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Circadian Biorhythms – Present Understanding and Status of Research

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ABSTRACT *The biological processes manifest in the form of endogenous and self-sustaining cyclic events called circadian rhythms. These events oscillate on a 24 hr scale under the influence of environmental cues and are monitored by an integrated system comprising circadian clocks, photoreceptors and transducers. Circadian clocks include central and peripheral clocks. While, the central clocks are closely associated with the suprchaismatic nuclei (SCN) of brain in vertebrates and neurons of brain in insects, the peripheral clocks are limited to peripheral tissues. The functions of photoreception and rhythm transduction are combined and performed by an integrated structure comprising pineal gland and retino-hypothalamic tract. The circadian clock mechanism resembles 'input-process-output model' in which light plays a key role in modulating the expression of circadian clock genes. Despite rapid advances in molecular and genetic basis of circadian mechanism, the 'clock-to-physiology cascade' still remains unresolved. It opens-up new vistas for further research in circadian biology.*

INTRODUCTION

Life originated in a cyclic environment. The earth and other planets adopted cyclic movement and the earth's atmosphere followed suit. Taking a cue from the environment, life too followed a cyclic route. The light played a key role in fine-tuning the cyclic behaviour of animals. The cyclicity of the world manifests in a variety of biological rhythms called circannual, circalunar and circadian rhythms. Circannual rhythms represent the longest and most pronounced cycles of the earth, brought about by its tilt on its own axis as it encircles the Sun in an elliptical path and are expressed as seasons, Other cyclic events of the earth, to which the living organisms are exposed are circa-lunar rhythms / circa-semi-lunar rhythms that repeats on monthly (~29 days) or fortnightly (14.8 days) basis, coinciding with the moon cycle. To quote a few examples; the bird migration, hibernation and aestivation in animals and seasonal changes in plants represent circannual rhythms, while breeding in several intertidal marine organisms represent the circalunar rhythms.

The most obvious rhythm of the earth is that of daily rhythm or circadian rhythm that expresses in the form of day and night cycles on a 24 hr periodicity. The life originated in such a cyclic geophysical world and the living organisms developed their own cyclicity in order to avail themselves of the offerings of the earth's environment and to survive and evolve in the cyclic world (Wallace et al., 1991). Most organisms possess time-keeping devises that follow 24 hr periodic scale to regulate their biological and behavioural events. These events oscillate with environmental rhythms and manifest on daily basis and are aptly called circadian rhythms (Latin: Circa = about; dien = day). The circadian rhythms arise internally in animals when they are placed under constant environmental conditions such as the light, temperature, humidity, barometric pressure, food and so on. Under such conditions, organisms show rhythmic behaviour on a roughly 24-hour basis. The actual duration of the rhythm is called free running time or tau. It varies from ~21 to 27 hr depending on the environmental conditions. For example, the sleep/ wake cycle in humans works on a free running time of 24 hours and 11 minutes (Campbell and Reece, 2005). Extensive studies in the last five decades revealed that a variety of organisms, such as the Arabidopsis (Johnson, 2001), Neurospora (Loros and Dunlap, 2001), Drosophila (Williams and Seghal, 2001; Hall, 2003), Zebrafish (Pando and Sassone-Corsi, 2002), mouse (Reppert and Weaver, 2000, 2001) and Cyanobacteria (Iwasaki and Kondo, 2004) show rhythmicity in their activities. The rhythmicity coordinates biological systems and synchronizes them with the external environment and its expressions are reflected in the form of molecular, behavioural and physiological events ubiquitously in all living organisms (Lamont and Amir, 2010). The rhythmic expressions result in behavioural responses like eating, sleeping, mating, hibernation, migration, cell division and regeneration in a variety of organisms including plants and microbes (Sharma, 2003). Many physiological processes such as the opening and closing of stomata, production of photosynthetic enzymes in plants and pulse rate, blood pressure, temperature, rate of cell division, blood cell count, alertness, urine composition, metabolic rate, sex drive and response to medication in humans and other mammals continue to oscillate in a circadian fashion (Campbell and Reece, 2005). The free running time of the rhythm, in respect of these events could be reset by exposing the organism to altered environmental cues, such as the light (Wallace et al., 1991).

