The Time of Your Life

By Paolo Sassone-Corsi, Ph.D.

Editor's Note: The circadian rhythm—the 24-hour cycle of the physiological processes of living beings—is instrumental in determining the sleeping and feeding patterns of all animals, including humans. Clear patterns of brain-wave activity, hormone production, cell regeneration, and other biological activities are linked to this daily cycle. Our author focuses on two relatively new areas of research—circadian genomics and epigenomics—and their potential for advancing medical insight.

Each morning we wake up from a night of sleep, and each day we eat our regularly timed meals, go through our normal routines, and fall asleep again for another night. This rhythm, so-called circadian—after the Latin words *circa diem* ("about a day")—underlies a wide variety of human physiological functions, including sleep-wake cycles, body temperature, hormone secretion, locomotor activity, and feeding behavior.

A simple look at other organisms reveals that circadian rhythms are remarkably conserved throughout evolution. Whether we consider the cyclic movements of leaves on a plant, the activities of a house cat, or the morning singing of birds, all follow a daily cycle that, being so natural and ancient, generally happens at a subconscious level. Over the past several decades, researchers have described a plethora of cyclic behaviors, metabolic rhythms, and physiological oscillations—all following a circadian pattern. Scientists have observed these behaviors in organisms as different as fungi, insects, unicellular protists, plants, cyanobacteria, vertebrates, and mammals. The rhythms are so wide-ranging that they include both the gravity-driven orientation of the photosynthetic flagellate *Euglena gracilis* and the social behavior of mammals in a group.

Why are circadian rhythms so omnipresent? The answer is straightforward. These biological cycles are based on the most ancient feature of our environment: the astronomical rotation of Earth on its axis, leading to the daylight-darkness cycle—the rhythmic repetition of days and nights.[1,2] This feature has remained immutable over a

billion years—although the length of the photoperiod has shortened somewhat over time.[1]

Scientists generally think that living beings have developed by adapting to the daylight-darkness cycle. My personal view is that, in addition to the adaptation process, life has developed *because* of the 24-hour light-dark cycle. Life-forms and their cellular, organismal, and molecular features would have been completely different on a planet with a longer or shorter light-dark cycle. Simple experiments on the small flowering plant *Arabidopsis thaliana* show that its size is reduced by half when subjected to light-dark cycles of 20 hours or 28 hours, corresponding to a planetary rotation that is only one-fifth slower or faster than Earth's.[3]

The role of the circadian clock appears to be so fundamental that, as shown in a number of studies, it has intimate links with the cell cycle.[4] This is nicely illustrated when we consider evolution's role in the process. Indeed, the cell division of a number of unicellular organisms, such as the green alga *Chlamydomonas reinhardtii*, the cyanobacterium *Synechococcus elongates,* and the dinoflagellate *Gonyaulax polyedra,* can be timed by a circadian mechanism. Also, disruption of the clock may have drastic health consequences. In humans, for example, night-shift workers have increased incidence of metabolic disorders.

In the past two decades the knowledge in the field of circadian biology has increased remarkably, such that today it is safe to claim that circadian rhythms represent possibly the ultimate example of systems biology. Some of these fairly recent findings, in my view, have prominently shaped our modern view of the field.

My First Encounter

I attended my very first conference on circadian rhythms more than 20 years ago. I was invited because, while working on the relationship between a messenger important in many biological processes and a gene (cyclic-AMP responsive element modulator, or CREM), my team stumbled on a clever molecular mechanism that allows expression to be cyclic in the pineal gland. Subsequently, we determined that CREM transcriptionally controls the gene encoding the serotonin N-acetyltransferase, an enzyme responsible for the rhythmic synthesis of the hormone melatonin from the pineal gland.

For the most part, the conference was a series of descriptive presentations about measuring circadian oscillations in a wide variety of organisms and physiological settings. Coming from the hard-core field of molecular transcription, I was fascinated by the spectacular variety of biological systems presented and intrigued by the obvious opportunities for mechanistic investigation. Most important, I found (and still find) the self-sustaining nature of circadian rhythmicity thought provoking. The field was on the verge of witnessing a series of conceptual transformations.

What is the evolutionary advantage of circadian clocks? They allow organisms to anticipate daily events (for example, food availability and predator pressure for animals, and sunrise for plants) rather than just reacting to them. Because the measure of time by circadian pacemakers is only approximate, their phase needs to be adjusted daily to stay in synchrony with geophysical time. Self-evident even to nonspecialists, light is the dominant entraining cue for all circadian timekeepers and is consequently considered the most critical *zeitgeber* (German for "time giver") for circadian physiology. In mammals, the anatomical structure that governs circadian rhythms is the suprachiasmatic nucleus (SCN), a small area in the brain consisting of approximately 15,000 neurons localized in the anterior hypothalamus.

