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are essential to antidepressant action. If ocular illumination has antidepressant effects beyond its circadian action—even at midday, an immediate energizing effect in fatigued winter depressives has been noted—will popliteal illumination provide a mimic?

Campbell and Murphy posit a potential advantage of popliteal illumination over illumination of the eyes for therapy: It can be administered while patients are asleep. Outside our dark bedrooms, nature's gradual, dim dawn signal often rises during the final hours of sleep, when the propensity for phase advances is greatest. Artificial simulations of dawn shift the melatonin rhythm, expediting wake-up, increasing morning alertness, and countering winter depression (*10*). Thus, bright light to open eyes is not a therapeutic *sine qua non*. The Campbell-Murphy method uses 13,000 lux of high illumination, similar to the amount used in postawakening bright light therapy (although the lux metric, adjusted for human photopic spectral sensitivity, may confuse dose specification). A comparison of popliteal illumination with dawn simulation at corresponding phases of sleep would clarify whether the two methods share parallel chronobiological and antidepressant effects. It is premature to assume therapeutic equivalence, especially since ocular light may well affect mood by trigger extracircadian mechanisms.

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11. The authors' research has been sponsored by a Department of Veterans Affairs Career Development Award (D.A.O.) and NIH grant MH-42931 (M.T.)

CELL BIOLOGY

Telomeres and Senescence: Ending the Debate

Titia de Lange

All's well that ends well, and so it goes for the decade-old debate on the role of telomere shortening in the senescence of cells. The dispute began in 1986 when Howard Cooke first glimpsed the DNA at the ends of human chromosomes (*1*). Curiously, the telomeric regions he examined, those capping the long arms of our sex chromosomes, were substantially shorter in somatic tissue than in germline cells. Knowing that telomeres were threatened by loss in each cell division unless their repeated sequences were replenished by telomerase, Cooke speculated that this enzyme [then just discovered in a ciliated protozoan (*2*)] might not be active in normal somatic human cells. The resulting erosion of telomeric DNA, now known to be a general feature of all human somatic tissues, could explain the shortening first witnessed in blood cells. He further speculated that the decline of the protective telomeric cap might eventually limit the ability of somatic cells to proliferate. Although this idea received considerable attention from students of human aging and cancer, it remained unproven and controversial. The doubt has now come to an end with a report on page 349 of this issue (*3*), describing direct evidence for a causal relation between telomere shortening and cellular senescence.

Critics of the idea that telomeric decline acts to count cell divisions for regulation of cellular life-span pointed to a wrinkle of murine origin. *Mus musculus*, the mouse species so favored for laboratory work, has telomeres that are three times longer than ours, yet its cells do not live three times as long (*4*). In a second apparent discrepancy, senescent human cells do not lose all telomeric DNA but carry residual telomeres similar in length to those of many other eukaryotes (*5*). How could incomplete loss of the telomeric DNA make such a crucial difference in the life of the cell?

Advocates of the model deployed their own arsenal of experimental evidence, predominantly of a correlative nature, including an impressive relation between the proliferative potential of primary human cells and the length of their telomeres (*6*) and the finding that telomerase activity was undetectable in most somatic tissues (*7*). Telomere-clock champions were also fortified by the consistent shortening of telomeres during cellular aging in culture and with aging of human tissues in vivo (*5*, *6*). Furthermore, the relation between telomere loss and senescence had been established by the finite life-span imposed on mutant yeast cells harboring a defunct telomere maintenance system (*9*).

Arguably, the most compelling data supporting the view that telomere loss eventually restrains the proliferation of human cells arose from human tumors and immortal cell lines. If telomere shortening curbs the number of divisions allotted to primary human cells, then immortalization should somehow liberate cells from this restraint. Indeed, immortalization of human cells is invariably accompanied by a key change in telomere dynamics involving either the activation of telomerase or an alternative mechanism that maintains telomeric DNA (*7*, *10*).

The tally of telomerase-positive human cancers is extensive, indicating that averting telomere loss is a common aspect of tumorigenesis, perhaps as frequent as mutations in the Rb and p53 pathways. The suggestion that telomerase activation is a mere side-effect of de-differentiation, or rapid proliferation, has not found an experimental foot-hold; stem cells have much lower telomerase activity than tumors, and many proliferating normal cells lack the enzyme altogether. Certainly, the simplest way to explain the prevalence of telomerase in human malignancies is to assume that telomere maintenance is a prerequisite for continued tumor growth; in other words, telomere shortening is a tumor-suppressing mechanism. This interpretation was recently assailed by studies of a knockout

The author is in the Laboratory for Cell Biology and Genetics, The Rockefeller University, New York, NY, 10021, USA. E-mail: delange@rockvax.rockefeller.edu

mouse lacking the essential RNA subunit of telomerase (*11*). Cells from these mice still could undergo malignant transformation and form tumors after transformation with viral oncoproteins, contrary to the predictions of the telomere-clock model. However, alternative mechanisms of telomere maintenance could have been activated in these cells.

