Tweaking the Human Circadian Clock with Light

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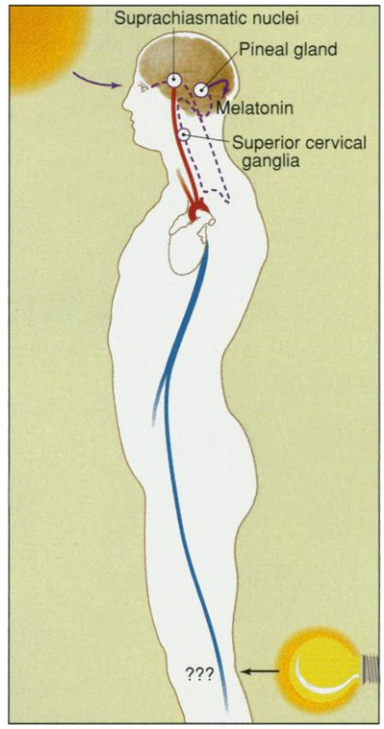
For more than two millennia, keen observers have noted the circadian rhythms of flowering plants that bloom only in the daytime or of trees with leaves that open during the day and close at night. Such movements are regulated by a clock within each organism (*1*), one that can be reset by light as days get shorter or longer.

Animals show strikingly similar circadian behavioral responses, driven by an internal clock and also reset (or entrained) by light (*2*). The time-keeping similarities between plants and animals have not been thought to extend to the light receptors (*3*). Information about chronobiological phototransduction in plants has blossomed, yet the primary visual light–sensitive system in animals—rod or cone photoreceptor molecules—may not be necessary for entrainment at all. But recently animals have started to seem more like plants. The fruit fly *Drosophila* has autonomous clocks throughout the body, each sensitive to its own intrinsic, as-yet-unidentified photoreceptors (*4*), and in a report on page 396 of this issue, Campbell and Murphy show that the endogenous clock of humans can be entrained by light application to an unexpected spot, the popliteal region—that is, the back of the knees (*5*).

The relation of light and other zeitgebers (time setters) to intrinsic biological rhythms is classically defined by phase response curves. These are constructed by exposing the organism to a zeitgeber at all phases of the endogenous rhythm and measuring the resulting effect on the phase of the cycle. With remarkable consistency, zeitgebers shift the rhythms by advancing the phase of the clock at certain parts of the circadian cycle and by delaying the phase when impinging on other parts of the cycle. The magnitude of such phase shifts is dose-dependent, the product of intensity × duration. The new phase response curves for extraretinal popliteal illumination in humans (*5*) conform to the classic pattern, with phase delays of body temperature and pineal melatonin secretion (outputs of the clock) occurring during the night, followed by phase advances early in the morning.

How is the entrainment signal carried from the knees to the endogenous clock? Presuming the conservation of chronobiological properties of plants and animals, one of us recently made a parsimonious proposal: that "humoral phototransduction" could subserve the zeitgeber effect (*6*) (see figure). This model postulates that heme moieties could serve as photoreceptors and that tetrapyrrole-based pigments, such as hemoglobin and bilirubin in mammals, act as counterparts to the tetrapyrrole-based, primary light-sensitive plant pigments of chlorophyll and phytochrome to mediate light's chronobiological effects. Hemoglobin and bilirubin could act in concert with the blood-borne nocturnal antioxidant melatonin to shift the phase of the clock. Such a model suggests a physiological function for light's known capacities to stimulate heme-based enzymes to form reactive gases, to cause dissociation of gases such as NO and CO from heme moieties, and to facilitate the destruction and excretion of unconjugated bilirubin.



Resetting the human clock. Light may shift the clock by: (i) a neural path (dashed line) from the eyes to the SCN, through the spinal cord, then ascending through the superior cervical ganglia and continuing to the pineal gland, where melatonin is secreted into the circulation or (ii) another pathway (red and blue) through the skin, possibly mediated through the blood.

The new results show that popliteal illumination with a visible-spectrum light source—originally developed to treat hyperbilirubinemia (or jaundice) in neonatal babies—can shift human circadian rhythms without any transduction of light through the eye. This result is consistent with, but does not prove, the idea that blood contains chronobiological photoreceptors (*5*). Further work will differentiate between light's effects on hemoglobin, bilirubin, the vascular tissue, and other components of the skin. Already we know from Campbell and Murphy's results that ultraviolet radiation, an obvious hazard to both eye and skin, is not required for the response.

In addition to its phase-shifting action, light exposure to the eyes immediately suppresses secretion of melatonin, a hormone that depresses body temperature, facilitates sleep onset, and—when administered in pill form at appropriate times of the day—induces phase advances or delays of the circadian system (*7*). The inhibitory signal originates at the suprachiasmatic nuclei (SCN), whose self-sustaining oscillations underlie the mammalian circadian pacemaker. The retinohypothalamic tract, which carries information from the eyes to the SCN, thus provides phase-shifting signals and inhibits melatonin secretion. Because melatonin is secreted when the phase response curve for light is active—in the "subjective" night—it will be important to ascertain whether popliteal illumination also directly inhibits melatonin secretory activity.

Ocular light therapy has been applied successfully for adjustment of aberrant sleep-wake cycles and alleviation of winter depression. When sleep occurs abnormally late (for example, with onset at 3 a.m. and awakening at 11 a.m.), light exposure at the end of the subjective night can elicit corrective advances, whereas early sleep (most common in the elderly) can be delayed by evening light (*8*). Winter depression has been blamed on the delayed sunrise at northerly latitudes, which might allow the circadian pacemaker to drift later relative to habitual sleep. Although depressed patients and normal individuals show similar melatonin secretion phases, morning light preferentially alleviates symptoms—the earlier, relative to evening melatonin onset, the better, as predicted by the phase response curve (*9*). On the other hand, evening light also has a mild benefit, which raises doubt that phase advances

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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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CELL BIOLOGY

Telomeres and Senescence: Ending the Debate

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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

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