible traffic problems.

The paper also reports on one reason why the proteasome might want to keep tight control over gating. With the help of yeast CP mutants that stay open, the authors compared the products of the proteasome when it was kept in a closed or an open state. The median length of peptides produced by the mutant CP was 40% larger than those produced by the wild type. The results, Finley says, confirmed their suspicions that the size of products is determined by competition between their ongoing degradation and their exit from the internal chamber of the proteasome.

The size of degradation products is not an idle question, because in mammals such a product may be incorporated into a class I histocompatibility molecule to be presented to the immune system as a potential antigen. Therefore, proteasome efficiency is likely to be important for immune system function. That makes the state of the channel important too, and may explain why the immune system churns out channel-opening proteins as it is revving up, Finley points out. "That's very consistent with our data, that you would want to open the channel" he says. "Because if the channel were closed and the peptides couldn't get out efficiently, then they would be overdigested, and when overdigested they would no longer be competent to be presented to the immune system."

Stay tuned. Finley says there are reasons to believe that there is a second mechanism for gating, which may be revealed by the use of full-length proteins rather than peptides in the in vitro proteasome assays. ■

Reference: Köhler, A., et al. 2001. *Molecular Cell.* 7:1143–1152.