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REVIEW

Molecular insights of Circadian Rhythm in Chilopoda

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The control of the sleep-wake cycle as well as several physiological and behavioral processes in living things depends on circadian rhythms. Circadian rhythms are regulated by internal biological clocks that coordinate with environmental stimuli like light and temperature. The significance of circadian rhythms in organisms is emphasized because they support the maintenance of equilibrium between internal and external environments. They have an impact on functions like cellular growth, hormone production, reproduction, and tissue regeneration. Melatonin, cortisol, vasopressin, acetylcholine, insulin, leptin, and body temperature all have an impact on the circadian rhythm. The transcriptional and translational feedback loops formed by a network of genes and proteins are thought to represent the biochemical basis of circadian rhythms. Retinal light exposure is the main factor in the circadian clock's regulation. It is said that Chilopods in particular are nocturnal creatures whose circadian cycles are impacted by environmental cues like light and temperature. Chilopods' circadian rhythms influence many facets of their physiology and behavior, and the timing of necrosis, apoptosis, and autophagy in connection to the day/night cycle has been researched in Chilopods. Also described are the experimental techniques and methodologies utilized to explore Chilopoda circadian cycles. These comprise field research and sample collecting, lab studies utilizing histological and histochemical procedures, scanning and transmission electron microscopy, and other staining techniques. The discussion of factors affecting Chilopoda circadian rhythms has a particular emphasis on light-dark cycles and entrainment. Chilopods synchronize their internal clocks with the external light-dark cycles, and exposure to light is essential for this process. The rate of physiological processes and the timing of circadian oscillations are also influenced by temperature, which is another element that affects Chilopoda circadian rhythms. This study gives a broad summary of the traits, biological basis, experimental approaches, and variables affecting Chilopoda circadian rhythms.

Key words: Chilopoda, circadian rhythm, environment cues, hormones, melatonin, day/night cycle

The "circadian cycle," also known as the circadian rhythm, is a physiological process that aids in controlling the sleep-wake cycle. An internal clock in the body runs on a 24-hour cycle and is necessary for basic activities and functions (Vitaterna *et al.*, 2001). They control a vast range of physiological and behavioural functions in living things, such as hormone production, metabolism, gene expression, and sleep-wake cycles. Internal biological clocks that regulate circadian cycles synchronize with the environment using cues like light and temperature (Takahashi *et al.*, 2017; Reddy *et al.*, 2005).

Overview of circadian rhythms

The Master Clock in the brain coordinates the circadian rhythms that the many bodily systems follow. Biological clocks have been used to describe the forms of repetitive biological processes known as biological rhythms. They are made up of specific molecules that interact with cells, acting as a circadian rhythm regulator in the process (Sollars *et al.*, 2015). Circadian rhythms are essential for upholding internal temporal order and synchronizing physiological functions with the outside world (Bass and Lazar, 2016). Circadian rhythm disturbances, including those brought on by shift work, jet lag, or sleep difficulties, can be harmful to one's health and well-being. Understanding circadian rhythms is crucial for optimizing therapies and treatments for different illnesses and enhancing human health in general (Archer and Oster, 2015).

Importance of circadian rhythms in organisms

To maintain a balance between the internal and external surroundings, multicellular organisms have internal biological clocks that control their behavioral and physiological processes. Numerous periodic rhythms, such as seasonal, monthly, and/or circadian rhythms, make up its structure. Reproduction, hormone synthesis and secretion, cell growth, and tissue regeneration are all influenced physiologically by the length of the day and night (Lipovsek *et al.*, 2004).

Animal brain cells react to light and darkness; the eye records these environmental changes and the cells that control the sleep-wake cycle get signals from these cells.

Melatonin, a hormone that initiates sleep, which is released more at night and less in the daytime, is one example of a hormone that affects the increase and decrease of the circadian rhythm. Cortisol causes an organism to become alert. Other hormones that affect attentiveness and the circadian rhythm include vasopressin, acetylcholine, insulin, and leptin (Mohawk *et al.*, 2012).

The circadian rhythm is influenced by body temperature, which rises during waking hours and falls during sleep. Circadian rhythms assist organisms in adjusting to environmental cues like temperature and light fluctuations, enabling them to anticipate and appropriately respond to daily and seasonal variations. For survival, reproduction, and effective energy use, this adaptability is essential (Takahashi, 2017; Hastings *et al.*, 2018).

