Computational and Structural Biotechnology Journal 13 (2015) 417–426

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

Mini Review

Circadian systems biology: When time matters

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ARTICLE INFO

Article history:
Received 31 May 2015
Received in revised form 9 July 2015
Accepted 10 July 2015
Available online 17 July 2015

Keywords:
Circadian clock
Cancer
Mathematical modelling
Chronobiology

ABSTRACT

The circadian clock is a powerful endogenous timing system, which allows organisms to fine-tune their physiology and behaviour to the geophysical time. The interplay of a distinct set of core-clock genes and proteins generates oscillations in expression of output target genes which temporally regulate numerous molecular and cellular processes. The study of the circadian timing at the organismal as well as at the cellular level outlines the field of chronobiology, which has been highly interdisciplinary ever since its origins. The development of high-throughput approaches enables the study of the clock at a systems level. In addition to experimental approaches, computational clock models exist which allow the analysis of rhythmic properties of the clock network. Such mathematical models aid mechanistic understanding and can be used to predict outcomes of distinct perturbations in clock components, thereby generating new hypotheses regarding the putative function of particular clock genes. Perturbations in the circadian timing system are linked to numerous molecular dysfunctions and may result in severe pathologies including cancer. A comprehensive knowledge regarding the mechanistic of the circadian system is crucial to develop new procedures to investigate pathologies associated with a deregulated clock.

In this manuscript we review the combination of experimental methodologies, bioinformatics and theoretical models that have been essential to explore this remarkable timing-system. Such an integrative and interdisciplinary approach may provide new strategies with regard to chronotherapeutic treatment and new insights concerning the restoration of the circadian timing in clock-associated diseases.

Contents

1. Introduction

The ability to adapt to and anticipate the light/dark cycles of the earth offered a survival advantage to many organisms that prevailed

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http://dx.doi.org/10.1016/j.csbj.2015.07.001

throughout evolution. As such, most organisms evolved an endogenous timing system—the circadian clock.

The circadian clock drives numerous physiological and behavioural processes, which consequently follow a rhythm of approximately 24 h and ensure an accurate adaptation to external daily rhythms [1]. In the last decades the field of chronobiology, which studies these biological rhythms, has boomed. As a consequence, theoretical and experimental tools were developed which fostered research and discovery in the field.

Circadian research has been extremely interdisciplinary and has attracted researchers from various scientific backgrounds. It was the astronomer Jean Jacques d'Ortous deMairan, who in 1729 provided evidence for the existence of circadian rhythms. He noticed that the daily leaf movements of the heliotrope plant, *Mimosa pudica*, persisted in constant darkness and suggested the existence of an endogenous time-generating mechanism, in line with the geophysical time [2]. However, the first evidence for a genetic basis of circadian rhythms was provided two centuries later, by Bünning. He reported that in common beans, the period lengths of the offspring ranged between the extremes of period lengths of the parent generation [3]. During the 20th century circadian rhythms were reported and further studied in other organisms as well, from cyanobacteria to humans (Fig. 1) [4].

Even though circadian research started centuries earlier, it was only in 1959 that, for the first time, Halberg used the term circadian (*circa* and *dies* "about a day") to describe the observed rhythms [3]. Shortly thereafter, in 1960, the Cold Spring Harbour Symposium on Biological Clocks [5] brought together researchers working on circadian rhythms, from experimental to rather mathematical and computational backgrounds. This event established the basis of circadian investigation and paved the way to chronobiology [3].

From thereafter, the search for the "clock" started. In 1971, Konopka and Benzer identified the first clock gene—*Period*—in *Drosophila melanogaster* by an EMS-induced mutant screen [6]. The following milestone in circadian rhythms research was the identification of the suprachiasmatic nucleus (SCN)—a brain region located in the hypothalamus—as the central pacemaker of the circadian clock, in 1990 [7].

Meanwhile, 14 core-clock genes [8] were identified in mammals and shown to form complex transcriptional/translational networks, that are able to drive oscillations in gene expression of output target genes, the clock-controlled genes. It became apparent that the circadian clock has systemic effects at the organismal level which are due to the intricate dynamics of this rather small number of core-clock genes generating a highly precise signal and leading to the propagation of a cascade of events which ultimately influence numerous cellular processes. Within these lines, our group and others have identified new clock-controlled genes and generated elaborate networks of circadian regulation for mammals [9–11].