Circadian rhythms are further broken down into routine cycles such as the diurnal (active during day), nocturnal (active during night) and crepuscular (active during dawn and dusk: eg, white tailed deer and some bats) rhythms. Nelson (2005) observed three more types of biorhythms, viz., infradian, ultradian and circatidal rhythms. The infradian rhythms are cycles longer than the duration of the day (> 24 hr); such as the reproductive cycles of many animals and menstrual cycle in humans. On the other hand the ultradian rhythms represent the cycles of shorter duration than the day (< 24 hr) that ranges from a few seconds to few hours, such as the 90 m-REM (Rapid Eye Movement) cycle, the 4 hr nasal cycle or 3 hr growth hormone production cycle. The circatidal rhythms represent the medium duration cycles (usually ~12 hr) that occur in marine organisms of the intertidal regions. This article was taken-up with a two-fold objective. Firstly, it attempts to take a closer look at the components of the circadian system and elucidate its genetic and molecular mechanisms. Secondly, it reviews the present status of research in chronobiology and suggests potential areas for further research in this emerging field.

Components of Circadian System

The circadian system is includes three components, namely the circadian clocks, photoreceptors and transducers. The three components are represented in the animal body either as separate organs or as a single integrated cohesive unit. In this system, the circadian clock generates the rhythmicity; the photoreceptor detects the light signals from the environment and creates a photic environment in the cell, while the transducer converts the rhythm so generated into overt physiological responses (Eskin, 1979).

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Circadian Clocks: All organisms, starting from the Cyanobacteria to humans, possess time-keeping devises called circadian clocks or biological clocks that regulate all biological events on 24 hr periodic scale (Hardin, 2005). The clocks are endogenous and self-sustaining oscillators responsible for the generation of the rhythms in two ways – first; exogenously by ambient environmental cues and second; endogenously by internal oscillators. By doing so, they regulate a wide variety of behavioural and metabolic events and modulate their time of occurrence during the day. Their key property is that they could be entrained or clock-shifted by external zeitgeber (time giver), a feature that enables organisms to keep track of time in their local environment. Obviously, they strengthen the innate adaptive abilities and fitness of animals and enhance their survivability under ever-changing environmental conditions such as the availability of food, light and mates (Decoursey, 2004; Paranjpe and Sharma, 2005). The free running period of the rhythm mentioned above actually relates to the time taken by the circadian clock for one cyclic event.

Two types of biological clocks, namely the central and peripheral clocks were identified in animals. While, the former are located in the central nervous system, the latter are distributed among the peripheral tissues (Giebultowicz, 2000; Balsalobre, 2002). In mammals, the central pacemaker is located in the suprachiasmatic nuclei (SCN), that constitute a mass of about 8000 cells in the hypothalamus of brain (Liu et al., 2007). The peripheral clocks are located in various organs such as the heart, liver, pineal gland and adipose tissue and their activities are synchronized by the SCN through the nervous and circulatory systems (Fukada and Okano, 2002; Shibata, 2004; Yaung, 2006; Zvonic et al., 2006). Similar clocks were also detected in several vertebrates including birds (Whitmore et al., 1998; Yamazaki et al., 2000; Diego et al., 2009).

The invertebrate clock system has been extensively studied. It consists of populations of oscillators located both within and outside the nervous system and it controls a variety of behavioural, developmental and physiological processes including locomotion, eclosion and retinal sensitivity (Sharma, 2003). Among invertebrates, insects provide an excellent model system with a multi-oscillatory circadian organization and posses a number of overt rhythmic activities such as ecdysis, foraging, courting, mating and oviposition (Brady, 1974). Such multiple overt rhythms suggest that the circadian behaviour is controlled by central and peripheral pacemakers (Giebultowicz, 1999; Saunders, 2002). More reports are available on the localization of central circadian clocks in Drosophila and Bombyx mori. In Drosophila, for example the circadian locomotory behaviour was reported to be controlled by a clock comprising 100 neurons, located in the ventrolateral regions of the brain (Kaneko et al., 2000; Blanchardon et al., 2001). Further, in Drosophila many different types of cells including those of the thorax, abdomen, antenna, legs, wings and testis etc., continue circadian cycles even in isolated conditions (Alberts et al., 2002). Additionally, several neural circuits and multiple clock neurons, each with a cell autonomous pacemaker capability and are stimulated by multiple sensory modalities (light, temperature, pheromones etc.) are involved in the regulation of the timing of eclosion, locomotory activity and other circadian behaviours in this insect (Chang, 2006). In Bombyx mori, the circadian pacemaker is located in the brain at dorsolateral protocerebrum which acts as the chief oscillator while the frontal ganglion, with an autonomous role in regulation plays an ancillary role and acts as a co-oscillator (Sehadova et al., 2004). In certain silk moths such as Hyalophora cecropia and Antheraea pernyi the central clocks are localized in the cerebral lobes of the brain (Truman and Riddiford, 1970; Truman, 1972; Sauman and Reppert, 1996). Similarly, circadian clocks were detected in the cerebral lobes of Drosophila melanogaster (Ewer et al., 1992), Caliphora vicina (Cymborowski et al., 1994) Culex pipens pipens (Chiba and Tomioka, 1987), Cydia pomonella (Gvakharia et al., 2000) and in the optic lobes of some cockroaches, crickets and certain other arthropods (Tomioka and Chiba, 1984; Waddel et al., 1990).

