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Circadian Clock: Exploiting the Biological Concept of Rhythm in Medicinal Plants for Human Welfare

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ABSTRACT

Biological rhythm is a common feature of almost all living beings that appears in different forms and sizes. Different terms have been coined to denote this periodicity, depending upon the duration of the cyclic event. As far as mechanism and modeling of rhythm is concerned, there is a conserved pattern seen so far. All the rhythms follow a simple model where, under the external input signals synchronized with environment, a central oscillator is entrained that leads to various cyclic physiological outputs. The photosynthetic organisms are not an exception to these biological clocks. Similar to various physiological outputs that plants show in rhythmic fashion, secondary metabolites' production are also hypothesized to follow circadian rhythm. These secondary metabolites are used by plants as defensive strategies but also used by humans as medicines, flavoring agents and recreational drugs. If such a circadian rhythm is followed in composition and/ or concentration of secondary metabolites of medicinal plants, their extraction strategies can be improvised in a way that the plants or their parts are harvested at the time when they produce maximum drug/ secondary metabolite.

Keywords: Biological clock, Central oscillator, Circadian clock, Entrainability, Rhythm, Zeitgebers

INTRODUCTION

Rhythm

Rhythm is the term given to any 'regular/ patterned recurring of any event' or simply the 'timed movement through space' (1). The presence of rhythm generates predictability and order. The word 'rhythm' originated from Greek word 'rhuthmos' or Latin 'rhythmus', which means any regular recurring motion or symmetry (2). The commonly used synonyms for 'rhythm' are 'pattern', 'regularity' or 'periodicity'. This periodicity is seen in both physical (e.g. sunrise and sunset) and biological systems (menstrual cycle in human females). Within the purview of this review, we focus on rhythm/ periodicity in biological systems.

Periodicity in Biological Systems

Periodicity is one of the common characteristic features of biological systems that appear in different forms and sizes. It is a well established fact now that biological systems possess a precise mechanism to measure and synchronize with environmental fluctuations via rhythms of definite time periodicity (3). The ubiquitous nature of biological rhythms that are present in many levels of complexity and organization, suggests their adaptive advantage. The rhythmic oscillations that operate with a nearly 24h cycle are called as circadian rhythms. The 'circadian' term was introduced by Franz Halberg in 1959 from latin words 'circa' (about) and 'dies' (day) (4). This rhythmic oscillation that closely matches with rotation of earth on its axis, influences wide range of physiological, developmental and behavioural aspects of life of nearly all the organisms ranging from simple unicellular prokaryotes to complex multicellular eukaryotes.

On either extreme of these circadian rhythms lie ultradian (<24h) and infradian (>24h) rhythms. The courtship song of *Drosophila* male (with a periodicity rate of 50-60s) (5) and hypocotyl circumnutations in stem elongation of *Arabidopsis* and *Sinapis* (with a periodicity rate of 25m to 8h) (6) are the examples of ultradian rhythms. Among infradian rhythms, menstrual cycle of higher primates presents a common example, with a periodicity of 25-35d (7). On more extreme side, lie the circannual rhythms that operate on annual rate e.g. flowering in plants and hibernating behaviour of animals (5, 8). In the present review, we will see how circadian rhythms are generated, operated and maintained in plants; to result in various cellular, physiological and developmental outputs. In addition, we will propose how we can utilize these rhythmic events of plants for human welfare.

Circadian Clock

Out of all the biological systems for time keeping, circadian clock is the most extensively studied one (9). By definition, an event that persists with a period of ~24h under constant conditions, even in the absence of external timing cues, is called as circadian rhythm (10).

Because of earth's rotation on its axis, various environmental fluctuations arise that are well observed in day-night cycle or diurnal cycle. Nearly all the living organisms on earth demonstrate the rhythmic oscillations in their physiology, development and behaviour in tune with these diurnal cycles. For example, sleep-wake cycle of animals, and photosynthetic machinery in plants. But these phenomenon are not purely environmental cue based, rather they operate even in the absence of these external environmental signals. And these endogenous oscillations give rise to what we call as 'biological clock'.

Historical Overview

The first experimental evidence of occurrence of endogenous rhythm in the absence of environmental cues came from the work of French astronomer Jean Jacques d'Ortous de Mairan in 1729, where he showed the closing and reopening of *Mimosa* leaves (sensitive plant) at night and day respectively, even in the constant darkness (11,8). These studies provided evidence that biological rhythms are might be driven by some endogenously operating cellular mechanisms. These circadian rhythms or clocks are proposed to confer adaptive advantages and overall enhanced fitness to the organisms (12, 13). Fundamental properties of Clock includes, persistent rhythm even in absence of environmental cycle, entrainment or synchronization to environmental cues, maintenance of period over a physiological permitted range of temperature, rhythmic physiological, developmental and cellular outputs (3).

a. Characteristics of a circadian rhythm

The fundamental characteristics that define circadian rhythms are: ***Endogenous circadian period*** i.e. persistence of rhythmic activity even in the absence of external environmental cues; ***Entrainability*** i.e. synchronization or adjustment with environmental cycles; ***Temperature compensation*** i.e. ability to maintain period over a wide physiologically permissible temperature range and, ***Rhythmic regulation*** of cellular, physiological and developmental activities within the life cycle of the organism (14).

b. Model of Biological clock

The conceptual classical model for working of biological clock comprises of three basic components i.e. input signals or Zeitgebers, the central oscillator and output pathways. The generation of rhythms is carried out by central oscillator, which is also called as system's pacemaker. But in order to be biologically significant, these rhythms should not only be generated but synchronized with external environment. For this, various input pathways transduce various time-keeping signals such changes in temperature or light intensity, from environment to oscillator. In synchronization to external stimuli and oscillator, output pathways are generated which complete this biological clock model (9) (Figure I).

