Cold Spring Harbor Symposia on Quantitative Biology

Biological Rhythms Workshop I: Introduction to Chronobiology

S. J. Kuhlman, S. R. Mackey and J. F. Duffy

Cold Spring Harb Symp Quant Biol 2007 72: 1-6
Access the most recent version at doi:10.1101/sqb.2007.72.059

Biological Rhythms Workshop I: Introduction to Chronobiology

S.J. KUHLMAN,* S.R. MACKEY,[†‡] AND J.F. DUFFY[§]

*Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724; [†]Department of Biology, The Center for Research on Biological Clocks, Texas A&M University, College Station, Texas 77843-3258; [§]Division of Sleep Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts 02115

In this chapter, we present a series of four articles derived from a Introductory Workshop on Biological Rhythms presented at the 72nd Annual Cold Spring Harbor Symposium on Quantitative Biology: Clocks and Rhythms. A diverse range of species, from cyanobacteria to humans, evolved endogenous biological clocks that allow for the anticipation of daily variations in light and temperature. The ability to anticipate environmental variation promotes optimal performance and survival. In the first article, Introduction to Chronobiology, we present a brief historical time line of how circadian concepts and terminology have emerged since the early observation of daily leaf movement in plants made by an astronomer in the 1700s. Workshop Part IA provides an overview of the molecular basis for rhythms generation in several key model organisms, Workshop Part IB focuses on how biology built a brain clock capable of coordinating the daily timing of essential brain and physiological processes, and Workshop Part IC gives key insight into how researchers study sleep and rhythms in humans.

INTRODUCTION

As a consequence of the Earth's rotation about its axis approximately every 24 hours, most organisms on this planet are subjected to predictable fluctuations of light and temperature. A diverse range of species, from cyanobacteria to humans, evolved endogenous biological clocks that allow for the anticipation of these daily variations. Thus, our internal physiology and function are fundamentally intertwined with this geophysical cycle. In fact, it was an astronomer, Jean-Jacques d'Ortous deMairan, rather than a biologist, who provided early insight into this evolutionary relationship between internal physiology and the geophysical cycle. deMairan (1729) made the observation that daily leaf movements in heliotrope plants continue in constant darkness. To emphasize the endogenous or self-sustained nature of biological clocks, Franz Halberg in 1959 coined the term *circadian* (Latin: *circa* = about; *dies* = day) to refer to daily rhythms that are truly endogenously generated, i.e., rhythms having a period of about 24 hours that continue to oscillate in the absence of any environmental input (Chandrashekaran 1998). Rhythm generation is now understood to be an intrinsic property of single cells, driven by an intracellular molecular oscillator based on transcriptional/posttranslational negative feedback loops. Under normal conditions, endogenous oscillations are synchronized to the environment, and it is generally thought that biological clocks provide an adaptive advantage by ensuring that an organism's internal biochemical and physiological processes, in addition to behavior, are optimally adapted to the local environment.

[‡]*Present address:* Department of Biology, St. Ambrose University, Davenport, Iowa 52803.

HISTORICAL TIME LINE

As early as 1729, it was documented that this daily rhythmic behavior was likely endogenously generated, and it was also near this time that Carl von Linne (1707–1778) constructed a "floral clock" noting the predictability of petal opening and closing times of various species of flowers (Chandrashekaran 1998). However, it was not until 200 years later that Erwin Bünning provided the first evidence for the genetic basis of circadian rhythms generation by demonstrating that period length is heritable in bean plants (Bünning 1935). Bünning (1936) also put forth an influential hypothesis that circadian oscillators can be used to measure seasonal changes in addition to measuring daily cycles and pointed out the adaptive significance of tracking seasonal changes. Thus, the field of circadian rhythms originated from keen observation of plants, and it was not until later that the first observations of endogenously driven rhythms in bacteria (Mitsui et al. 1986), single-cell eukaryotes (Sweeney and Hastings 1957), insects (Beling 1929), birds (Kramer 1952), rodents (Richter 1922), primates (Simpson and Gaibraith 1906), and humans (Aschoff and Wever 1962) were discovered.

