Leland N. Edmunds, Jr.

Cellular and Molecular Bases of Biological Clocks

Models and Mechanisms for Circadian Timekeeping

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Preface

As Bünning (1973) noted in his prefatory remarks, to suggest the existence of an endogenous diurnal rhythm was generally regarded even as late as 1955 as subscribing to a mystical or metaphysical notion. One might argue justifiably that the field of biological rhythms emerged as a discipline (often designated as chronobiology) with the Symposium on Biological Clocks held in 1960 at Cold Spring Harbor, New York. The tens of thousands of papers that have been published since this important meeting attest to the fact that the periodic behavior of living systems, rather than being in some sense pathological—or at the very least abnormal—and confined to a very small number of organisms and tissues, is the normal state of affairs, the rule rather than the exception.

A particularly intriguing class of biological periodicity is reflected in the large number of well-documented persisting circadian rhythms, having periods of approximately 24 hours, that occur at every level of eukaryotic organization. Despite their importance, many reviews of rhythmic phenomena stop short of a detailed consideration of these characteristically longer-period rhythms, perhaps because a convincing explanation of this very same property, together with that of temperature compensation of the free-running period, has not as yet yielded to experimental analysis. Small wonder then that a lead editorial in *Nature* some years ago (231:97–98, 1971) somewhat plaintively queried why so little was known about the biological clock and suggested that it was time to wind it up. Present-day grant review panels, some 15 years later, seem to echo this theme even more stridently!

Nevertheless, progress has been made. The Dahlem Conference on The Molecular Basis of Circadian Rhythms, held in Berlin over a decade ago (Hastings and Schweiger, 1976), admirably summarized in its unique format the most exciting current trends at the time (membranes were hot) and is still quite useful as a reference work. This monograph attempts to update this treatment and consider some of the more provocative developments in the field (as, for example, those obtained with the powerful approach of molecular genetics). Its title, nevertheless, is a bit presumptuous. We still cannot clearly delineate the molecular basis of circadian rhythmicity,

and we will have to expand our treatment somewhat to include progress made at the cellular and biochemical levels as well.

The layout of the book emulates that of a kind of sextet: the outer two movements, consisting of a brief introduction to circadian rhythms and a final section treating general theoretical and applied aspects, flank an inner quartet of chapters comprising the core of the book. The first of these chapters (chapter 2) surveys circadian organization at the cellular level and describes the most important eukaryotic microorganisms that have served as experimental material for the biochemical and molecular analysis to follow. Chapter 3 discusses in some detail the interaction of cell division cycles and circadian oscillators. Chapter 4 treats the results obtained by several major experimental lines of attack on circadian clock mechanisms. Finally, the various biochemical and molecular models for circadian oscillators that have been constructed on the basis of the data presented earlier are outlined in chapter 5, along with their formal predictions and the degree to which they have been validated or disconfirmed.

Where appropriate, ultradian and other noncircadian periodicities are discussed (such as the glycolytic cycle—perhaps the best understood biochemical oscillator, and for that reason, instructive as a possible mechanism whereby longer periods might be generated), and parallels are drawn to the recent explosion of work on neuronal oscillators (Berridge et al., 1979; Carpenter, 1981, 1982). Although the chapters are designed to be read sequentially, particularly by the potential initiate to the field, it is unlikely that they can compete successfully with a good concert, movie, or home videotape. Consequently, those readers at least somewhat versed in this arcane area should also find it profitable to skip among the various sections. To this end, there is extensive cross-referencing and a rather large bibliography is appended (literature search ended June 1987) with name and date citations' being given in the text.

In a work of this scope, sins of omission and commission unfortunately are inevitable. I will appreciate learning of such errors, distortions and misinterpretations. I thank all those authors who have given me permission to use their published illustrations; Dr. Colin S. Pittendrigh, who introduced me to the field; the National Science Foundation, which has supported my own research throughout the years; Prof. Régis Calvayrac, Directeur de la Laboratoire des Membranes Biologiques, Université Paris VII, who provided a home away from home for the finishing touches on the manuscript; Dr. Charles F. Ehret, who read the entire work; and Dr. Danielle L. Lavel-Martin, who aided in the production of the manuscript. I am grateful to all my students in my course on Biological Clocks over the past 20 years who helped me justify my existence, particularly those special classes at the University of Tel Aviv and the Université Paris VII who endured shortened versions of this monograph during my tenure as visiting professor there.

Stony Brook, New York — Leland N. Edmunds, Jr.