A notable feature of insect time-monitoring system is the presence of peripheral clocks. A piece of epidermis of cockroach, cultured in vitro, showed circadian rhythmicity in cuticle secretion (Weber, 1995). Even isolated cells of many other insects also displayed self-sustained circadian oscillations in their metabolic activities (Plautz et al., 1997). Another line of evidence indicated the existence of organ-specific and tissue-specific multi-oscillatory circadian systems that are controlled by clock genes and their products (Hall, 1995; Giebultowicz, 2000). Recent microarray studies revealed the presence of cell-specific circadian genes (CCGs) that are implicated in the control of behavioural and physiological responses in insects (Ptitsyn et al., 2006). Though the existence of peripheral oscillators throughout the body of Drosophila is doubtful and they are reportedly restricted to certain pockets of the nervous system (Plautz et al., 1997; Giebultowicz, 2000, 2001). Most of the circadian clocks work in cell autonomous fashion and even isolated single cells can keep track of time and act as timing centers in complex multi-cellular organisms (Michel et al., 1993; Welsh et al., 1995).

Photoreceptors and Rhythm Transducers: In the mammalian circadian systems, the functions of photoreception and rhythm transduction are combined. The combined function is performed by an integrated structure called 'pineal-retino-hypothalamic tract'. In this structure, the pineal gland (also called 'the penis of brain') is connected to the SCN through a nerve ganglion in the neck region and forms an integrated clock system. In this mechanism, the pineal gland produces melatonin in response to light-dark cycle of the environment and releases it at regular intervals in a rhythmic fashion that acts as a chemical zeitgeber and reset the clock located in the SCN (Falcon, 1999; Christopher et al., 2006). The primary input to the SCN is the light detected by a third class ocular photoreceptor system that comprises a subset of blue-light photosensitive retinal ganglion cells expressing the photopigment melanopsin (Pierson and Foster, 2009). Intrinsically, melanopsin-expressing ganglion cells provide retinal input via the retino-hypothalamic tract to the SCN that synchronizes the circadian oscillator to the external day-light cycle (Warren et al., 2003). At cellular level a group of flavoproteins called cryptochromes act as intracellular photoreceptors which provide necessary input from light sources for the operation of circadian clocks (Alberts et al., 2002).

In non-mammalian vertebrates (birds, reptiles, amphibians and fishes), the pineal gland establishes contiguity with retina and hypothalamic supra-chaismatic nucleus on one hand and with the peripheral oscillators on the other and coordinate circadian behaviour. Additionally, it is known to contain extra-retinal photoreceptors which work in conjunction with those in the eye and mediate light effects on physiology and behaviour. While in birds and reptiles it acts as an ancillary photoreceptor, in amphibians and fishes it works as a main photoreceptor (Diego et al., 2009). More interestingly, in birds and some other lower vertebrates, the pineal gland serves as a rhythm generating system much like that of biological clock (Arendt, 1995). Obviously, the pineal gland contains clock-cum-photoreceptive unit besides acting as a melatonin generating system. Interestingly, the ultimate stage in the evolution of circadian system in mammals is that the pineal gland lost the functions of rhythm generation and photoreception, which it did in all other vertebrates (Diego et al., 2009). What is clear to date is that the pineal gland transduces information pertaining to light-dark cycles to body physiology and to the rhythmic machinery through its hormone, melatonin (Arendt, 1995).