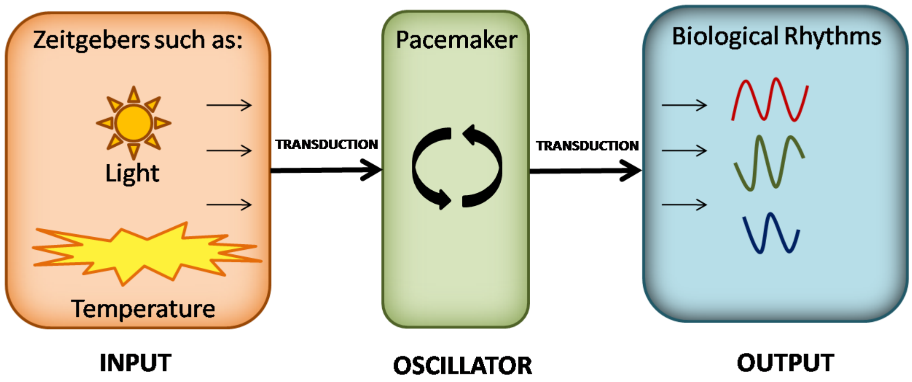


Figure-I: Simple model of biological clock comprising of three basic components i.e. Input signals (Zeitgebers), endogenous central oscillator and output pathways i.e. rhythms.

c. Mechanism of Circadian clock

Various genetic and molecular approaches have revealed that conserved regulatory mechanisms operate the clock functions from mammals to insects and plants to fungi (3) except cyanobacteria oscillator which rely on circadian pattern of ATPase activity (15) and protein phosphorylation (16). The common mechanism involves transcriptional auto-regulatory loops at the core of central oscillator with positive and negative components (17, 18). Considering these similarities, let us discuss the clock mechanism of *Arabidopsis*. The components of *Arabidopsis* clock include two single MYB transcription factors CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) (19) and LATE ELONGATED HYPOCOTYL (LHY) (20) as well as pseudo-response regulator TIMING OF CAB EXPRESSION 1 (TOC1) (21). The rhythmicity of *Arabidopsis* clock is regulated by one of the transcriptional loop which is defined by reciprocal regulation among these oscillator components (22). LHY and CCA1 function as negative regulator of TOC1 which in turn directly or indirectly activates LHY, CCA1 expression (23) (Figure II). This CCA1, LHY-TOC1 reciprocal regulation is important for clock functioning but does not explain all the rhythmicity in *Arabidopsis* (24, 25). However, still there is lot more to understand the complete mechanism of transcriptional circuit for the regulation of rhythmicity at oscillator core. Besides transcription, the clock regulation at post-transcriptional level, post-translational level and the chromatin dynamics have also been worked out (3), but details are beyond the purview of this article.

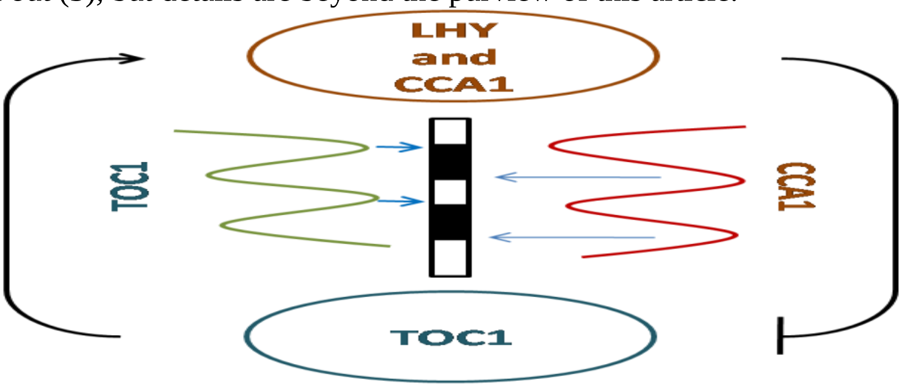
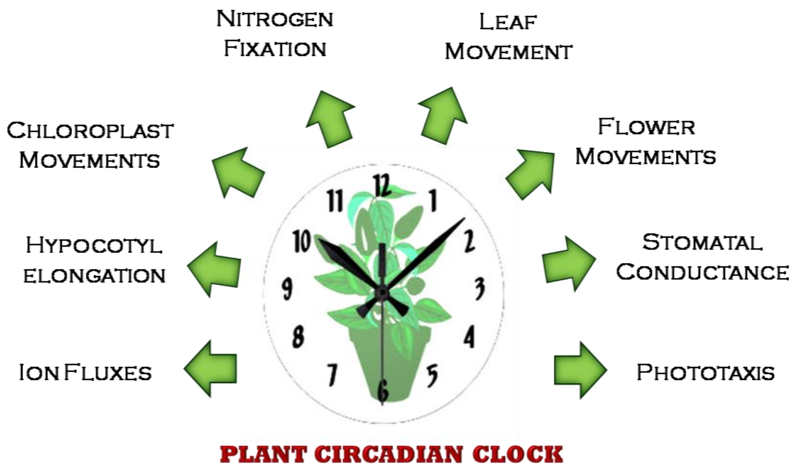