A breakthrough in understanding the genetic basis for rhythms generation was made by Ronald Konopka and Seymour Benzer (1971) using a mutant screen in *Drosophila melanogaster*. Mutagenized flies were examined for the persistence of two circadian behaviors: pupal eclosion and locomotor activity. Flies displayed one of three categorical mutant phenotypes: a lengthening of circadian period, a shortening of period, or arrhythmia. All phenotypes were complemented by a single locus, now referred to as the *Period* gene. Shortly after this discovery in fruit flies, the *Frequency* gene was shown to be

essential for rhythms in conidiation to persist in the filamentous fungus *Neurospora crassa* (Feldman and Hoyle 1973). These surprising results showed that single-gene mutations could disrupt a complex behavior and, together with the wonderful discovery of a heritable timing mutation in hamsters by Martin Ralph and Michael Menaker (1988), provided the rationale for pursuing a large-scale mutant screen in mice (Vitaterna et al. 1994). Subsequently, using a variety of strategies, dozens of clock genes have been discovered in both prokaryotic and eukaryotic systems, including cyanobacteria, fungi, plants, insects, and mammals. Even human rhythms are potently altered by clock gene mutations (Jones et al. 1999). The specific clock gene players in these diverse systems are described in more detail in Part IA. A striking common principle emerges from examining these diverse clock gene systems, i.e., all organisms seem to have evolved transcriptional/posttranslational feedback loops to ensure high-amplitude, near 24-hour, rhythms generation. Furthermore, the identification of specific clock genes has led to the development of real-time bioluminescent and fluorescent reporters that have the spatial resolution to track rhythmicity at the level of the functional unit of rhythms generation, i.e., the single cell (Hastings et al. 2005). Such resolution is essential to understand how individual oscillators are coupled within a population of rhythmic cells.

In the midst of this clock gene explosion, however, there is an important lesson to be learned from a pivotal experiment conducted using clock components of cyanobacteria. In 2005, Nakajima et al. (2005) reconstituted a circadian oscillator in a test tube using only cyanobacterial proteins and ATP. The lesson: It is possible to construct a near 24-hour oscillator in the absence of gene transcription.

It is clear from the work of many talented and dedicated scientists that most organisms inherit the innate ability to keep track of time on a 24-hour scale. How do organisms use their timing devices? What are the negative consequences if organisms are no longer able to effectively use their clocks, such as in disease or aging? We know from the work of Karl von Frisch and Beling that bees use their clocks to visit flowers at the appropriate time of day so that they can feed when the flower is open. Work from Kramer shows that birds use their biological clock during migration to help compensate for the changing position of the sun throughout the day. For an inspiring account of these classic studies, we recommend reading Chapter 1 in Moore-Ede et al. (1982). Additionally, DeCoursey et al. (2000) showed that chipmunks use an innate biological clock to properly time foraging to avoid predation. More recently, studies on monarch butterflies by Steve Reppert (2006) revealed that the circadian clock likely participates in initiating migration by tracking seasonal changes. Future studies in the field of circadian rhythms will continue to explore the wonderful and creative ways in which organisms use their biological clocks to coordinate internal function and navigate through the environment (for mammalian review, see Buijs and Kalsbeek 2001).

CIRCADIAN TERMINOLOGY AND GENERAL METHODS

Here, we define some commonly used terms in the field. "Black-box" experimental designs are frequently used to probe the mechanisms underlying clock function (Moore-Ede et al. 1982). For example, deMairan (1729) used a black-box approach in his heliotrope plant study. The plant was treated as a system whose internal components were unknown (i.e., black box), but whose function was studied by assaying the observable output of leaf movement in response to perturbations caused by environmental inputs (the light/dark cycle). To a large extent, the following terminology developed over the years to precisely report the results of black-box experiments.

Observable, or measurable, output rhythms of the circadian timing system, such as leaf movements, are defined as *overt outputs*. In the case of animals, wheel-running activity and levels of circulating hormones in the blood are two commonly assayed overt outputs. In addition, a system can be composed of multiple oscillators, in which the output signal of one oscillator influences the circadian properties of another oscillator. The output signal can then be referred to as a *coupling signal*. In practice, the distinction between an overt rhythm and coupling signal may depend on the experimenter's perspective.

