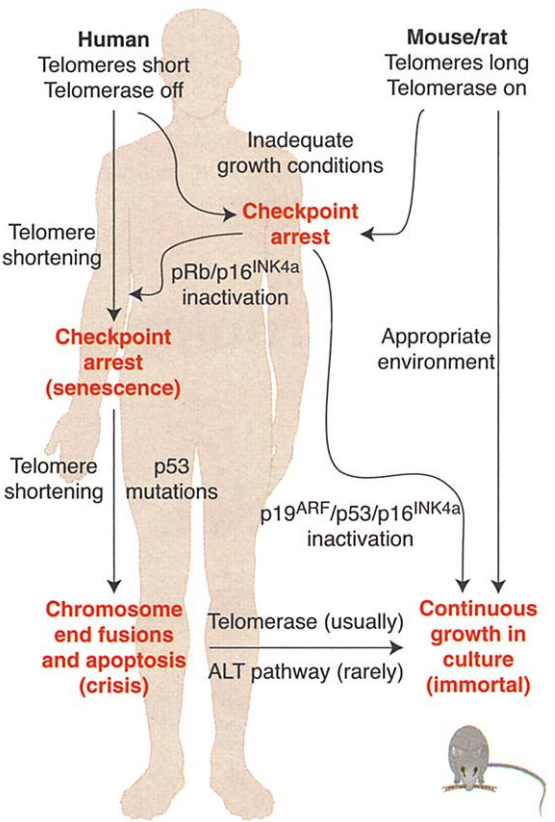
PERSPECTIVES: AGING

# When Do Telomeres Matter?

Jerry W. Shay and Woodring E. Wright

Some cultured mammalian cells do not proliferate indefinitely but rather, after a certain number of doublings, permanently stop dividing, a process called replicative senescence. Human cells use shortening of telomeres (the ends of chromosomes) with each cell division to count the number of cell divisions they have undergone, and stop dividing after 50 to 90 doublings. Rodent cells do not seem to have a cell division counting mechanism. So what causes rodent cells to stop dividing after 10 to 15 doublings? A clue comes from studies by the Raff and Lloyd groups at the MRC Laboratory for Molecular Cell Biology in London. On pages 868 and 872 of this issue, they present persuasive evidence that, under the correct culture conditions, normal rat oligodendrocyte precursor cells (*1*) and rat Schwann cells (*2*) continue to divide indefinitely. These findings are of obvious practical importance because they establish the conditions necessary for successful propagation of the supportive cells of the nervous system. However, they also help to resolve a feature of replicative senescence that has been widely misinterpreted. The authors provide direct evidence that what has been called cellular senescence in cultured rodent cells in fact may be a response to inappropriate culture conditions.




**The long and short of aging.** Most rodent cells contain the enzyme telomerase and have long telomeres. Under the appropriate tissue culture conditions, rodent cells are capable of continuous growth (*1*, *2*, *8*). However, an inadequate culture environment (for example, that in which appropriate survival factors are missing or where different cell types cannot interact) may result in DNA damage or other stresses that induce arrest of cell division at cell cycle checkpoints. The spontaneous inactivation of $p19^{ARF}$, p53, and perhaps $p16^{INK4a}$ under standard culture conditions frequently enables normal rodent cells to grow continuously. Human fibroblasts have short telomeres and are usually telomerase silent. As is the case with rodent cells, inadequate culture conditions may induce human cells to activate checkpoint pathways that lead to their early (and telomere-independent) growth arrest (*9*, *10*). In contrast to rodent cells, bypass of the p53 and/or $pRb/p16^{INK4a}$ checkpoints is insufficient to immortalize human fibroblasts but does prolong their lifespan. Continuous telomere shortening of human fibroblasts leads to chromosome fusions, crisis, and apoptosis. Only a rare human cell (1 in 10 million) can bypass crisis either through telomerase reactivation or through ALT (the rare alternative pathway for telomere lengthening). Although methods for counting the number of cell divisions do not limit the growth of cultured rodent cells, in human cells telomere shortening does provide a record of the number of doublings and does control replicative senescence.