Systems biology approaches are crucial to investigate the clock on a systems-wide level as shown in Fig. 2. The usage of large-scale component identification using genome-wide technologies, and the development and implementation of *in silico* mathematical models, able to integrate multiple levels of data and generate predictions and testable hypotheses, are the key building blocks in systems biology which are being transferred to circadian systems biology. Nevertheless, targeted smaller scale experimental approaches are essential to validate results gained from systems biology approaches. Since the 1990s, genomics, proteomics and metabolomics data generation dramatically developed. High-throughput methods such as microarrays, next-generation sequencing and mass spectrometry now allow for the global analysis of cells, tissues and organisms and require models and tools to analyse such massive datasets [12,13]. In parallel, mathematical models were developed and are widely used to simulate the complexity of processes involved in the generation of circadian rhythms [14].

In this review, we focus on what started as a rather unusual, not wide accepted field of research and succeeded in demonstrating that time matters and that deregulation of the temporal system has severe consequences in disease and therapy. Nevertheless, it is important to point out that the circadian system influences many other fields apart from medicine which include agriculture, psychology, ecology and environmental biology [15–18].

In the following, we provide an overview of the circadian clock, with a focus on the mammalian clock, the established methods to study it experimentally, as well as theoretically and the consequences, at the organism level, of the failure of this remarkable time-generating system.

2. Basic structure of the circadian system: the core-clock

The mammalian circadian clock is hierarchically organized in three main components: input signalling pathways, the main pacemaker (or central oscillator) and output signalling pathways.

Signals received from the environment are communicated via the input pathway to the main pacemaker. These signals, named zeitgebers or timing cues, are used to synchronize the pacemaker oscillations with the solar day–night cycle. Light is consequently the strongest zeitgeber, but also temperature, noise, food, exercise and melatonin can act as zeitgebers [19].

The central oscillator is formed by two clusters of neurons (~100,000 neurons in each cluster in humans) and is located in the hypothalamus above the optic chiasm, thus named Suprachiasmatic Nucleus (SCN) [1].

Light signals are received by the retinal cells which transmit the input to the SCN via the retino-hypothalamic tract. In the SCN, calcium influx occurs in response to the interaction of glutamate with NMDA (N-Methyl-D-aspartate) receptors, leading to the activation of IP3 (inositol triphosphate) and ryanodine receptors or acting directly on posttranscriptional mechanisms [20]. Upon signal reception, the pacemaker generates and sustains rhythms that are subsequently diffused to the peripheral organs via output pathways such as the glucocorticoid pathway [21]. Peripheral oscillators exist within the different tissues throughout the organism being present in most cells (Fig. 1) and regulate various biochemical processes [1,22].

Several key characteristics were defined to describe the circadian clock: it is self-sustained or endogenous—the circadian rhythm persists even in the absence of environmental inputs; it is entrainable—the oscillator can be reset or phase shifted by exposure to different time cues (e.g. light) which allows synchronization to the external light/dark cycle; it is temperature compensated—the period of circadian rhythms changes only slightly under different temperatures within the organism's physiological range; and it is able to transmit a time-signal to peripheral oscillators and reset those to the prevailing zeitgeber [23].

The endogenous mechanism that generates sustained oscillations—in peripheral and SCN cells—is constituted by a gene regulatory network. A set of 14 genes forms the core-network of the mammalian circadian clock, the core-clock network (CCN), that accounts for the generation of circadian rhythms within individual cells [19] (Fig. 1, lower panel). These elements are necessary for the robust generation of oscillations, which can occur in the absence of external inputs. This characteristic justifies the designation of core-clock and was first demonstrated computationally [14]. These genes are members of the *Per* (period), *Cry* (cryptochrome), *Bmal* (brain and muscle ARNT-like protein), *Clock* (circadian locomotor output cycles kaput, NPAS2 in neuronal tissue), *Ror* (RAR-related orphan receptor) and *Rev-Erb* (nuclear receptor, reverse strand of ERBA) gene and protein families. All elements in this network interact via positive and negative transcriptional and translational feedback loops [14].

During the early time of the circadian day, the heterodimer complex CLOCK/BMAL1 is formed and regulates the transcription of all other genes within the CCN. This is achieved via binding of the CLOCK/BMAL1 complex to E-Box sequences within the promoter regions of the target genes, *Ror*, *Rev-Erb*, *Per* and *Cry* [14,19].

The network can be seen as the interconnection of two larger loops, the PER/CRY (PC) loop and the REV-ERB/*Bmal*/ROR (RBR) loop [14]. In