Molecular basis of Circadian Rhythm in Chilopoda

A network of genes and proteins that form coupled transcriptional and translational feedback loops controls circadian rhythms. The primary molecular building blocks of circadian clocks are clock genes like PERIOD and CRYPTOCHROME as well as transcription factors like CLOCK and BMAL1. These elements work together rhythmically to create and keep the circadian oscillations (Partch *et al.*, 2014; Green *et al.*, 2012). The primary input that controls the phase of this central Suprachaismatic nucleus pacemaker and enables the circadian clock to synchronize with the natural light-dark cycle includes retinal light exposure. Ambient light levels have a significant impact on physiology, allowing organisms to synchronize their internal and external processes to the time of day and to coordinate their physiological and behavioral activities (Casper and Gladanac, 2014).

Many individual circadian oscillators dispersed throughout the brain and peripheral tissues are synchronized by signals coming from the SCN. The intracellular clock system, which is made up of positive and negative transcriptional-translational feedback loops, has been the molecular foundation of such circadian oscillations. It creates a circadian rhythmicity of numerous genes by cyclic messenger ribonucleic acid and protein levels of essential clock elements (Casper *et al.*, 2014; Reppert *et al.*, 2001).

A Comprehensive Overview of Circadian Rhythm in Chilopod

Myriapoda is a class of terrestrial invertebrates that may be found in most woods, meadows, and even hot deserts. They play a critical part in soil disintegration by assisting in the breakdown of dead plant and animal matter. They are considered bio-indicators of the environment as a consequence (Sonakowska *et al.*, 2014). Two significant lineages of myriapods, herbivorous millipedes (*Diplopoda*) and carnivorous Chilopods including, two smaller clusters Symphyla, which superficially resemble little white Chilopods, and the tiny Pauropoda. They have a multi-segmented trunk made up of very identical segments and lack a distinct thorax or abdomen (Chipman *et al.*, 2014).

Characteristics of Circadian Rhythm in Chilopods

Chilopods' daily activity patterns were discovered through early study, which suggested that they have circadian rhythms. The circadian biology of Chilopods was first understood through observations of their locomotion, eating patterns, and reproductive cycles (Koilraj *et al.*, 2000). Chilopods are sensitive to environmental cues such as light and temperature, and these factors can affect their circadian rhythms. As a result, they are more likely to be active during the night when it is more relaxed and darker. Numerous behavioral expressions of Chilopoda circadian cycles, such as daily activity patterns and aging behavior, have been studied. Environmental signals including temperature and light-dark cycles are essential for bringing in and regulating Chilopoda circadian rhythms (Szymczak *et al.*, 1989). Chilopoda circadian rhythms can have an impact on the animal's homeostasis or the ecological niches they occupy (Rost- Roszkowska *et al.*, 2016). The synthesis and secretion of several chemicals, cell proliferation, and tissue/organ regeneration are just a few of the physiological responses to the duration of the day and night cycle that can occur. It has been established that an insect's nervous system and digestive system act as an internal biological clock that regulates several physiological processes, including feeding, hormone synthesis, reproduction, and other activities (Wang *et al.*, 2013). The ability to compare circadian rhythms among several arthropod groups is provided by Chilopods. Understanding the evolutionary conservation and divergence of circadian clocks can be accomplished by contrasting the chemical elements and clock mechanisms found in Chilopods with those found in other animals.

A few species of Chilopods also showed the evidence of apoptosis and necrosis, in the structure and ultrastructure of the endodermal area of the digestive system were examined. As a result, an examination of the timing of necrosis and apoptosis concerning the day/night cycle was carried out. It has been proved that apoptosis is the specific kind of cell death that initiates genetically predetermined cell death. Numerous elements and circumstances can cause apoptosis (Norbury *et al.*, 2001; Elmore *et al.*, 2007). Apoptosis is a sort of cell death, yet there is no inflammatory response. When apoptotic cells fail to release their organelles and contents into the extracellular space surrounding nearby tissues, where they would otherwise be phagocytized by neighboring cells such as macrophages or parenchymal cells and inflammation is triggered (Kurosaka *et al.*, 2003). Apoptosis and circadian rhythms have been linked in the literature to date in vertebrates. Myriapods are dependent on the day/night cycle for survival, and as a result, their use in studies examining the interactions between circadian rhythms and mechanisms involved in maintaining homeostasis is advantageous (Park *et al.*, 2009).

Circadian Clock Mechanisms in Chilopods

The growth cycle and behavior of myriapod invertebrates, such as Chilopods are coordinated with the day/night cycle. Chilopods are nocturnal animals, meaning they are active at night and sleep during the day. Clock genes, transcription factors, and protein regulators are some of the molecular elements of the Chilopoda circadian clock (Partch *et al.*, 2014). Chilopods have been shown to have orthologs of key clock genes like period (per), timeless (tim), and clock (clk). Over the course of a day, these genes' expression and activity fluctuate as a result of their interaction in autoregulatory feedback loops. This activity pattern is controlled by their circadian rhythms, which are biological processes that affect behavior, physiology, and other functions over a 24-hour cycle (Reppert *et al.*, 2001).