Replicative senescence in human cells (most of which lack the enzyme telomerase that prevents loss of telomeres) is caused by shortening of the telomeres with successive cell divisions (*3*). Once telomeres become sufficiently short, they presumably lose the ability to mask the end of the chromosome and prevent it from being recognized as a broken DNA molecule. This results in activation of the p53-dependent damage checkpoint, which induces growth arrest of the cells (*3*). There is now compelling evidence that cultured rodent cells do not use the shortening of telomeres to time their cessation of division after 10 to 15 doublings (*4*). The telomeres of mouse and rat cells are 5 to 10 times as long as those of human cells. Their telomeres are so long that although rodent cells engineered to lack telomerase show telomere shortening, they show no decrease in the number of doublings before division ceases (*5*). Although telomerase is active in the rodent neural cells studied by the Raff and Lloyd groups, the presence of telomerase activity does not explain why the authors succeeded in growing these cells indefinitely.

If telomere shortening is not inducing rodent cellular senescence, then what is? A core concept of modern theories of aging is that longer-lived species have evolved more efficient maintenance and repair capacities in order to better fend off the damage that accompanies the passage of time. Mouse and rat cells repair DNA damage far less efficiently than do human cells (*6*) and are much more sensitive to a variety of agents that produce oxidative stress (*7*). This has led to the hypothesis that the foreign environment of tissue culture—which has two rather than three dimensions, lacks most extracellular matrix molecules, has 20% oxygen, and contains a distorted mixture of nutrients, trace elements, hormones, and serum—might induce a level of stress that can be tolerated by human fibroblasts but causes progressive damage and eventual growth arrest of mouse fibroblasts (*4*). The authors of the new studies are to be congratulated for discovering the appropriate culture conditions that permit the indefinite growth of rat oligodendrocyte precursor cells and Schwann cells. They also deserve a round of applause for demonstrating that these cells do not have any intrinsic method for counting cell divisions that could prompt their entry into replicative senescence. The use of the term cellular senescence to describe the growth arrest of cultured rodent cells reflects a historical misapplication of terminology rather than any underlying molecular pathway.

In spite of their success, it is clear that the "appropriate" culture conditions are still not perfect. The new papers report increases in cell cycle checkpoint factors, such as $p19^{ARF}$ and $p16^{INK4a}$ for cultured Schwann cells (*2*), and $p19^{ARF}$, $p18^{INK4a}$, and the Cip/Kip family of cyclin-dependent kinase inhibitors (p21, p27, and p57)

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for oligodendrocyte precursor cells (*1*). These increases do not result in growth arrest, apparently because there is a compensatory increase in the expression of positive growth mediators such as Cdk2, Cdk4, and cyclins D1, D3, and E. The indefinite growth observed by the investigators probably reflects a combination of culture components that minimize "stress," including the right hormones and other factors that are able to balance the stresses that do occur. The cultured neural cells remained diploid (that is, they retained both sets of chromosomes) and, in contrast to most established rodent cell lines, their cell cycle checkpoints were activated normally in response to insults such as irradiation or the overexpression of Ras.

It is widely believed that replicative senescence evolved to limit the number of available cell divisions, and that it thus behaves as a brake against the accumulation of the multiple mutations needed for a cell to become malignant. A 70-kg man who lives for 80 years has to be 14,000 times as resistant to developing cancer as a 0.2-kg rat that lives for 2 years: (70 kg ÷ 0.2 kg) × (80 years ÷ 2 years) = 14,000. The results of the two new studies (*1*, *2*) support the notion that a cell's conventional arsenal—which includes DNA repair pathways and antioxidant enzymes—is adequate to protect against the accumulation of mutations and development of cancer during the short life of small organisms. In contrast, larger and longer-lived species had to evolve replicative senescence to ensure that they would have the greatly increased protection that their longevity necessitated (*4*). Thus, although some might interpret the indefinite growth of normal rat oligodendroglial precursor cells and Schwann cells as a feature unique to only specific cell types, we think it more likely that this unfettered growth represents a fundamental biological difference between normal human cells (which count cell divisions) and normal rodent cells (which do not). Appreciating this difference will be essential for designing and interpreting experiments that investigate how replicative senescence, telomeres, and telomerase are involved in aging and cancer.