A rhythm is considered to be circadian if the oscillation has a period of approximately 24 hours and continues in constant conditions, such as constant light (referred to as LL) or constant darkness (DD) (Fig. 1A). The inability of a rhythm to continue under constant conditions implies that the rhythm is driven by external time cues (or a *zeitgeber*, German for time-giver), rather than generated internally. It is important to keep in mind that when rhythmicity is measured at the tissue or population level, the loss of an overt rhythm due to experimental perturbation may not be the result of loss of rhythmicity per se. Rather, individual oscillations may continue, but they are not observed due to phase differences among oscillating units (Fig. 2).

The time needed for one circadian oscillation to occur under constant conditions is known as the *free-running period* (FRP). Under normal conditions, circadian rhythms are not free-running, rather, they are synchronized to the local environment. The process by which a rhythm synchronizes to an external cycle is referred to as *entrainment*. Interestingly, although circadian rhythms persist under constant conditions, the FRP can vary slightly in response to changing light intensities. Diurnal organisms display a slightly shorter FRP (i.e., faster clock) in higher light intensities than they do in low light; nocturnal organisms exhibit the inverse response with a longer FRP (i.e., slower clock) in high light than in dim light. This phenomenon, originally described by Jürgen Aschoff, is known as "Aschoff's Rule" (Aschoff and Wever 1962). FRP can also vary based on the light history that the organism experienced before being placed under constant conditions. The influence of light history on FRP is referred to as an *aftereffect*. Clearly, when assaying

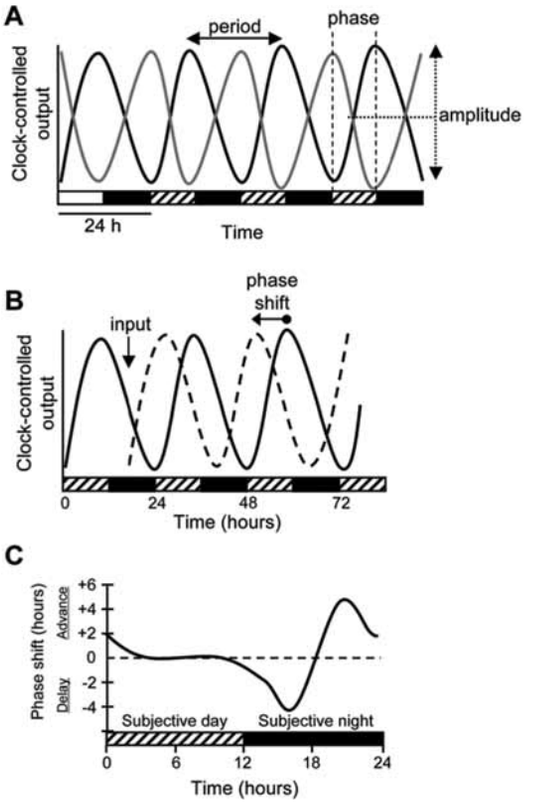

Figure 1. Properties of a circadian rhythm. (*A*) Behavioral circadian rhythms can be entrained by external stimuli, such as a light/dark cycle, and will persist with a near 24-hour period in the absence of environmental cues, such as constant darkness. Properties of the rhythm that are commonly measured are period, phase, and amplitude. Period is the duration of time to complete one cycle. It is typically measured from peak to peak, but it can be measured from any specific position on the curve. Phase is the relative position on the curve (e.g., the peak) in reference to a particular time, such as time placed in constant darkness. Amplitude is the measurement of the recorded output from the midline of the curve to either the peak or trough. (*B*) The phase of a circadian rhythm can be reset by the stimuli to which it entrains. In this case, exposure to a stimulus (input) rapidly lowers the level of the rhythmic variable (*dashed line*), which recovers to a rhythm with a shifted phase as compared to the curve that did not receive the input (*solid line*). (*X* axis) Time in the light/dark cycle is depicted by alternating white and black bars, and time under constant conditions is depicted by alternating hatched and black bars. Level of clock-controlled output is displayed on the *Y* axis. (*C*) Phase-response curves measure the magnitude and direction of phase-dependent responses to brief exposures of an external stimulus. The *X* axis represents the circadian time at which a light pulse is applied to an organism; the *Y* axis shows the change in phase of the circadian-controlled output in response to the light pulse. Positive shifts are indicated as advances, and negative phase shifts are delays. A brief light pulse given to an organism during the subjective day (the organism's own internal day) produces little to no phase response, light in the early subjective night (the organism's own internal early night) produces phase-delay shifts, and light in the late subjective night produce phase-advance shifts.

overt rhythms and calculating the FRP, the influence of these factors should be taken into consideration (see also Part IC, Definition of Sleep).