Circadian clock mechanism – an 'input-process-output model' The circadian clock mechanism can be viewed as an 'input-process-output' model, much like that of an industry. In this

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mechanism, the light is the major source of input, while the process involves a complex molecular and genetic mechanism and the output manifests in the form of overt physiological and behavioural events.

Light as a source of input: Circadian rhythms in insects are modulated by a wide variety of environmental factors such as the light, food, temperature, relative humidity etc., (Damiola et al., 2000; Weinert, 2001; Sharma, 2001; Stokkan et al., 2001; Kita et al., 2002). However, light is the chief environmental cue that acts as a principal zeitgeber (time giver) for circadian rhythms (Peschel et al., 2009). The most important role of light is that it synchronizes circadian rhythms and sets the physiological events of organisms to occur at an appropriate time – a process called entrainment or clock-shifting. Because of this reason, the free running time of the rhythm is either delayed or advanced over the normal 24 hr pattern (Tomioka and Sakamoto, 2006). The impact of light on circadian rhythms has been elucidated in several insects including Drosophila (Konopka et al., 1989; Stanewsky, 2002; Hardin, 2004), cockroach (Page and Barrett, 1989) and cricket (Tomioka and Chiba, 1989) under altered photoperiodic conditions such as 12:12 hr light-dark cycle (LD), continuous light (LL) and continuous dark (DD). It appears that circadian clocks of several insect species are labile and that the free running time of the rhythm undergoes changes due to light regimes experienced during pre-adult development (Weinert, 2001; Sharma, 2001). Such long lasting changes in the free running time are termed after-effects and seems to be displayed by a variety of insect species. For instance, the blowfly, Calliphora vicina showed longer free running periods when the newly emerged flies are transferred from LD to DD condition (Kenny and Saunders, 1991). In the fruit fly Drosophila, relatively longer free running periods were observed in the flies reared under LD 12:12 hr or LD 8:16 hr than those reared under LL, LD 20:4 hr or LD 16: 8 hr. Similarly, the cockroach exposed to LD 8:14 hr and LD 8:18 hr and the cricket exposed to LD12:12 hr and LD 4:20 hr showed significantly longer free running periods and the cricket exposed to LD 16:8 hr and LD 20:4 hr showed shorter free running periods (Page et al., 2001; Koga et al., 2005). Our recent studies on biochemical rhythms in the silkworm, Bombyx mori have substantiated the role of light in altering the free running time of the circadian rhythms in its tissues such as the silk gland, fat body, muscle and haemolymph (Sailaja and Sivaprasad, 2010a, 2010b, 2011; Sailaja et al., 2011; Sivaprasad and Sailaja, 2011) and digestive system (Bhuvanewari and Sivaprasad, 2012a, 2012b, 2013; Bhuvanewari et al., 2013a, 2013b) further substantiate the role of photoperiod as a major source of input for the expression of circadian rhythms in insects.

The process involves clock genes and their products: Extensive genetic and molecular studies during the last three decades on model organisms such as the bacteria, fungi, fruit flies and mice have provided in-depth understanding of the genetic and molecular mechanisms underlying the circadian clocks. This obviously involves 'molecular gears' that are triggered by clock genes at the cellular level and the basic mechanism appears to be identical in all eukaryotes (Glossop and Hardin, 2002). The underlying mechanism involves multiple feedback loops (with positive and negative limbs) comprising clock genes, transcripts and their products (transcriptional activators and repressors) that oscillate with near 24-hr periodicity (Hall, 1998; Stanewsky, 2002; Glossop et al., 2003). It has been established that some protein molecules that are synthesized at regular intervals, acts as transcriptional factors and accumulate during first half of the circadian cycle but decline during its second half due to self-inhibition of their own production through feedback controls (Campbell and Reece, 2005). Current research provides detailed account on the circadian clock mechanism in some model organisms such as the fruit flies and silkworms among insects and mice among mammals.