The gene believed to control circadian rhythms in Chilopods is called the Period. Discovering the molecular mechanisms underlying Chilopods' circadian cycles has been the focus of recent research. Insights into the central regulatory network have been gained because of the identification and characterization of genes and proteins involved in the circadian clock mechanism (Meuti and Denlinger, 2013). Researchers discover the essential components of the circadian clock system by analyzing the expression patterns of clock genes. The study adds to our knowledge of the evolutionary conservation and divergence of circadian clocks across other arthropod species and adds an understanding of the molecular basis of Chilopoda circadian rhythms (Reppert and Weaver, 2001).

Experimental Methods and Approaches

During the literature study using PubMed, three papers were selected to investigate how the Circadian rhythm plays a role in determining whether autophagy in the midgut epithelium of Chilopods is influenced by the day/night cycle(Rost-Roszkowska, *et al.*, 2014), The occurrence of apoptosis and necrosis in the midgut of Chilopods throughout the circadian cycle (Rost-Roszkowska *et al.*, 2016) and Studying the ultrastructure of the salivary glands in *Lithobius forficatus* (a species of Chilopoda) in relation to seasonal and circadian rhythms(Kaminska *et al.*, 2016). Various experimental approaches were used for their investigation.

Field study and collection of samples.

Adult *Lithobius forficatus* Chilopoda samples were gathered from various places in southern Poland. Different times of the day and seasons were used to isolate the salivary glands of male and female Chilopods in order to study the ultrastructure of the salivary gland in relation to circadian cycles. L. forficatus adult specimens were procured from southern Poland and central/southern Bohemia, whereas S. cingulata specimens were acquired from pet stores and rose in plastic containers. Throughout the day/night cycle, the animals' midguts were fixed every six hours for analysis to investigate apoptosis and necrosis in the midgut epithelium of the two species as well as to study autophagy in the midgut epithelial cells of the two species in association with their circadian cycle.

Laboratory Studies and Behavioral Assays

Samples were processed for analysis utilizing histological and histochemical techniques. After being fixed and coated in gold, the isolated salivary glands underwent scanning electron microscopy (SEM). Decapitated specimens were preserved, dried, and embedded in epoxy resin for light microscopy and transmission electron microscopy (TEM), respectively. For morphological observation, semi-thin and ultra- thin slices were prepared and dyed. The salivary glands contain a variety of substances, including proteins, lipids, mucopolysaccharides, carbohydrates, and more, all of which can be found using histochemical techniques. These procedures required staining the sample with particular chemicals and then examining it via transmission electron microscopy or light microscopy. Overall, the salivary glands of *Lithobius forficatus* were examined to better understand their structural and biochemical properties using a mix of SEM, TEM, and histochemical investigation (Kaminska, *et al.*, 2016).

For investigating the signs of apoptosis and necrosis in the midgut during circadian cycle the specimens were beheaded, fixed in glutaraldehyde and osmium tetroxide, dehydrated, and then embedded in epoxy resin for microscopic analysis. For light microscopy, semi-thin and ultrathin slices were cut and stained with methylene blue, while lead citrate and uranyl acetate were used for transmission electron microscopy. The percentage of midgut epithelial cells that exhibited apoptosis and necrosis was calculated by counting the quantity of these cells. A TUNEL assay was also carried out (terminal deoxynucleotidyl transferase- mediated dUTP nick end labeling). Cryostat pieces of the Chilopoda bodies were cut, embedded, and mounted on slides without fixation. After applying a permeabilization solution, the slides were stained with the TUNEL reaction mixture. The material was cleaned before being marked with DAPI and seen using a fluorescence microscope (Rost-Roszkowska *et al.*, 2016).

In another study, autophagy in the midgut epithelial cells of two species of Chilopods, *Lithobius forficatus* and *Scolopendra cingulata* was examined where the specimens were severed from their heads and preserved in glutaraldehyde and osmium tetroxide for microscopic examination. After dehydrating, the material was sectioned and then encased in epoxy resin. Methylene blue was used to stain semi-thin sections for light microscopy, and uranyl acetate and lead citrate were used to stain ultra-thin sections for transmission electron microscopy. Specimens were gathered at specific periods and were stained using histochemical methods. Acid phosphatase staining and mono-dansyl-cadaverine (MDC) labeling were applied to produce cryosections. Using a buffer solution that included naphthol phosphate and Fast Red Violet LB, acid phosphatase staining was carried out. Cryosections were stained with MDC, which particularly stains autolysosomes, by incubating them in a solution of MDC. A fluorescent microscope was used to examine the stained sections (Rost-Roszkowska *et al.*, 2014).