Figure-II: Negative feedback loop between LHY/CCA1 and TOC1 in *Arabidopsis* circadian clock which contributes to antiphasic oscillations between LHY/CCA1 (Peak during dawn) and TOC1 (Peak during dusk). White and black boxes represent day and night respectively. Arrow indicates activation whereas path ending with a perpendicular dash represents repression.

d. Outputs of the plant biological rhytmicity

A large number of biological processes are controlled by circadian rhythms in plants. Examples among circadian-controlled physiological functions include rhythmicity in leaf movement, photosynthetic capacity, stomatal conductance, cell Division, hypocotyls elongation, phototaxis, ion fluxes, flower movements, nitrogen fixation, chloroplast movements and many more (8) (Figure III). A large number of genes have also been reported whose expression depends on circadian rhythm (26, 27). Presumably, the benefit of having a biological clock lies in the fact that it organizes a temporal program within the organism that cues various physiological processes to occur at a specific phase in relation to daily environmental cycle.



DISCUSSION

Circadian Regulation of Plant Immunity

The observation that many of the plant defense related genes are modulated in a circadian manner (28) suggests that plant immunity is regulated by circadian clock. Some of the recent reports to support this view include Wang *et. al.* (2011) (29), who have shown the role of circadian rhythms in defense against microbial pathogens; Bhardwaj *et al.* (2011) (30), who have reported the circadian regulation of interaction of *Arabidopsis* with *P. syringae* strain; and Goodspeed *et al.*(2012) (31), who have shown that resistance to herbivores is also regulated in a circadian fashion. Additionally, a large number of metabolites have also been shown to exhibit rhythms in plants' roots and leaves (32). These metabolites are known to have anti-herbivore properties suggesting that differential oscillation of these metabolites may enhance plant defense against herbivores with various diurnal rhythms. Thus, it provides an adaptive advantage to plants in defense against various pathogens.

Figure-III: Some of the key biological outputs in plants that are reported to involve circadian clock.

Table-I: Glossary of commonly used terms in plant chronobiology

TERM	DEFINITION
Clock	Entire circadian system, sometimes used only for oscillator
Circadian	About a day i.e. ~24h
Input pathway	Information from the environment such as temperature and light, that are transduced to oscillator
Oscillator	The autonomous timekeeper of cell, responsible for generation of self-sustained rhythms
Output	Linker of oscillator to biological processes it controls
Pacemaker	Central oscillator, synonym of oscillator only
Entrainment	Synchronization of oscillator with environmental cues
Rhythm	Regular pattern or oscillation of a process
Free-running rhythm	Self-sustained oscillations operating under constant conditions.
Phase	State of one rhythm relative to another reference rhythm
Phase-shift	A displacement in timing phase of an oscillation
Amplitude	Difference between mean value and maximum/ minimum of a sinusoidal oscillation

Future Prospects: Exploiting Plant Defensive Strategies for Human Welfare

Based on the above discussion, it is clear that plants do possess the rhythmicity in their physiological outcomes, just like most of other organisms. And if so, the secondary metabolites that are produced in plants as a by-product of their metabolism cannot be an exception. Thus, it is hypothesized that the composition and/ or concentration of these secondary metabolites cannot remain constant throughout the diurnal cycle and varies with time. These secondary metabolites are the chemicals that plants produce as one of their defensive strategies against various environmental threats. The secondary metabolites produced by medicinal plants are used as drugs for human use as well. Hence there seems to be a rhythmic fashion of production of these drugs by medicinal plants also. If we can precisely dissect out the plant circadian rhythm in terms of production of these secondary metabolites of medicinal value, we will be able to extract these compounds more efficiently. Thus, the biological phenomenon of clock if further investigated in medicinal plants, we, the human beings would be able to use these plants more efficiently as we would be aware of the specific time when they produce these secondary metabolites in higher quantities. Probably, such work would provide a scientific basis to our traditional practices where specific medicinal plants are used at specific time of the day for efficient results e.g. Brahmi (Bacopa monnieri) is preferred to be taken in morning hours. In modern medicines also, the time of administration of a drug at specific time alters the resulting effect which is termed as chronotherapy. It provides another evidence of use of chronobiology in pharmacokinetics (33). The chronopharmacognosy is the term introduced to the branch of science (34) which links chronobiology and pharmacognosy. This link between basic and applied sciences can serve for better eco-industrial prospects in near future.

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