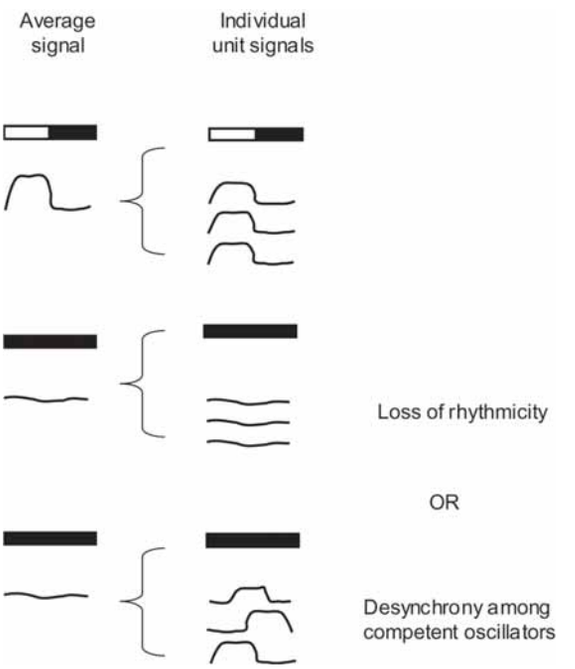

Figure 2. Importance of assaying rhythms at a single-cell resolution. To be considered circadian, a rhythm must be maintained under constant conditions. When examining rhythms at the tissue level in which the tissue is composed of multiple cells, it is important to realize that the absence of rhythmicity under constant conditions could be due to two possibilities: either the loss of rhythmicity (indicating the rhythm is not circadian) or desynchrony among oscillators. In the later case, individual rhythms may actually be circadian; however, the assay has insufficient resolution to demonstrate the presence of rhythmicity. To distinguish between the two possibilities, assays sensitive to the functional unit of rhythms generation must be used.

In addition to being endogenously generated, there are two other properties of circadian rhythms that are often the focus of experimental studies: (1) the ability of the rhythm to be reset, or phase-shifted, by transient exposure to time cues such as light and (2) temperature-compensation, defined below (Pittendrigh 1981; Dunlap et al. 2004).

Phase-shifting and the Phase-response Curve

The phase of a rhythm can be shifted (or reset) by transient exposure to certain environmental cues (Fig. 1B). Because FRPs are close to, but not exactly, 24 hours, the rhythm must be reset each day in order to avoid falling out of phase with the external environmental cycle. As discussed earlier, many organisms use the daily fluctuation of light and darkness as the resetting signal to entrain their rhythms to the 24-hour day. An early study by Hastings and Sweeney (1958) in the dinoflagellate *Gonyaulax polyhedra* demonstrated a property common among circadian systems: The biological clock is not equally sensitive to light at all times of day. As has been shown subsequently

for many organisms that use light as an entraining signal, light exposure in the early subjective night produces a phase-delay shift (i.e., the rhythm is delayed to a later hour), whereas light exposure in the late subjective night produces a phase-advance shift (the rhythm is advanced to an earlier hour), and light during the subjective day produces very small or no phase shifts. Thus, the response to light is dependent on the organism's internal sense of time. The magnitude and direction of these responses can be plotted with respect to the organism's own circadian phase at which the light was presented. Such a plot is referred to as a *phase-response curve* (PRC) (Fig. 1C).

It is important to realize that there are cases in which it is possible that exposure to an external cue will simply transiently perturb the level of the rhythm being assayed, without leading to a persistent shift in the rhythm. This condition is referred to as *masking*. Mechanistically, masking can arise because sensory pathways may act on a specific behavior independent of the clock (for an example in mammals, see Part IB).

Temperature-compensation

For a biological clock to be reliable in a natural setting, its period should *not* change much despite changes in ambient temperature. Morning events should occur at essentially the same time, independent of weather conditions the night before. This property, termed temperature-compensation, stems from the observation that the value of the FRP changes very little during different temperatures within the organism's physiological range. The effect of changes in temperature on the rate of most biochemical reactions is measured by a Q_{10} value, which is defined as the ratio of the rate of a given process at one temperature to the rate at a temperature 10°C lower. The Q_{10} value of the period of a circadian rhythm remains near 1, as opposed to other known biochemical reactions that have Q_{10} values of 2 or 3.

