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# Science

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SPECIAL ISSUE

## STAYING ON TRACK

How circadian rhythms influence physiology and health

# ON THE CLOCK

## Our bodies' internal timepieces drive daily rhythms and influence health

PHOTO: JOE BELCOVSON/SALK INSTITUTE

*By* **L. Bryan Ray** *and* **John Travis**

Benjamin Franklin, the sage of colonial America, advised that "Early to bed and early to rise, makes a man healthy, wealthy, and wise." Recent studies in circadian biology bear him out. Staying in synchrony with the 24-hour light-dark cycle of Earth does indeed provide benefits, if not to the pocketbook, at least to health and brain function.

Circadian biology is the study of the biochemical clocks that keep time in our brains and most cells in our bodies. Evidence is accumulating that misalignment of these clocks with the daily light-dark cycle of our environment can have profound effects on physiology, raising the risk of disease. At the same time, modern society generates pressures that tend to push activity and sleep out of sync with circadian biology. From extended work hours and shift work, to frequent air travel across time zones, to consumption of digital information late at night on electronic screens emitting "daylight" cues, many of us are subject to some amount of circadian disruption—including scientists whose research demands work at night. Learning how to have a healthy life despite these circadian disruptions will require a new understanding of how biological clocks influence physiological processes, which could ultimately lead to new applications in circadian medicine.

**INSIDE**



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**It matters not only what, but when you eat. These mice ate the same amounts of a high-fat diet, but the thinner one was restricted to eating during the active phase of its circadian cycle.**

PERSPECTIVE

# Circadian clocks: Not your grandfather's clock

Fred W. Turek[1,2,3]*

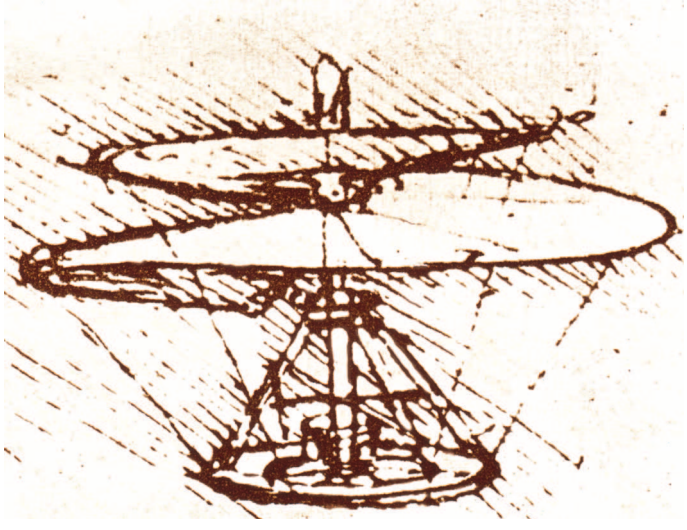
**The last 20 years have seen the rapid evolution of our understanding of the molecular genes and networks that enable almost all forms of life to generate 24-hour—or circadian—rhythms. One finding has been particularly exciting: that the molecular circadian clock resides in almost all of the cells of the body and that the clock regulates the timing of many cellular and signaling pathways associated with multiple disease states. Such advances represent a new frontier for medicine: circadian medicine.**

The first time a biomedical researcher sees the locomotor activity record of a rodent (e.g., mouse or hamster) that is "free running" without time cues such as a light-dark cycle, they are awestruck at the precise timing of the animal's daily activity: an almost invariant period close to 24 hours [i.e., circadian, from the Latin words circa (about) and dies (day)], day after day, week after week, month after month. What is the internal timing mechanism that enables a living organism to keep such precise track of the geophysical day imposed by the rotation of the earth even when external entraining factors (primarily the 24-hour light-dark cycle) have been removed? Indeed, the precision of the rhythmic expression of a physiological or behavioral rhythm that varies as little as 1 to 2 min over 24 hours for months is so remarkable it led to a school of thought in the 1950s and '60s that concluded that an organism could not keep such accurate time, so the timing must in fact be coming from some unknown physical timing cue impinging on life from the cosmos.