Abbreviations

LL	continuous illumination
DD	continuous darkness
LD	light-dark cycle
LD: x,y	light-dark cycle comprising x hours of light and y hours of dark
WC: x,y	temperature cycle comprising x hours of warmer and y hours of colder temperatures (°C)
T	period of an LD cycle or other periodic *Zeitgeber* (environmental cue)
τ	average period of a free-running rhythm in constant conditions
ϕ	phase of a rhythm
ϕ_r	phase reference point, or phase marker
$+\Delta\phi, -\Delta\phi$	change (advance, delay) in phase (phase shift)
ZT	environmental (*Zeitgeber*) time (where ZT 0 corresponds to the onset of light)
CT	circadian time (CT 0 indicates the phase point of a free-running rhythm that has been normalized to 24 hours and corresponds to that occurring at the onset of light in a LD: 12,12 reference cycle)
PRC	phase-response curve [plot of the phase shift of a free-running circadian rhythm engendered by a perturbing light (or other) signal as a function of the circadian time at which it was applied]
g	average generation (doubling) time of a population of cells
ss	average step-size, or factorial increase in cell concentration (plateau to plateau) after a phased, or synchronized division step
CAM	crassulacean acid metabolism
CAP	compound action potential
CDC	cell division cycle

G_q	fundamental quantal cell cycle
PA	photosynthetic activity
PC	photosynthetic capacity
PSI(II)	photosystem I (II)
SCN	suprachiasmatic nuclei
AC	adenyl cyclase
ANISO	anisomycin
cAMP	cyclic AMP
CAP	chloramphenicol
CCCP	carbonyl cyanide *m*-chlorophenyl hydrazone
CPZ	chlorpromazine
CHX	cycloheximide
DCCD	*N*, *N'*-dicyclohexylcarbodiimide
DCMU	diuron, 3-(3,4-)dichlorophenyl 1)-1, 1-dimethyl urea
DES	diethylstilbestrol
DNP	dinitrophenol
FCCP	*p*-trifluoromethoxyphenylhydrazone
FUdR	5-fluoro-2'-deoxyuridine
MDH	malate dehydrogenase
NAD(H)	nicotinamide adenine dinucleotide
NADP(H)	nicotinamide adenine dinucleotide phosphate
PDE	phosphodiesterase
PEPC	phosphoenolpyruvate carboxylase
PFK	phosphofructokinase
PUR	puromycin
TFP	trifluoperazine
VIP	vasoactive intestinal polypeptide
VP	vasopressin

I trifle with my papers from time to time;
It is one of the lesser frailities.

Horace

Were I to await perfection,
My book would never be finished.

Tai T'ung, 13th Century Chinese scholar;
The Six Scripts: Principles of Chinese Writing

A clock is a clock is a clock.

Science News, 1983, 124(22) : 346

Contents

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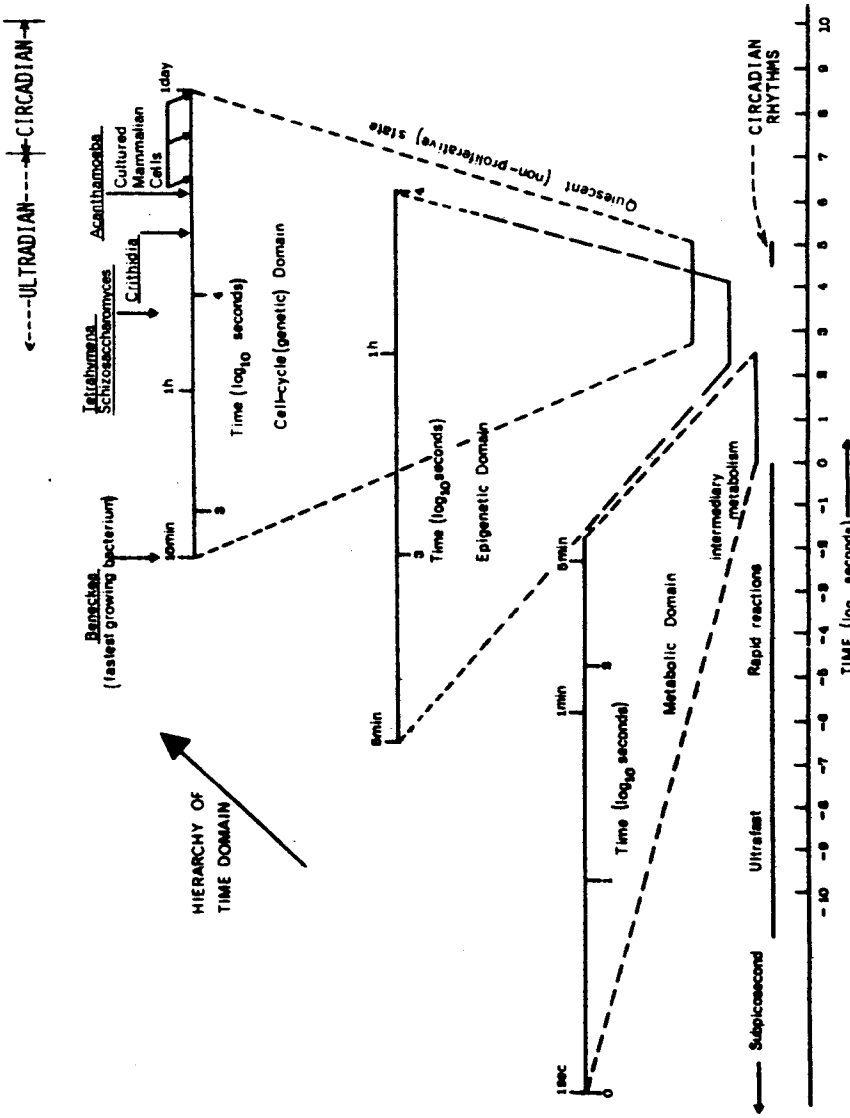
Introduction

1.1 Temporal Organization

As Pittendrigh noted in 1961 in an engaging essay, biologists are confronted with a continuously reproducing and evolving set of highly organized living systems. An organism that has thrived by differential reproductive success is said to be "adapted," and its adaptation is reflected in its total organization. This organization is strongly history-dependent, having arisen through the twin processes of natural selection and adaptation. Biological problems, therefore, pivot on the complexities of biological organization.