Studies on Drosophila melanogaster have greatly advanced our understanding of circadian rhythms at molecular level and it involves at least seven clock genes; per (period), tim (timeless), cyc (cycle), dbt (double time), cry (cryptochrome), vri (vrille) and pdp-1 (Paradomain protein-1) (Rosato and Kyriacau, 2001). The expression of clock genes is subjected to positive as well as negative regulation through appropriate loops. The positive loop includes transcriptional activators like CLOCK (CLK) and CYCLE (CYC), while the negative loop includes transcriptional repressors like TIM (or TIMELESS) and PER (or PERIOD). The detailed mechanism is given by Alberts et al., (2002) in their famous book; 'Molecular Biology of the Cell' (pp. 420-422). A transcriptional feedback loop with a built-in time delay mechanism appears to be the heart of the circadian clock mechanism. This built-in mechanism involves the accumulation of certain key gene products switches off transcription their absence switches on transcription of clock genes. Accordingly, two proteins, namely TIM and PER coded by tim and per genes respectively, accumulate in the cytosol and depending on the light signals provided by the intracellular flavoproteins, the TIM gets degraded and resets the clock. In the absence of light, the TIM joins with PER and forms a hererodimer, which is transported to the nucleus and inhibits the expression of a number of clock genes including tim and per creating a feedback loop that causes the levels of TIM and PER to rise and fall. Two other proteins, namely the CLK and CYC also form hero-dimers and activate the transcription of per and tim genes, whose products (PER and TIM) are then translocated into the nucleus at a specific times of the day and inhibit the transcription of clk and cyc genes (William and Sehgal, 2001; Hardin, 2005). In turn the levels of CLK- CYC complex are regulated by products of vri and pdp-1 genes (VRI & PDP-1). In addition, the clock mechanism is regulated by another clock protein namely, DBT (short for double-time), coded by a dbt gene. This protein acts as a kinase and regulates the functioning of the clock indirectly by phosphorylating and degrading the free PER protein monomers in the cytosol. This process delays the periodic accumulation of TIM and PER, which is crucial to the functioning of the clock. Clock-shifting or entrainment occurs in response to new light-dark cycles. Light synchronizes the clock to the environment through multiple photoreceptors; most notably by an intracellular photo-protein called CRY (CRYPTOCHROME), which is coded by cry gene (Krishnan et al., 2001; Allada and Emery, 2009).

Following the advances made in Drosophila, the circadian clock mechanism of the silkmoths Antherae pernyi and Bombyx mori has been extensively studied (Sauman and Reppert, 1996; Sahadeva et al., 2004). The process involves proteins like CRY, PER, CYC and DBT that are coded by respective clock genes-cry, per, cyc and dbt. While the CRY participates in light perception, PER, CYC and DBT drive the core oscillator. Though, the details of many products are not known, the Bombyx mori cycle (BmCyc) and clock genes (BmClk) have been sequenced and it is known to express in many tissues such as the fat body, head, silk gland and mid-gut (Markova et al., 2003). The findings in fruit flies and silk moths were further reinforced by the detection of per homologues in the testes and vas deferens of codling moth, Cydia pomonella (Gvakharia et al., 2000). Fruitfly and silkworm clock genes have functional homologues in vertebrates and the basic principles of their interactions via feedback loops are conserved from insects to mammals (Glossop et al., 1999; Reppert and Weaver, 2000). Among mammals, the molecular and genetic mechanism of circadian clocks has been studied in mice. The mechanism is basically similar to that of insects and involves a two-way transcriptional feedback loop in which different molecules like CLOCK, BMAL-1, PER-1, PER-2, CRY-1, CRY-2 have major roles. The central feature of clock mechanism in mice is that the CLOCK / BMAL-1 hetero-dimer acts as transcriptional activator (positive element) while the PER / CRY protein light-sensitive complex acts as transcription repressor (negative element). It appears that the CLK / BMAL-1 hetero-dimer is the key component of the mammalian molecular clock, while PER 1, the product of per

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gene, being light-sensitive resets the mammalian molecular clock (Paranjpe and Sharma, 2005).