By the 1970s, the overriding consensus in the field was that the "clocks" regulating circadian rhythms in essentially all forms of life were located within the organism. In mammals, an apparent master circadian clock was found to be located in an area of the anterior hypothalamus, the suprachiasmatic nucleus (SCN), because lesioning the SCN abolished circadian rhythms (*1*, *2*). Because even unicellular organisms generate circadian rhythms and because studying mutations in the DNA of flies, fungi, or plants revealed that single genes somehow regulate the period of the clock, it was clear that circadian rhythms were generated by intracellular events. However, the fundamental mechanisms by which genes, proteins, and cellular processes could produce precise circadian oscillations was unknown, and no circadian clock genes had been identified in higher organisms. Investigators from that early era who now find themselves old enough to be grandfathers or grandmothers could not have predicted how rapidly the field would advance to uncover the fundamental molecular mechanisms and networks of the clock system that we now know regulates thousands of circadian rhythms at the cellular, metabolic, physiological, and behavioral levels. Nor did we ever imagine that this molecular clock would be embedded in a vast array of cellular processes that underlie health and disease. The circadian clock we know today is indeed not your "grandfather's clock."

As discussed in detail in the four review articles in this special section of *Science*, dozens of circadian clock genes are now known to regulate the cycling of mRNAs and proteins through transcriptional and translational feedback loops. The rapid pace of the discovery of the molecular clock was aided by the finding that the core clock genes are remarkably conserved across species as diverse as flies, mice, and humans, allowing for the integration of genetic and molecular discoveries across model species and humans. The use of a chemical mutagen coupled with phenotypic screening led to the discovery of the *Clock* mutant mouse, which expressed an abnormally long circadian period of 27 to 28 hours (*3*). When this first mammalian clock gene was cloned in 1997, it was a pleasant surprise to find that a homologous gene, when mutated in the fly, also altered the circadian period (*4*). Later in 1997, mammalian homologs of the first circadian clock gene cloned in the fly in 1984 (*per*) were found in mice and humans (*5*). Binding partners of the CLOCK and PER proteins (BMAL1 and CRY, respectively) were soon discovered, and the scaffold of the driver (CLOCK-BMAL1) and repressor (PER-CRY) limbs of the transcriptional-translational feedback oscillator was now in place to allow an in-depth understanding of the core molecular clock. The pace of discovery surrounding circadian clocks was featured by *Science* in their year-end Breakthrough of the Year: The Runners-Up selections in back-to-back years (1997 and 1998) when it was noted that "a volley of rapid-fire discoveries revealed the stunning universality of the clock's working" (*6*).

Although the discovery of the core clock genes and proteins was of great importance in the field, a corollary discovery had an equal impact and was not expected: The clock genes are expressed in almost all of the cells of the body not just the SCN. Furthermore, expression of the core circadian clock genes is cyclical, and the molecular clock regulated the timing of the



SHEILA TERRY/SCIENCE SOURCE

**Fig. 1. Leonardo da Vinci's helical air screw.** A blueprint, albeit an imperfect and incomplete one, of how circadian disorganization at the cellular level can be linked to pathophysiological states is available and awaits explanation that could lead to a new era for medicine: circadian medicine. Leonardo da Vinci drew the blueprint of the helicopter about 450 years before a helicopter was even built and could fly. The ongoing and expected rapid advances in circadian medicine should allow the present circadian blueprint to go from the bench to the bedside to standard clinical care and a healthy lifestyle in a period of time that is an order of magnitude less than that needed to go from da Vinci's blueprint to flight.

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expression of hundreds—if not thousands—of "clock-controlled genes" (CCGs). It is estimated that almost half of the genes in the mouse genome oscillate with a circadian period in one or more organs of the body and that the cycling RNAs and proteins vary in different organs (*7*). Many of the CCGs are part of key cellular and signaling pathways that regulate metabolism and immune, hormonal, and neural functions. Thus, circadian synchronization within the cell and between organ systems of the whole organism is critical to health and well-being, and the breakdown of this 24-hour temporal order could lead to pathological conditions at many levels of organization.

