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Circadian Clocks

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Preface

The human body functions as a 24-h machine: remarkably, this machine keeps going with a *circa* 24-h rhythm in sleeping and waking, in physiologies such as blood pressure and cortisol production, in cognitive functions, and indeed also in expression of circa 10–20 % of the genome in any given cell. The circadian (from the Latin "*circa diem*" or about a day) clock controls all of these processes with a molecular mechanism that is pervasive, as we now know that essentially every cell of our body is oscillating. Furthermore, our cells apparently utilize a circadian clock mechanism with a similar molecular makeup. The recent years have witnessed an enormous progress in our understanding of the mechanistic and genetic basis of this regulation, which we have tried to highlight in this volume.

The circadian clock is relevant for heath—clock gene mutants show reduced fitness, increased cancer susceptibility and metabolic diseases. In addition, drug efficacy and toxicity often vary with time of day with huge implications for therapeutic strategies. The intention of this book is to provide the reader with a comprehensive and contemporary overview about the molecular, cellular and system-wide principles of circadian clock regulation. In keeping with the focus of the *Handbook of Experimental Pharmacology* series, emphasis is placed on methods as well as the importance of circadian clocks for the timing of therapeutic interventions. Despite the decades-old practice of administration of cortisol on the morning, chronopharmacology and chronotherapy are still mostly at an experimental level. Thus, knowledge about the widespread impact of circadian clocks should be invaluable for a broad readership not only in basic science but also in translational and clinical medicine.

This book contains four topical sections. Part I is devoted to describing our current knowledge about the molecular and cellular bases of circadian clocks. In the first chapter, the readers learn about clock genes and the intracellular genetic network that generates ~24-h rhythms on the molecular level. The second chapter focuses on how the circadian clock is using epigenetic mechanisms to regulate the circadian expression of as many as 10 % of cellular transcripts. The following two chapters focus on the hierarchy of mammalian circadian organization: the clock in the brain is the master pacemaker, often controlling daily timing in peripheral

tissues. The mechanisms of these synchronization processes within tissues and organisms are discussed.

Part II of the book is devoted to describing how and what is controlled by the circadian clock. The general term for this is *outputs* of the clock. Here, we will cover sleep, metabolism, hormone levels and mood-related behaviors that are especially relevant to pharmacology. In recent years, the reciprocal control of metabolic processes and the circadian system emerged, which is the focus of the first chapter of this part. This connection has been elucidated both on a molecular basis and also in epidemiological studies. Several common themes will emerge including the feedbacks between clocks and the clock output systems as well as the balance between local and tissue-specific clocks and the system-wide control of circadian functions. Concerning human behavior, there is nothing more disparate than the states of sleep and wakefulness; the reader will learn that the timing of these states is profoundly governed by the circadian clocks and its associated genes (see also Part III, Roenneberg et al.). Single point mutations in clock genes can dramatically alter sleep behavior. Disruption of temporal organization—clock gene mutations or shift work—can lead to health problems and behavioral disorders related to mood alterations. The last chapter in this section discusses these connections and possible *pharmacological* interventions such as light or lithium therapy.

The aim of Part III is to discuss the implications of a circadian system for pharmacology. The first chapter reviews studies from the past several decades that describe daily changes in drug absorption, distribution, metabolism, and excretion. In addition, drug efficacy is controlled by the circadian system due to daily changes in the levels and functionality of many drug targets. The second chapter exemplifies these principles for anticancer therapy, where chronotherapy is relatively advanced. This may be based on the fact that cancer cells have less synchronized circadian clocks. Modulating or strengthening the molecular clock by pharmacological intervention is a strategy that is addressed in one of the contributions in this section. High-throughput screening approaches for small molecules that are capable of pharmacological modulation of the molecular clock are described—this may develop into a valuable approach for both scientific and therapeutic purposes. The last chapter in this section focuses on the role of light for the synchronization of the human clock to our environment (entrainment). Light is the primary synchronizer (*zeitgeber*), and novel light-sensitive cells in the retina mediate entrainment, which is conceptually and epidemiologically analyzed. In shift work, as well as in everyday working life, the dissociation of internal and external time leads to health problems, suggesting the need for intervention strategies that use light as though it were a prescription drug.