Output pathways of circadian clocks: It is believed that circadian clocks and their precisely timed rhythmic activities confer greater adaptive advantage and enable organisms to survive under extreme environmental conditions by a mechanism called entrainment or clock-shifting, a feature that expresses in physiological, biochemical and behavioural parameters (Pittendrigh, 1993;; Giebultowicz, 2001; Sharma, 2003). In mammals too, the transcriptional factors show circadian rhythmicity both in the central (SCN) and peripheral (liver) clocks (Ripperger et al., 2000). Essentially, this mechanism links the clocks with physiological outputs through a variety of molecules including transcriptional factors (Brown and Schibler, 1999; Jackson et al., 2001). For instance, the information on locomotory activity rhythm is transmitted to effector organs by a pigment dispersing factor (PDF), coded by pdf gene located in the per- expressing neurons of Drosophila (Renn et al., 1999; Park et al., 2000). Similarly, another protein called juvenile hormone binding protein and some other gene products control feeding behaviour in this insect (Lorenz et al., 1989; So et al., 2000). Another peptide called pigment dispersing hormone (PDH), a homolog of pigment dispersal factor of Drosophila, plays an identical role in the tobacco hornworm, Manduca sexta (Homberg et al., 1991; Wise et al., 2002). Similarly, a peptide molecule, namely [Arg^7]-corazonin has been identified as an output messenger of the circadian clock mechanism in Periplanata americana, Bombyx mori, Locusta migratoria and several other insects (Veenstra, 1989; Hua et al., 2000; Wise et al., 2002; Shao Qi-Miao et al., 2003). Though its functional role still remains uncertain, presumably, it is known to acts as a potent cardioaccelerator in cockroach (Veenstra, 1989) and help in melanization (body colouration) in locust (Hua et al., 2000) and spinning activity in silkworm (Tanaka et al., 2002). In addition, some other corazonin-like molecules (eg. His^7-corazonin and homologs of casein kinase) were also detected in the brain-suboesophageal ganglion (BR-SG) complex of silkworm and other lepidopterans- a fact that reflects the prevalence of two distinct corazonin output pathways triggered independently by variations in light and dark cues, even in the same species (Wise et al., 2002; Shaoqi et al., 2003).

Status of research in insect chronobiology

Insect Chronobiology, the scientific study of biological rhythms in insects, has emerged as an interesting and potential area of research in modern biology at international and national levels.

International Level: At international level, attempts were made to elucidate the molecular and genetic mechanism of circadian clocks in Drosophila, Bombyx mori and other insects. Prominently, three lines of approach are discernable in research in this field. The first approach focussed on identification, isolation and cloning of circadian clock genes in Drosophila (Konopka and Benzer, 1971; Allada et al., 2009). Subsequently, the homologues of Drosophila circadian clock genes were identified, isolated and cloned in Bombyx mori and other silkworms (Reppert et al., 1994; Sauman and Reppert,1996; Sehadova et al., 2004). The second approach was concerned with the analyzing the expression patterns of the clock genes and identification of their products such as the PER (Period), TIM (Timeless), CRY (cryptochrome), CYC (Cycle), DBT (Double Time) and their mechanism (Williams and Seghal, 2001; Hall, 2003). The third approach dealt with the localization of endogenous pace makers or circadian clocks or oscillators (Sehadova et al., 2004; Reppert, 2006).

National Level: In the last two decades no significant work has been initiated on insect circadian biology. However, several Indian researchers initiated investigations in this field, in collaboration with researchers abroad. The studies were more or less similar to those carried out at international level and focused on identification of clock genes, elucidation of their expression patterns and the role of gene products (Sharma, 2003; Sathyanarayana, 2004). However, no significant work has been initiated on insect circadian biology, more specifically with reference to the physiological and biochemical parameters of metamorphosing silkworm, except for our preliminary investigations on circadian biochemical rhythms in B. mori. Our reports on the biochemical basis of circadian rhythms in this insect throw some light on the expression patterns of the circadian clock mechanism. More importantly, the circadian studies on protein and amino acid profiles (Sailaja and Sivaprasad, 2010a, 2010b, 2011; Sailaja et al., 2011; Sivaprasad and Sailaja, 2011) and those on digestive enzymes and substrates (Bhuvanewari and Sivaprasad, 2012a, 2012b, 2013; Bhuvanewari et al., 2013a, 2013b) have highlighted the correlation between the gene expression timings vis-à-vis the photoperiod and the rhythmic changes in the synthesis and secretion of tissue proteins and digestive enzymes in B. mori.

Conclusion: Though the insect circadian mechanisms have been dissected up to the level of clock genes and output molecules, the actual mechanism of 'clock-to-physiology cascade' still remains unresolved. Though, the genotype of the rhythm has been traced, its phenotype has not been elucidated. Obviously, though the genetic and molecular basis of the circadian rhythm has been unraveled, its peripheral physiological and biochemical manifestations have not been elucidated to the satisfactory level, though some preliminary studies carried out from our laboratory (cited in the text) were originally intended to fill this gap in insect chronobiology. Nevertheless, much needs to be done in this field of emerging biology.

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