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PERSPECTIVES: SEMICONDUCTORS

# Toward Functional Spintronics

**Hideo Ohno**

The enormous success of semiconductor electronics we have been witnessing is solely based on the charge carried by electrons. The other key property of the electron, its spin, has been completely ignored in semiconductors but is used in information storage by magnetic materials. Researchers are now investigating ways to use both properties simultaneously in semiconductors (*1*). This field is often called semiconductor spintronics because it is expected to lead to the integration of processing and storage capabilities thus far carried out separately and to quantum information processing that exploits the quantum nature of the spin state (*2*). A key advance has been the synthesis of magnetic semiconductors based on III-V compounds (such as GaAs used in transistors and lasers), which may enable the integration of magnetism into existing semiconductor devices (*3*). However, practical application of spintronics requires room-temperature ferromagnetism in semiconductors. Making such materials represents a substantial challenge for materials science.

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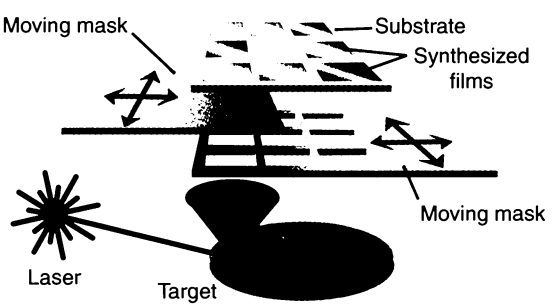


**Schematic diagram of the combinatorial laser ablation apparatus used by Matsumoto *et al.*** The method allows the formation of a series of thin films with varying elemental composition under virtually identical conditions. Without such methods, one needs to prepare thin films one by one while trying to keep the preparation conditions the same, which is time consuming and difficult.

On page 854 of this issue, Matsumoto *et al.* report the discovery of just such a material (*4*). Their room-temperature ferromagnetic semiconductor is based on anatase, one of the forms of titanium dioxide, doped with a few percent of cobalt. The material is made by laser ablation in high vacuum. Its band gap is wide enough ($E_g$ = 3.2 eV) not to absorb any light at visible wavelengths, and the semiconductor is therefore completely transparent.

Ferromagnetic semiconductors such as europium oxide have been known for decades, and III-V– and II-VI–based ferromagnetic semiconductors were reported recently. But all of them are ferromagnetic only below room temperature and have a band gap in the infrared; that is, they are opaque. Initial results about room-temperature ferromagnetism in semiconductors were reported recently by two other groups (*5*, *6*).

The semiconductor reported by Matsumoto *et al.* (*4*) provides considerable flexibility in designing circuits and storage, particularly for devices with displays. Semiconductors can be used to build circuits, but when they are ferromagnetic, they may also be used to build magnetic storage devices called magnetic random access memories (*7*). The working dimension in semiconductor circuits and magnetic storage becomes smaller and smaller as the quest for ever higher speed and density continues. However, we cannot reduce the dimensions of visual user interfaces at the same rate for obvious reasons. The ideal scenario would be the integration of the electronic circuits and magnetic storage with the user interface in a single flat panel display, leading to a truly electronic paper. Especially when the display itself needs thin film transistors to drive individual elements (as is the case in liquid crystal displays, where opaque transistors block some of the fluorescent backlight), the full integration of circuit-storage-display by a transparent semiconductors is highly desirable.

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