Finally, Part IV of this book is devoted to systems biology approaches to our understanding of circadian clocks. In general, our field has relied on models to enhance our conceptual understanding of the highly complex circadian system. The iterative approach of improving models with data from high throughput approaches and feeding back the results for experiments suggested therein—in essence, modern systems biology—is developing into a major tool in our chronobiology repertoire.

In the first chapter of this section, the principles of rhythm generation will be described from a mathematical perspective. It will become clear that feedback loops and coupling are fundamental concepts of oscillating systems. How these fundamentals are used to create rhythms that regulate, for example, transcription at many different times of day is highlighted in the second chapter of this part. The last chapters again help to appreciate the pervasiveness of circadian regulation by focusing on genome- and proteome-wide studies that uncovered circadian rhythms almost everywhere.

This volume adds up to an up-to-date review on the state of chronobiology, particularly with respect to molecular processes. It should be of special interest to chronobiologists, pharmacologists, and any scientists who is concerned with excellent protocols and methods.

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Part IV Systems Biology of Circadian Clocks

Part I
Molecular and Cellular Basis of Circadian Clocks

Molecular Components of the Mammalian Circadian Clock

Ethan D. Buhr and Joseph S. Takahashi

Abstract Mammals synchronize their circadian activity primarily to the cycles of light and darkness in the environment. This is achieved by ocular photoreception relaying signals to the suprachiasmatic nucleus (SCN) in the hypothalamus. Signals from the SCN cause the synchronization of independent circadian clocks throughout the body to appropriate phases. Signals that can entrain these peripheral clocks include humoral signals, metabolic factors, and body temperature. At the level of individual tissues, thousands of genes are brought to unique phases through the actions of a local transcription/translation-based feedback oscillator and systemic cues. In this molecular clock, the proteins CLOCK and BMAL1 cause the transcription of genes which ultimately feedback and inhibit CLOCK and BMAL1 transcriptional activity. Finally, there are also other molecular circadian oscillators which can act independently of the transcription-based clock in all species which have been tested.

Keywords Circadian • Clock • Molecular

1 Introduction

As the sun sets, nocturnal rodents begin to forage, nocturnal birds of prey begin their hunt while diurnal birds of prey sleep, filamentous fungi begin their daily production of spores, and cyanobacteria begin nitrogen fixation in an environment

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of low O_2 after the day's photosynthesis. As the sun rises the next morning, many plants have positioned their leaves to catch the first rays of light, and many humans sit motionless in cars on a nearby gridlocked highway. It is now understood that the obedience to temporal niches in these and all organisms is governed by a molecular circadian clock. These clocks are not driven by sunlight but are rather synchronized by the 24-h patterns of light and temperature produced by the earth's rotation. The term circadian is derived from "circa" which means "approximately" and "dies" which means "day." A fundamental feature of all circadian rhythms is their persistence in the absence of any environmental cues. This ability of clocks to "free-run" in constant conditions at periods slightly different than 24 h, but yet synchronize, or "entrain," to certain cyclic environmental factors allows organisms to anticipate cyclic changes in the environment. Another fundamental feature of circadian clocks is the ability to be buffered against inappropriate signals and to be persistent under stable ambient conditions. This robust nature of biological clocks is well illustrated in the temperature compensation observed in all molecular and behavioral circadian rhythms. Here temperature compensation refers to the rate of the clock being nearly constant at any stable temperature which is physiologically permissive. The significance of temperature compensation is especially evident in poikilothermic animals that contain clocks that need to maintain 24-h rhythmicity in a wide range of temperatures. Combined, the robust oscillations of the molecular clocks (running at slightly different rates in different organisms) and their unique susceptibility to specific environmental oscillations contribute to and fine-tune the wide diversity of temporal niches observed in nature.