To resolve the controversy, Bodnar *et al.* tested the effect of inappropriate activation of telomerase in normal human cells (*3*). Their approach was made possible by the discovery of the reverse transcriptase subunit of telomerase (TRT) from two unicellular eukaryotes (*12*) and by the subsequent identification of a human ortholog (hTRT) (*13*). Most somatic human cells do not express this reverse transcriptase but contain all the other components of the enzyme so that expression of the missing hTRT component leads to reconstitution of enzyme activity (*14*). The task of Bodnar *et al.* was therefore straightforward: they simply examined the proliferative potential of primary human cells that were forced to express telomerase from a transfected hTRT gene.

Their results were strikingly unequivocal. Activation of telomerase resulted in the unscheduled addition of the drones of TTAGGG repeats that normally cap human chromosome ends. Cells with such artificially elongated telomeres showed a spectacular alteration in their growth potential. Whereas the primary cells used in the experiment normally senesced after a well-defined number of cell divisions, the telomerase-positive cells missed their senescence cue and continued to divide. At the time of submission of the work, many of the cell lines had proceeded for 20 population doublings beyond their normal senescence point and not only continued to grow vigorously but also showed a normal karyotype and maintained a youthful morphology. Similar results were obtained with three different primary cell types (retinal epithelial cells, foreskin fibroblasts, and vascular endothelial cells) attesting to the universal role of telomere shortening in the senescence of human cells.

These data support Cooke's original speculation (*1*) and the subsequent proposal of the telomere-clock model (*15*). Human telomeres are programmed to undergo gradual shortening by about 100 bp per cell division and when several kilobases of the telomeric DNA are lost, cells stop dividing and senesce. How telomere shortening is detected, and what the downstream signaling pathway is, will need to be addressed. However, there is now little doubt that this process has the makings of a powerful tumor suppressor system, creating a barrier to any cell that has escaped normal growth control by its environment.

But what about mice with their long telomeres and the fact that knockout mice lacking the telomerase RNA gene still spawn transformed cells? The answer to these questions is not clear at this stage. However, it seems very unlikely that mice use telomeres as a tumor suppressor system and perhaps with good reason. Since the telomere barrier to proliferation does not manifest itself until many cell divisions have passed, this mechanism may not be useful for a small animal in which a 2-cm mass of misplaced cells could be life-threatening. Such a small tumor would usually not affect people adversely. Other tumor suppressor pathways show subtle differences between mice and men as well (for example, BRCA-1 and Rb).

As Bodnar *et al.* point out, the ability to rejuvenate human cells with telomerase will create significant new opportunities for basic and applied interests. Will the manipulation of telomerase allow alteration of cellular life-span in vivo? Time will tell, but resetting the telomere clock may come at a price, since the induction of cellular immortalization might increase the rate of tumorigenesis in the altered cell population.

The implications for cancer research are also profound. The new data indicate that activation of telomerase in human tumors bypasses cellular senescence and is thus a requirement for tumor progression. As such, the molecular basis for telomerase de-regulation in tumors should be of immediate interest; the prediction is that one or more tumor suppressor genes prevents activation of telomerase in normal human cells.

Finally, the results should strengthen the determination of those who are searching for telomerase inhibitors as potential anti-cancer agents. However, the role of telomerase in human tumors deserves further testing and some other concerns remain, including worries about the protection afforded by long tumor telomeres and the possibility that other telomere maintenance systems might take over once telomerase is out of commission. As human telomeres have only just advanced as prime pieces in the field of oncogenetics, there is no telling what the end-game will be like.

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SURFACE CHEMISTRY

A New Opportunity in Silicon-Based Microelectronics

John T. Yates Jr.

The silicon surface designated by crystallographers as the (100) face provides the foundation for the silicon-based microelectronics industry and has been the subject of a wide range of investigations that make use of almost the entire arsenal of experimental methods in surface science (*1*). One small portion of the research on this important silicon surface concerns its chemical reactivity with organic molecules, which recently led to a major advance that is destined to open new technological opportunities in microelectronics (*2*). With the knowledge gained, it may be possible to bond a wide range of useful organic molecules directly to the dangling bonds on the surface.

Clean Si(100) is a reconstructed surface in which pairs of atoms dimerize to produce ordered rows of silicon dimers in (2×1) unit cells, as first postulated by Schlier and Farnsworth almost 40 years ago on the basis of low-energy electron diffraction (LEED) patterns (*3*). These dimers, now routinely observed by scanning tunneling microscopy (STM), present a pair of dangling bonds (exhibiting partial p character) at the surface, and the high reactivity of these "free radical" sites has been demonstrated repeatedly by means of surface electronic spectroscopies as well as by observations of chemisorption on the surface (*1*). More than 10 years ago, we and others found that

The author is in the Departments of Chemistry and Physics, and the Surface Science Center, University of Pittsburgh, Pittsburgh, PA 15260, USA. E-mail: jyates@mvs.cis.pitt.edu