It is important to note that temperature-compensation is not the same as temperature insensitivity. Temperature itself can be can be a strong zeitgeber in some organisms (Liu et al. 1998). Individual reactions within the clock are undoubtedly affected by changes in temperature, but the system as a whole is buffered such that the output of rhythmic behavior does not show large variations in its FRP.

Concept of Clock

What is a circadian clock and how has the concept of a "clock" facilitated research? As described in one account (Chandrashekaran 1998), the now widespread use of the term clock was in part inspired by Gustav Kramer's studies on time compensation of the sun compass in birds. Shortly thereafter in 1960, the Cold Spring Harbor Symposium of Quantitative Biology was boldly titled "Biological Clocks." Today, the term "clock" is used pervasively to emphasize the endogenous nature of rhythms generation, that circadian rhythms are innate rather than learned phenomena, and importantly, to imply that a primary function of circadian rhythms is to measure time (Pittendrigh 1961; Moore-Ede et al. 1982). It is this conceptual framework that prompted the design of shifted-schedule or jet-lag experiments and helps us understand the relationship between seasonal adaptation and circadian rhythms generation.

Conceptually, the components of a circadian clock can be broken down into three basic elements: an input pathway, a pacemaker, and an output pathway (Fig. 3). At the heart of a circadian clock is the pacemaker, a central oscillator or a network of coupled oscillators, that is entrainable and has the ability to synchronize downstream targets. The temporal information produced by the pacemaker is interpreted by the output pathways, which then regulate the timing of metabolic and behavioral processes. For the oscillator to maintain synchrony with the environment, input pathways must relay external timing cues to the pacemaker. It is important to realize that conceptually, one can discuss these three elements as distinct entities; however, in biological terms, one protein or physiological process can subserve multiple roles.

The 2007 Cold Spring Harbor 72nd Symposium on Clocks and Rhythms included presentations that covered

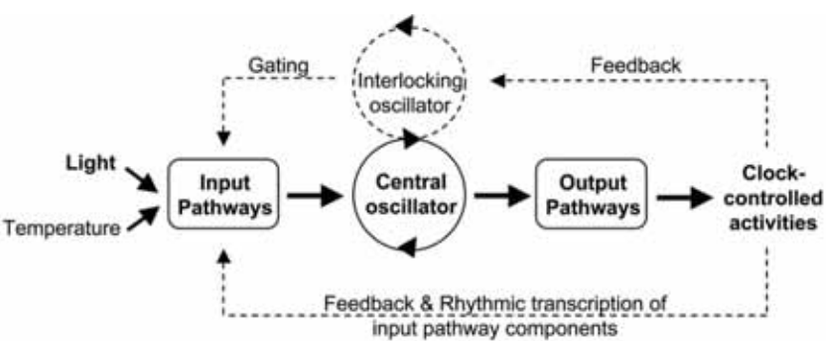

Figure 3. Representation of circadian clock divisions. A circadian clock can be depicted as having input pathways, a central oscillator (or pacemaker), and output pathways. The central oscillator produces the endogenous biological rhythm and can be synchronized with the environment via input pathways through cues such as light or temperature. Output pathways convey the clock's rhythms to downstream targets and drive overtly rhythmic activities. Some circadian systems consist of more elaborate pathways (shown as *dashed lines*) that include multiple, interlocking oscillators and positive or negative feedback from clock-controlled activities to oscillator and/or input components. (Modified, with permission, from Gardner et al. 2006 [© The Biochemical Society].)

an amazingly diverse range of organisms (from bacteria to humans) and technical approaches. Despite the diversity, there are fundamental concepts that bind the field together. In the following chapter, we use the three-division framework of input, rhythm generator/pacemaker, and output to introduce the reader to key circadian molecular players and physiological processes. The goal of this workshop is to concisely present the unifying concepts of the field in order to foster lively discussion and critical evaluation of the data and strategies used in the study of biological clocks and rhythms. A list of abbreviations used within this Workshop Review is provided below (Table 1).

ACKNOWLEDGMENTS

We thank Drs. Marty Zatz, Mirjam Münch, and Sean Cain for comments on earlier versions of the manuscript.

