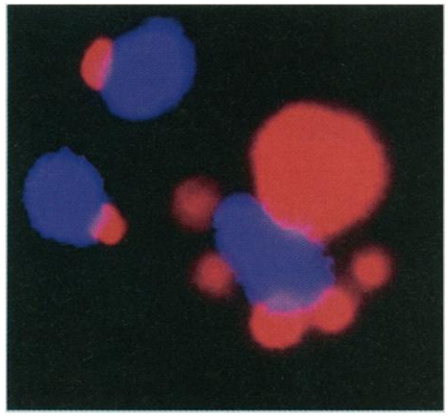
CELL BIOLOGY

Do Fateful Circles of DNA Cause Cells to Grow Old?

Ordinarily when we think of aging, it's the outward signs that come to mind: wrinkles, graying hair, and withering muscles. But if Leonard Guarente's hunch is right, time may leave a much more telling mark in the nuclei of our cells.

In work described in the 26 December issue of *Cell*, Guarente and David Sinclair, molecular biologists at the Massachusetts Institute of Technology (MIT) in Cambridge, have linked aging and senescence in yeast to the buildup in the nucleus of circles of DNA that have popped out of the chromosomes and copy themselves each time the yeast cells replicate. The researchers propose that when enough of these circles accumulate, they clog the nucleus and prevent the cell from reading or replicating its genome, causing it to stop dividing and ultimately to die. "It's a fairly precise timing mechanism, and the effect on the cell will be quite gradual, which is how we recognize aging," Guarente explains.

No one has yet found these aberrant DNA circles in mammalian cells. But "all cells have the potential to suffer these circle pop-out events," comments David Shore, a molecular geneticist at the University of Geneva in Switzerland. "There's obviously the tantalizing suggestion that this [mechanism] may be related to senescence in other organisms." If that proves to be the case, then yeast should provide a useful model not only for learning about aging in humans but also for assessing ways to slow it down.



D. SINCLAIR AND L. GUARENTE/MIT

Generation gap. An aged yeast nucleus (blue) has a swelled, fragmented nucleolus (red), unlike nuclei of too-young cells (left).

Guarente discovered the circle buildup while using the budding yeast, *Saccharomyces cerevisiae*, to study Werner's syndrome, a hereditary disease in which people age prematurely and often die before reaching age 50. No one knows how defects in the Werner's syndrome gene cause premature aging. But the human gene, discovered over a year and a half ago (*Science*, 12 April 1996, pp. 193 and 258), has a yeast equivalent, called *SGS1*, which made it possible for Guarente to address the problem in that much simpler organism.

Not only does *SGS1* look like the human gene, but it also influences the rate of aging, the MIT team found. Cells of budding yeast can reproduce by mating, but more often they bud off daughter cells asexually. Normal cells can repeat the process about 25 times before they gradually enlarge and become unable to mate sexually with other yeast cells, telltale signs of old age. But the researchers found that the *SGS1* mutants aged prematurely; they stopped budding and became sterile after only about nine rounds of budding (*Science*, 29 August 1997, p. 1313).

At the time, Guarente and his colleagues noted an intriguing change in a small structure within the nucleus, known as the nucleolus. The nucleolus is where the RNAs that help make up the cell's protein factories, the ribosomes, are transcribed from the ribosomal DNA. Normally compact and crescent-shaped, the nucleoli of the mutant cells had become enlarged and fragmented. Now he knows the cause of the structural changes.

With Sinclair, Guarente found that as mother cells replicate their DNA prior to budding, they also replicate small circles of ribosomal DNA. The researchers suspect that the first circle probably arises by accident because of the highly repetitive nature of ribosomal DNA. Such DNA is more likely to be mishandled and excised by the cell's DNA-processing machinery. "It's an enormous task to prevent a circle from forming," Guarente suggests. But once formed, circles can replicate with the rest of the yeast cell DNA.

By adding a marker to DNA that causes cells containing the circles to turn pink, the researchers also showed that the circles almost always stay in the mother cell. As a result, they accumulate over time, eventually reaching the point where this excess DNA equals the total yeast genome. "It changes the morphology of the nucleus," says David Finkelstein, a yeast biologist at the National Institute on Aging in Bethesda, Maryland. Indeed, so great is the burden that the nucleolus seems to burst.

The circles are seen in both normal cells and the *SGS1* mutants, but they accumulate much faster in the mutant cells, suggesting that they may be linked to the rate of aging. In addition, when the researchers took a length of DNA that, under specific conditions, releases a DNA circle and added it to another yeast strain, they found that its life-span decreased by 40%. "What [they have] found is that the ribosomal DNA is amplified and keeps building up," says Finkelstein. "Aesthetically, it's a very pleasing observation." In a different yeast strain, the researchers retarded the formation of the ribosomal DNA circles and extended the life-span by 25%.

Guarente suspects that certain proteins normally hold circle formation in check and thereby slow aging. One is the SGS1 protein itself, which is concentrated in the nucleolus. Another is a set of molecules called SIR proteins, which inactivate or "silence" entire regions of genes whenever they bind to a chromosome. In earlier work, Guarente's group had shown that the SIR proteins gradually migrate to the nucleolus as yeast cells age. They also found that in a long-lived SIR mutant, this migration occurred earlier than usual, suggesting that the earlier shift delayed aging-related processes there.

But Guarente and Sinclair haven't figured out how any of these proteins might control circle formation. And they have yet to prove that the circles are actually causing the cells to age, for example, by disrupting normal replication and transcription. Furthermore, although Guarente thinks human tissues with actively dividing cells could suffer a similar fate, Finkelstein is skeptical. "That he sees this phenomenon in yeast doesn't necessarily mean that's what happening in humans," he cautions.

But others are deeply intrigued. "They've really come up with the clearest molecular mechanism for aging that anyone has come up with yet," says Shore.

While yeast have very little repetitive DNA outside the ribosomal genes, humans have lots. That DNA might give rise to circles as it replicates, and the yeast work suggests that the circles of any kind of DNA that can replicate could have an aging effect. "This could be the tip of the iceberg," notes Lawrence Loeb, a molecular biologist at the University of Washington, Seattle.

The next step is to try to find out whether human cells do, in fact, accumulate circles. But that could be quite a challenge, he and Guarente note, because they don't know what kind of cells to look at. Nevertheless, says Shore, the idea "is screaming out to be tested in humans."

–Elizabeth Pennisi