However, the circadian clock governs rhythmicity within an organism far beyond the sleep: activity cycle. In humans and most mammals, there are ~24-h rhythms in body temperature, blood pressure, circulating hormones, metabolism, retinal electroretinogram (ERG) responses, as well as a host of other physiological parameters (Aschoff 1983; Green et al. 2008; Cameron et al. 2008; Eckel-Mahan and Storm 2009). Importantly, these rhythms persist in the absence of light–dark cycles and in many cases in the absence of sleep–wake cycles. On the other side of the coin, a number of human diseases display a circadian component, and in some cases, human disorders and diseases have been shown to occur as a consequence of faulty circadian clocks. This is evident in sleep disorders such as delayed sleep phase syndrome (DSPS) and advanced sleep phase syndrome (ASPS) in which insomnia or hypersomnia result from a misalignment of one's internal time and desired sleep schedule (Reid and Zee 2009). In familial ASPS (FASPS), the disorder cosegregates both with a mutation in the core circadian clock gene *PER2* and independently with a mutation in the PER2-phosphorylating kinase, CK1 δ (Toh et al. 2001; Xu et al. 2005). Intriguingly, transgenic mice engineered to carry the same single amino acid change in PER2 observed in FASPS patients recapitulate the human symptoms of a shortened period (Xu et al. 2007). Although these mutations are likely not the end of the story for these disorders, they give insight into the way molecular clocks affect human well-being. Jet lag and shift work sleep disorder are other examples of health issues where the internal circadian clock is desynchronized from the environmental rhythms. In addition to sleep-related

disorders, circadian clocks are also directly linked with feeding and cellular metabolism, and a number of metabolic complications may result from miscommunication with the circadian clock and metabolic pathways (Green et al. 2008). For example, loss of function of the clock gene, *Bmal1*, in pancreatic beta cells can lead to hypoinsulinemia and diabetes (Marcheva et al. 2010). Finally, some health conditions show evidence of influence of the circadian clock or a circadian clock-controlled process. For example, myocardial infarction and asthma episodes show strong nocturnal or early morning incidence (Muller et al. 1985; Stephenson 2007). Also, susceptibility to UV light-induced skin cancer and chemotherapy treatments varies greatly across the circadian cycle in mice (Gaddameedhi et al. 2011; Gorbacheva et al. 2005).

In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus is the master circadian clock for the entire body (Stephan and Zucker 1972; Moore and Eichler 1972; Slat et al. 2013). However, the SCN is more accurately described as a "master synchronizer" than a strict pacemaker. Most tissues and cell types have been found to display circadian patterns of gene expression when isolated from the SCN (Balsalobre et al. 1998; Tosini and Menaker 1996; Yamazaki et al. 2000; Abe et al. 2002; Brown and Azzi 2013). Therefore, the SCN serves to synchronize the individual cells of the body to a uniform internal time more like the conductor of an orchestra rather than the generator of the tempo themselves. The mammalian SCN is entrained to light cycles in the environment by photoreceptors found exclusively in the eyes (Nelson and Zucker 1981). The SCN then relays phase information to the rest of the brain and body via a combination of neural, humoral, and systemic signals which will be discussed in more detail later. Light information influencing the SCN's phase, the molecular clock within the SCN, and the SCN's ability to set the phase of behavior and physiology throughout the body constitute the three necessary components for a circadian system to be beneficial to an organism (1) environmental input, (2) a self-sustained oscillator, and (3) an output mechanism.

2 Mechanism of the Molecular Circadian Clock

2.1 Transcriptional Feedback Circuits

The molecular clock mechanism in mammals is currently understood as a transcriptional feedback loop involving at least ten genes (Fig. 1). The genes *Clock* and *Bmal1* (or *Mop3*) encode bHLH-PAS (basic helix–loop–helix; *Per-Arnt-Single-minded*, named after proteins in which the domains were first characterized) proteins that form the positive limb of the feedback circuit [reviewed in Lowrey and Takahashi (2011)]. The CLOCK/BMAL1 heterodimer initiates the transcription by binding to specific DNA elements, E-boxes (5′-CACGTG-3′), and E′-boxes (5′-CACGTT-3′) in the promoters of target genes (Gekakis et al. 1998; Yoo et al. 2005; Ohno et al. 2007). This set of activated genes includes members of the