Table 1. List of Abbreviations

5HT	serotonin neurotransmitter
ARAS	ascending reticular activating system
Arc	arcuate nucleus
AVP	arginine vasopressin
BF	basal forebrain
bHLH	basic helix-loop-helix
BMAL1	brain and muscle ARNT-like protein 1
C-box	Clock box
CAMK1	calcium/calmodulin-dependent protein kinase 1
CBS	CCA1-binding site
CCA1	CIRCADIAN CLOCK ASSOCIATED 1
CikA	circadian input kinase
CK(1, 2)	casein kinase (1, 2)
CLK	CLOCK
CO	CONSTANS
COP1	CONSTITUTIVELY PHOTOMORPHOGENIC 1
CRE	cyclic-AMP response element
CREB	CRE binding
CRY	CRYPTOCHROME
CYC	CYCLE
DBMIB	2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone
DBT	DOUBLE-TIME
DD	constant darkness
DET	DE-ETIOLATED 1
DIC	differential interference contrast
DMH	dorsal medial hypothalamus
DMV	dorsal motor nucleus of the vagus
DR	dorsal raphenuclei
EE	evening element
EEG	electroencephalogram
ELF	EARLY FLOWERING
FDR	fast-delayed rectifier
FFT	fast Fourier transform
FLO	FRQ-less oscillator
FRH	FRQ-interacting RNA helicase
FRP	free-running period
FRQ	FREQUENCY
FT	FLOWERING LOCUS T
GABA	γ-aminobutyric acid
GFP	green fluorescent protein
GHT	geniculohypothalamic tract
GI	GIGANTEA
GnRH	gonadotropin-releasing hormone
GRP	gastrin-releasing peptide
HAT	histone acetyltransferase
hPVN	hypothalamic paraventricular neurons
Hz	hertz (cycles per second)
IGL	intergeniculate leaflet
ipRGC	intrinsically photosensitive retinal ganglion cells
IPSP	inhibitory postsynaptic potential
JET	JETLAG
KaiC~P	phosphorylated KaiC
LabA	low amplitude and bright
LC	locus coeruleus
LD	light/dark cycle
LdpA	light-dependent period
LH	lateral hypothalamus
LHY	LATE ELONGATED HYPOCOTYL
LL	constant light
LUX	LUX ARRHYTHMO
NE	norepinephrine neurotransmitter
NREM	nonrapid eye movement
PACAP	pituitary adenylate-cyclase-activating polypeptide
PAS	PER-ARNT-SIMS
PDF	pigment-dispersing factor
PDP1ε	PAR DOMAIN PROTEIN 1ε
PER	PERIOD
Pex	period extender
PHY	PHYTOCHROME
PP1(2A)	protein phosphatase 1 (2A)
PPT	pedunculopontine nuclei
PRD-4	Period-4
PRR	pseudo-response regulator
REM	rapid eye movement
RHT	retinohypothalamic tract
RORE	ROR element
RpaA	regulator of phycobiliosome-associated
SasA	*Synechococcus* adaptive sensor
SCN	suprachiasmatic nucleus
SGG	SHAGGY
sPVZ	subparaventricular zone
SPY	SPINDLY
SWA	slow-wave activity
SWS	slow-wave sleep
TIC	TIME FOR COFFEE
TIM	TIMELESS
TOC1	TIMING OF CAB EXPRESSION 1
TMN	tuberomammilary neurons
TTX	tetrodotoxin
VIP	vasoactive polypeptide
VLP	ventrolateral preoptic nucleus
VP-box	VRI/PDP1ε box
vPVN	paraventricular nucleus of the hypothalamus
VRI	VRILLE
VVD	VIVID
WC	WHITE COLLAR
WCC	WHITE COLLAR COMPLEX
ZTL	ZEITLUPE

REFERENCES

Aschoff J. and Wever R. 1962. Spontanperiodik des menschen bei ausschluss aller zeitgeber. *Naturwissenschaften* **49:** 337.

Beling I. 1929. Uber das zeitgedachtnis der bienen. *Z. Vgl. Physiol.* **9:** 259.

Buijs R.M. and Kalsbeek A. 2001. Hypothalamic integration of central and peripheral clocks. *Nat. Rev. Neurosci.* **2:** 521.