Factors Influencing Chilopoda Circadian Rhythms

Light Dark Cycles and Entrainment

Circadian rhythms in Chilopods are trained and controlled by light-dark cycles. The daily activity patterns of these organisms are timed to the surrounding light-dark cycles. Their physiological and behavioral functions, including their patterns of locomotion and eating, are influenced by the presence or absence of light cues. According to studies, Chilopods have photoreceptors that may entrain their internal clocks depending on the length and intensity of light exposure. For instance, studies on the

Chilopoda *Lithobius variegatus* have shown that synchronized circadian rhythms in locomotor activity depend on light-dark cycles (Tuf *et al.*, 2006; Haacker, 1967).

Temperature effect on Chilopods Circadian rhythms

Another significant factor that affects Chilopoda circadian cycles is temperature. The rate at which the physiological processes occur and the timing and potency of circadian actions, directly impacted by temperature changes. Chilopods are ectothermic species, which means that the environment affects how hot or cold they are. According to studies, changes in temperature can affect Chilopods' metabolic and locomotor rates, which in turn affect their circadian rhythms. The time and duration of locomotor activity patterns may be affected by temperature fluctuations, according to study on the Chilopoda *Scolopendra subspinipes*. It has already been shown that sudden decreases in temperature cause millipedes to become extremely active (Cloudsley-Thompson, 1951; Cloudsley-Thompson, 1949). By actively seeking appropriate temperature microhabitats that enhance their physiological processes and circadian patterns, Chilopods can display behavioral thermoregulation.

Role of social cues and environmental factors.

Chilopods' circadian rhythms have a substantial functional impact on their ecology and behavior. The physiological processes and periodic patterns of activity are essential to many aspects of Chilopoda life. Chilopods engage in a variety of activities that are coordinated with their circadian rhythms, including foraging, predator avoidance, and mating. Internal clocks that are educated by environmental cues, particularly light-dark cycles, have an impact on the timing of these actions (John Koilraj *et al.*, 1999). Chilopods benefit from the circadian rhythms' ability to adapt. It enables them to tailor their behavior patterns to the resources that are available and any potential risks in their surroundings. Chilopods can take advantage of ideal conditions for pursuing prey or avoiding predators by being active at various times of the day or night. This adaptation helps improve their foraging efficiency and survival (Tanaka and Watari, 2020).

CONCLUSION

Chilopods' circadian rhythms have a big impact on how they behave and how they live in the environment. The physiological processes and periodic patterns of activity are essential to many aspects of Chilopoda life.

In summary, this research paper offers an in-depth study of Chilopoda circadian rhythm, concentrating on their traits and clock mechanisms. Chilopods, members of the Myriapoda class, are important bioindicators in the ecosystem and aid in soil decomposition. Herbivorous millipedes (Diplopoda) and carnivorous Chilopods are the two important lineages of Chilopods. both have circadian rhythms that are regulated by environmental cues including light and temperature. The study emphasizes how temperature and light-dark cycles are key environmental signals in controlling Chilopoda circadian rhythms. The length of the day and night cycle has an impact on Chilopoda activity patterns, aging behavior, and physiological responses. The results show that Chilopods have an internal biological clock that involves their neurological and digestive systems and controls a variety of physiological processes.

Furthermore, the study focuses on clock genes such period, timeless, and clock to examine the molecular mechanisms underpinning Chilopoda circadian cycles. Chilopods' circadian rhythm is influenced by the auto-regulatory feedback loops that cause these genes' expression and activity to change during the day. Understanding the molecular foundations of Chilopoda circadian rhythms offers insights into how circadian clocks have evolved to be both conserved and divergent among arthropods. The impact of the day/night cycle on autophagy, apoptosis, necrosis, and the ultrastructure of Chilopods was examined using experimental techniques and methodologies. Chilopoda samples were gathered for field investigations from numerous places, at various times of the day, and throughout the seasons. The salivary glands, midgut epithelium, and cellular processes were examined in laboratory research using histological, histochemical, scanning electron microscopy, transmission electron microscopy, and other techniques.

Chilopoda circadian rhythms are influenced by a number of variables, including temperature, social cues, and light-dark cycles. Chilopods synchronize their daily activities to the local light-dark cycles, and changes in temperature can have an effect on their metabolic and locomotors rates and, as a result, their circadian rhythms. Chilopods also modify their behavior to improve foraging effectiveness, predator avoidance, and mating by using their circadian rhythms. By illuminating their traits, clock mechanisms, and the factors that affect their circadian cycles, Chilopods' circadian rhythms may now be better understood. The results serve as a foundation for further study in this area and add to the body of knowledge in the field of circadian biology. Understanding the complex circadian rhythms of Chilopods helps to understand the better ecological and behavioral adaptations of these intriguing creatures.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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