For decades scientists have considered this central pacemaker to be the unique circadian clock controlling all daily behavior, metabolism, and physiology.[1 5] SCN neurons are able to self-sustain rhythmicity for weeks even when isolated in a culture dish. Their plasticity is also remarkable: The SCN is reset every day by the light-dark cycle, and thereby undergoes seasonal variations corresponding to the changes in the photoperiod. Thus, SCN neurons are wired to oscillate (to repeatedly move in one direction and back many times), but they receive the light signal through specialized retinal neurons and via the retinal-hypothalamic tract (RHT), thereby insuring their timely adjustment to the changing photoperiod and environment.[5] The SCN's role as the master clock is demonstrated by grafting experiments: Normal SCN grafted into a genetically arrhythmic animal can restore circadian rhythmicity.

Clocks Everywhere!

One discovery that has deeply affected the field of circadian biology during the past 15 years is that oscillators are present in most tissues. The thinking for decades was that the SCN alone directs all circadian body functions, but more recent findings reveal that the liver, spleen, muscle, and other body functions all have their own internal clock. My research team first described this finding in a vertebrate,[6] extending previous observations made in *Drosophila*.[7,8] Soon afterward, this finding was confirmed in mammals.[2,9] In *Drosophila* and zebra fish, light-dark cycles can directly entrain all oscillators, a scenario that is possible only in organisms in which at least some photons reach the internal organs.[6,7]

The evolution of larger and thus opaque organisms necessitated the development of a different, nonphotic (in addition to photic) communication system. In mammals, we see this in the organization of neuroendocrine circuits that convey the timing information from the SCN to the entire organism via direct and indirect signaling pathways. The SCN thereby functions as a master pacemaker, a kind of orchestra director that hierarchically coordinates the subsidiary oscillators located in peripheral tissues.

This notion was further illustrated by an experiment in which fibroblasts (a type of cell that plays a critical role in wound healing) that originated from a mutant mouse (and thereby had a faster clock) took on the rhythm of a host mouse when grafted as a

subcutaneous implant.[10] Additional evidence demonstrated the presence of circadian oscillators even in established cell lines: In cultured fibroblasts the endogenous clock system needed a simple serum shock to be resynchronized,[11] while the pacemaker of zebra fish's embryonic cells started ticking upon exposure to a short pulse of light.[2] Together, these findings significantly extended our view of circadian organization at the whole-organism level. They also underscored the fact that circadian-clock functions are not the prerequisite of a relatively small number of SCN neurons, as scientists thought for decades, but instead are common features of most cells.

Yet, more than a decade after these discoveries, some fundamental questions remain unanswered. Specifically, how do SCN neurons communicate and synchronize with the periphery? Are peripheral clocks in different tissues somewhat connected in an SCN-independent manner? Is there any functional feedback from peripheral oscillators back to the SCN?

Solving these points will be highly valuable for biomedical research. As the highly orchestrated network of clocks is based on cascades of signaling pathways, studies by several laboratories focused on understanding how clocks lead to the activation of transcriptional programs that define the unique circadian features of a given tissue. The important surprise came when transcriptional array profiles demonstrated that the clock controls a remarkable fraction of the genome.

Circadian Genomics and Epigenomics

Since the original discovery of the period (*per*) gene in the fly by Ronald Konopka and Seymour Benzer more than 40 years ago, the analysis of clock genes and their relationships and functions has kept an increasing number of researchers busy.[12] At the heart of the molecular network that constitutes the circadian clock are factors involved in turning "on" or "off"' transcription organized in feedback loops. This organization ensures cycles of oscillatory gene expression and the control of a remarkable fraction of the genome. Various studies have established that at least 10 percent of all expressed genes in any tissue are under circadian regulation.[13] Additional levels of circadian regulation implicate parallel and intertwined regulatory loops and the control by the cell of clock proteins stability. Moreover, scientists anticipate that tissue-specific transcriptional regulators contribute or intersect with the clock machinery.

The unexpectedly high proportion of circadian transcripts suggests that the clock machinery may direct widespread events of cyclic chromatin remodeling, which is the dynamic modification of chromatin architecture to allow access of condensed genomic DNA. This consequently affects the cycles of transcriptional activation and repression. Remarkably, a recent analysis covering 14 types of mouse tissues identified approximately 10,000 known genes showing circadian oscillations in at least one tissue. These findings underscore the presence of molecular interplays between the core clockwork, which can be assumed to be common to all tissues, and cell-specific transcriptional systems. Taking into consideration the recent view of the mammalian

circadian clock as a transcriptional network, through which the oscillator acquires plasticity and robustness, it is reasonable to speculate that the clock network contributes to physiological responses by intersecting with cell-specific transcriptional pathways.[13]

Considering the thousands of genes regulated in a circadian manner, researchers have questioned how the complex organization of chromatin copes with the task of controlling harmonic oscillations. A number of studies have revealed that several chromatin dynamics contribute to circadian function, rendering specific genomic loci either active (open) or silenced (closed) for transcription.[13] Specifically, we have found that the clock machinery is itself essential for circadian control of chromatin dynamics.