Bünning E. 1935. Zur kenntis der erblichen tagesperiodiztat bei den primarblattern von *Phaseolus multiflorus*. *Jahrb. Wiss. Bot.* **81:** 411.

———. 1936. Die endogene tagesrhythmik als grundlage der photoperiodischen reaktion. *Ber. Dtsch. Bot. Ges.* **54:** 590.

Chandrashekaran M.K. 1998. Biological rhythms research: A personal account. *J. Biosci.* **23:** 545.

DeCoursey P.J., Walker J.K., and Smith S.A. 2000. A circadian pacemaker in free-living chipmunks: Essential for survival? *J. Comp. Physiol. A* **186:** 169.

deMairan J. 1729. Observation botanique. *Histoire de L'Academie Royale des Sciences,* p. 35.

Dunlap J.C., Loros J.J., and DeCoursey P.J. 2004. *Chronobiology: Biological timekeeping*. Sinauer Associates, Sunderland, Massachusetts.

Feldman J.F. and Hoyle M.N. 1973. Isolation of circadian clock mutants of *Neurospora crassa*. *Genetics* **75:** 605.

Gardner M.J., Hubbard K.E., Hotta C.T., Dodd A.N., and Webb A.A. 2006. How plants tell the time. *Biochem. J.* **397:** 15.

Hastings J.W. and Sweeney B.A. 1958. A persistent diurnal rhythm of luminescence in *Gonyaulax polyedra*. *Biol. Bull.* **115:** 440.

Hastings M.H., Reddy A.B., McMahon D.G., and Maywood E.S. 2005. Analysis of circadian mechanisms in the suprachiasmatic nucleus by transgenesis and biolistic transfection. *Methods Enzymol.* **393:** 579.

Jones C.R., Campbell S.S., Zone S.E., Cooper F., DeSano A., Murphy P.J., Jones B., Czajkowski L., and Ptáček L.J. 1999. Familial advanced sleep-phase syndrome: A short-period circadian rhythm variant in humans. *Nat. Med.* **5:** 1062.

Konopka R.J. and Benzer S. 1971. Clock mutants of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci.* **68:** 2112.

Kramer G. 1952. Experiments on bird orientation. *Naturwissenschaften* **94:** 265.

Liu Y., Merrow M., Loros J.J., and Dunlap J.C. 1998. How temperature changes reset a circadian oscillator. *Science* **281:** 825.

Mitsui A., Kumazawa S., Takahashi A., Ikemoto H., Cao S., and Arai T. 1986. Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically (letter). *Nature* **323**: 720.

Moore-Ede M.C., Sulzman F.M., and Fuller C.A. 1982. *The clocks that time us*. Harvard University Press, Cambridge, Massachusetts.

Nakajima M., Imai K., Ito H., Nishiwaki T., Murayama Y., Iwasaki H., Oyama T., and Kondo T. 2005. Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation in vitro. *Science* **308:** 414.

Pittendrigh C.S. 1961. Circadian rhythms and the organization of living systems. *Cold Spring Harbor Symp. Quant. Biol.* **25:** 159.

———. 1981. Circadian systems: General perspective and entrainment. In *Handbook of behavioral neurobiology: Biological rhythms* (ed. J. Aschoff), pp. 57 and 95. Plenum Press, New York.

Ralph M.R. and Menaker M. 1988. A mutation of the circadian system in golden hamsters. *Science* **241:** 1225.

Reppert S.M. 2006. A colorful model of the circadian clock. *Cell* **124:** 233.

Richter C.P. 1922. A behavioristic study of the activity of the rat. *Comp. Psychol. Monogr.* **1:** 1.

Simpson S. and Gaibraith J.J. 1906. Observations on the normal temperature of the monkey and its diurnal variation, and on the effect of changes in the daily routine on this variation. *Trans. R. Soc. Edinb.* **45:** 65.

Sweeney B.M. and Hastings J.W. 1957. Characteristics of the diurnal rhythm of luminescence in *Gonyaulax polyedra*. *J. Cell. Comp. Physiol.* **49:** 115.

Vitaterna M.H., King D.P., Chang A.M., Kornhauser J.M., Lowrey P.L., McDonald J.D., Dove W.F., Pinto L.H., Turek F.W., and Takahashi J.S. 1994. Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. *Science* **264:** 719.