It is almost self-evident that the spatial organization and the functioning of living forms are inextricably intertwined. Of equal importance, however, is the temporal dimension: at the physiological level, for example, not only must the *right* amount of the *right* substance be at the *right* place, but also this must occur at the *right* time (Halberg, 1960). This is true also for the organism itself, which often must be positioned in time in favorable biotic or physical conditions. Since the environment is highly periodic with respect to many of its variables, it would not be surprising (indeed, it would be essential) for the organism to adapt to these cyclicities.

Organisms can and do measure astronomical time in some manner—as opposed to the purely private timekeeping reflected in such variable-period physiological rhythms (see Section 1.3) as heartbeat and alpha-brain waves. This is demonstrated explicitly by four categories comprising diverse phenomena occurring throughout the animal and plant kingdoms: (1) persistent rhythms, having daily (circadian), tidal, lunar (monthly), and yearly (circannual) periods, (2) the *Zeitgedächtnis*, or time sense, of bees and humans, (3) seasonal photoperiodism, wherein many organisms perform a certain function at a specific time of the year by what may be essentially a daily measurement of the length of the day (or night), and (4) celestial orientation and navigation, in which the sun, moon, or stars are used as direction givers, implying a timing system to compensate for their continuously, but predictably, shifting positions (Bünning, 1973; Palmer et al., 1976; Brady, 1982). All four types of timekeeping, or functional bio-


←---ULTRADIAN---+-CIRCADIAN-→
Bacteria (fastest growing bacterium)
Tetrahymena Schizosaccharomyces
Crithidia
Acanthamoeba
Cultured Mammalian Cells
1day
1h
20min
8
4
Time (log_{10} seconds)
Cell-cycle (genetic) Domain
Quiescent (non-proliferative) state
1h
4
8min
3
Time (log_{10} seconds)
Epigenetic Domain
8min
1min
8
Time (log_{10} seconds)
Metabolic Domain
1sec
0
intermediary metabolism
Rapid reactions
Ultrafast
Subpicosecond
CIRCADIAN RHYTHMS
HIERARCHY OF TIME DOMAIN
TIME (log_{10} seconds)
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10

chronometry, have external correlates (generated by the movements of the earth, moon, and sun) to which the organism has adapted. Although the last three kinds commonly are found only in higher organisms and probably are relatively recent, more sophisticated variations on a more ancient evolutionary theme, the first category of persistent rhythms, are displayed commonly in most, if not all, eukaryotic (but not prokaryotic) unicells (however, see Section 6.1.1). An understanding of the physiological and biochemical bases of these simpler clocks, therefore, may be crucial to the elucidation of the higher-level phenomena.

The underlying biological clocks that generate the foregoing types of rhythmicity all possess considerably longer periods than those that give rise to ultrafast and rapid chemical reactions, to biochemical rhythmicities of intermediary metabolism (e.g., glycolytic oscillations), and to those rhythms (such as cellular respiration) commonly observed in the epigenetic and genetic time domains (Lloyd et al., 1982b; see Section 1.3). The time domain, therefore, of circadian rhythms—the primary subject matter of this review—lies at the interface (Fig. 1.1) between the upper border of the genetic domain comprising cell division cycles and those even longer periodicities in the temporal hierarchy of living systems (*cf.* Fig. 17, p. 72 in Ehret, 1974).

1.2 General Properties of Circadian Rhythms

Circadian rhythmicities, having a period of about 1 day, have been documented throughout the plant and animal kingdoms at every level of eukaryotic organization. Their general characteristics are summarized in Figure 1.2. Typically, they can be synchronized (entrained) by imposed diurnal light or temperature cycles to precise 24-h periods and can be predictably phase-shifted by single light and temperature signals. Yet they are able to free-run for long timespans as persisting rhythms under conditions held constant with respect to most environmental time cues *(Zeitgeber)*, with a natural period close to but seldom exactly 24 h. (Unless otherwise noted, we will always use the term "circadian" in this restricted sense.) Furthermore, the free-running period (τ) is remarkably well compensated for changes in the ambient temperature within the physiological range, as might be expected of an accurately functioning oscillator or clock.

FIGURE 1.1. Time domains of living systems. Note that the domain of circadian rhythms, which display periodicities of about a day, lies at the interface between ultradian rhythms (having periods less than 24 h) and longer infradian rhythms (with periods greater than 24 h), and encroaches upon the (genetic) domain of the cell division cycle. (Adapted from Lloyd et al., 1982b, with permission of Academic Press)