***"Thus, circadian synchronization within the cell and between organ systems of the whole organism is critical to health and well-being, and the breakdown of this 24-hour temporal order could lead to pathological conditions at many levels of organization."***

This rapid evolution of circadian biology has led to unanticipated new avenues for understanding health and disease etiology. Although disrupted circadian rhythms, including disturbed sleep-wake cycles, were thought to be symptomatic of diseases, there is a growing awareness that this disruption may contribute to the development, progression, and severity of disease states, including depression, neurological disorders, obesity, diabetes, cardiovascular disease, and gastrointestinal disorders.

Circadian disorder can also occur when humans "voluntarily" disrupt the normal phase relation between their internal circadian clock(s) and the solar day. For many years it has been known that shift workers live in a chronic state of circadian misalignment and that they have an increased prevalence of many adverse health outcomes (*8*). Similarly, individuals flying across multiple time zones experience the malaise of jet lag, as it may take up to a week for the internal clock to synchronize with the local time. More recently, investigators have focused on a form of chronic circadian misalignment, referred to as "social jet lag," whereby circadian disruption that is equivalent to traveling across 2 to 4 time zones twice a week occurs in individuals who change the timing of their sleep-wake cycle during the week to conform to work or school compared with weekends when there are fewer restrictions on sleep or wake time. An increase in body mass index correlated with the magnitude of social jet lag has been found in large epidemiological studies (*9*).

The implication of the misalignment of the timing of food intake has received considerable attention since it was discovered that mice eating at the "wrong" time of day (during the light period for nocturnal mice) gain more weight than mice eating at the "right" time of day (during the dark period for mice), despite taking in similar amounts of calories and showing the same amount of activity (*10*). In addition, the normal rhythm of food intake in *Clock* mutant mice, as well as in mice fed a high-fat diet, is disrupted, such that mice eat about the same amount during the day as they do during the night and gain more weight than control animals (*11*, *12*). It is particularly noteworthy that the temporal patterns of sleep and feeding behaviors, although they have little effect on the SCN pacemaker, can entrain circadian oscillations in peripheral organs; this leads to desynchronization between central and peripheral oscillators, the health implications of which could well be involved in the etiology of multiple disease states.

A major issue that the circadian biomedical community faces today is how to bring to clinical practice the enormous advances in linking the molecular clock mechanisms to the numerous cellular and signaling pathways associated with many disease states. Although some progress has been made in influencing clinical care practitioners to take into account circadian changes in pharmacokinetics when determining optimal time of day and dose for drug treatment (*13*), there has been little progress in considering circadian disorganization as a clinically relevant risk factor and/or a contributor to the etiology of the disease state. There is a consensus in the circadian community about the need for biomarkers to assess circadian function, including molecular, cellular, and physiological signals, in health and disease (*14*); however, this is unlikely to occur until we can easily and routinely measure, in some depth, the overall internal circadian organization of an individual over the 24-hour day. For now, no single time point of biomarkers at even the "omics" level (e.g., transcriptomics, metabolomics, or proteomics) (*15*) is sufficient to differentiate and quantify the exact nature of disease-relevant circadian reprograming or disruptions that may have occurred (e.g., loss of rhythmicity of certain pathways and internal desynchrony among organs).

It is anticipated that rapid advances in systems biology, information technologies, and the development of a new generation of wearable or implantable biosensors will make it possible in the future to monitor hundreds—if not thousands—of circadian oscillating biomarkers in body fluids on a routine basis. Data could then be made available via remote-monitoring medical servers to physicians and other health care providers with the tools to continuously track in real time the entire "circadianome" of the individual. These data could be integrated with precision medicine initiatives, which will not be "precise" without the consideration of the circadian profile over time in healthy individuals or of changes that occur with the progression of a particular disease or disorder. Just as Leonardo da Vinci sketched the blueprint of a helicopter more than 400 years before one was actually built that could fly (Fig. 1), we have the blueprint of the molecular circadian clock, albeit an incomplete and imperfect one, to bring the advances in our understanding of the circadian clock system and its relation to multiple physiological and pathophysiological cellular pathways to application in standard clinical care. Given that advances in the circadian field and their implications for medicine are anticipated to continue to evolve at a rapid pace, it should not take 400 years to fully integrate the complex circadian organization that exists within health and disease states into a new frontier for medicine: circadian medicine.

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