This finding provided a gateway to search for other components of the circadian chromatin complexes.[13] One of these is MLL1, an enzyme implicated in some forms of cancer, that dictates the recruitment to chromatin of the clock machinery thereby targeting circadian genes.[14]

As soon as the first chromatin circadian regulators were identified, the search for the counterbalancing enzymes was open. The discovery that the activity of SIRT1—a longevity-associated enzyme belonging to a family of nicotinamide adenine dinucleotide (NAD^+) activated deacetylases—oscillates in a circadian fashion established the first molecular and conceptual link between the circadian clock and metabolism.[15, 16] SIRT1

demonstrates an oscillation in activity, impinging back on the circadian clock. The discovery of circadian-directed sirtuin activity spurred hypotheses as to whether metabolites such as NAD^+ themselves serve a predominant role in the cellular link between metabolism and the circadian clock.[15, 16]

The Metabolic Clock

Intuitively, circadian physiology implies that a considerable fraction of cellular metabolism is cyclic. Also, the analysis of mice mutants for clock proteins has revealed a number of metabolic defects. Indeed, metabolome analyses by mass spectrometry have shown that about 50 percent of all metabolites oscillate in a given tissue. Yet, the question that we have been addressing is as follows: What is the molecular link between clock-driven control and the oscillation of a given metabolite? In this respect, the example of NAD^+ is paradigmatic.

Indeed, circadian oscillation of SIRT1 activity suggested that cellular NAD^+ levels may oscillate. This is indeed the case, and the way this regulation is achieved is conceptually revealing. The circadian clock controls the expression of the gene encoding nicotinamide phosphoribosyltransferase (NAMPT), a key rate-limiting enzyme in the salvage pathway of NAD^+ biosynthesis.[17,18] The clock machinery is recruited to the NAMPT promoter in a time-dependent manner. The oscillatory expression of NAMPT is abolished in mice mutated in clock function, leading to drastically reduced and nonoscillatory levels of NAD^+. These results make a compelling case for the existence of an interlocking,

classical, transcriptional feedback loop that controls the circadian clock with an enzymatic loop wherein SIRT1 regulates the levels of its own cofactor.[17,18]

More recently, we have questioned whether a nutritional challenge would modify the genomic and metabolomic circadian profile. The nutritional implications of this approach are multiple, especially in a modern society with an endless availability of food. Mice that are fed a high-fat diet (HFD) experience a drastic reprogramming of the circadian clock. Genes that normally would oscillate stop doing so; in addition, many genes whose expression profile is normally noncyclic start to oscillate.[19] The HFD-induced reprogramming pushes the liver to acquire a new circadian homeostasis that implicates genes of the inflammasome and heat-shock response. In this sense, the example of NAMPT and NAD^+ is again very revealing. Under HFD, the oscillation of both NAMPT and NAD^+ is abolished because the clock machinery cannot be recruited to chromatin. This illustrates that different nutrition strategies directly "talk" to chromatin remodelers, resulting in a reprogramming of genomic functions.[19]

The Next Phase

Mysteries in circadian biology remain. The intrinsic, fundamental role played by the circadian clock in a large array of biological functions illustrates that much more will be unraveled in the upcoming years. Specifically, we predict that the clock will be found to play a key role in the host-pathogen relationship, in the inflammatory response to infection, and in the disturbances caused by tumoral growth. Based on our current

knowledge, it will be critical to decipher the role that specific epigenetic regulators have in controlling the circadian epigenome.

Recent findings stress the role of two HDACs of the sirtuin family, SIRT1 and SIRT6, in partitioning the circadian genome in functional subdomains.[20] This partitioning leads to a segregation of cellular metabolism, again underscoring the intimate link with homeostasis.[20] Finally, the role of circadian metabolism in neurons is likely to reveal yet-unexplored regulation pathways that may help us decipher the relationship that the circadian clock has with the sleep-wake cycle.

Bio

Paolo Sassone-Corsi, Ph.D., is Donald Bren Professor and director of the Center for Epigenetics and Metabolism at the University of California, Irvine. Before his move to California in 2006, he was director of research at the National Center for Scientific Research (CNRS) in Strasbourg, France. Sassone-Corsi's major interest is concentrated on the mechanisms of signal transduction able to modulate nuclear functions and, in particular gene expression, chromatin remodeling, and epigenetic control. He has received many awards, including the EMBO Gold Medal, the Charles-Leopold Mayer Prize of the Academie des Sciences (France), and the Ipsen Award for Endocrinology (U.S.). He received his Ph.D. in genetics from the University of Naples, Italy.

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