

The Evolutionary Pathways of the Circadian Rhythms through Phylogenetical Analysis of Basal Circadian Genes

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Abstract. Circadian rhythm is the endogenous clock in organisms that regulates the performance of various physiological and metabolic events in accordance with the periodic oscillating changes in the environment, especially the periodic light-dark cycle. The clock has endowed organisms with the ability in anticipating environmental changes allowing them to adjust their survival strategies accordingly, promoting their selective fitness. However, the evolutionary path and the emergence of such an intricate and vital system remain elusive. The article aims to analyse the molecular architecture and components of the circadian clock among three kingdoms of plants, animals, fungi, and their unicellular ancestors, revealing the possible emergence of the circadian clock from the primordial circadian rhythm of prokaryotes to complicated rhythms seen in multicellular organisms. In comparative genetic analyses of the circadian clocks, researchers have identified homologs in the circadian genes of multicellular organisms with their unicellular ancestors, indicating prior emergence of the circadian clock than multicellularity. In addition, comparative genetic studies among fungi, animal, and plant circadian clocks implied that the emergence of circadian rhythms across the kingdoms resulted from convergent evolution due to the significant selective advantages concomitant with the circadian clock. Furthermore, the article also reviewed methods of gene transferring laterally, including horizontal gene transfer and endosymbiotic gene transfer, which may explain the overall similarities in the transcription-translation feedback mechanism among the many circadian rhythms. However, while genetic transfer among distantly related organisms enhanced biodiversity and biological innovations in nature, whether the horizontal changes of genetic materials contribute to the similar feedback loop of the circadian clock still requires further research to determine.

Keywords: Circadian rhythm, evolution, phylogenetic, molecular analysis.

1. Introduction

The circadian cycle is a fundamental part of all organisms, with the daily oscillation in physiology and behaviour exhibited from simple-unicellular bacteria to complex-multicellular organisms. Such endogenic cycles provide effective strategies for organisms to adjust and optimise their physiological responses based on the 24-hour cycle of environmental change by controlling endogenous events such as metabolism, sleep-wake cycle, hormonal cycle, and more. The history of discovering the circadian rhythm can be traced back to the early 18th century when the French scientist de Mairan conducted the earliest known investigation on the circadian clock by observing the daily oscillation behaviour of leaves in the plant species *Mimosa pudica*. He also has noticed that the circadian pattern persists even in constant dark conditions. Still, a detailed and comprehensive analysis of the molecular architecture of the clock was not established until the mid-20th century.

Contributed by its significance in manipulating the organism's behaviour, the complex network coordinated by numerous genes, proteins, and transcription factors is believed to be highly conservative in evolution, indicating the possibility of tracing back these circadian genes of complex organisms to their first appearance in the phylogenetic tree. The evolution of the circadian rhythm has always been an intriguing topic of scientific research. The epic evolution of circadian rhythm might begin with the serendipitous adaptations of prokaryotes in responding to the light-dark cycle via regulating the internal physiological processes, including metabolism and reproduction, as a means of anticipating and preparing periodic environmental changes. Another hypothesis suggests that the circadian rhythm may have arisen from the need to coordinate circadian timings according to the

daily fluctuation as a metabolic byproduct and whether these cyclic oscillations led to the emergence of circadian rhythms [1]. From the evolution of circadian rhythm in primordial prokaryotes, these organisms gained a more potent selective advantage over other competing organisms, making the gene pass on, both via vertical gene transfer, passing the gene to the offspring, and horizontal gene transfer, genetic transformation among unrelated organisms [2]. One of the earliest life forms on the earth, cyanobacteria, has been found to possess a circadian cycle that synchronises its metabolic and physiological processes with the day-night cycle, marking the very first emergence of circadian rhythm in the epic of evolution. Researchers have indicated the cyanobacterial gene clusters of cyanobacteria strand Synchronous kaiABC regulating the circadian feedback cycle as researchers observed a rhythmic expression of genes when applying a luciferase reporter system [3]. Further studies on cyanobacteria circadian rhythms indicate an increased fitness in cyanobacteria strands in conditions with regular lighting than constant light since the circadian clock allows these bacteria to adjust and anticipate correspondingly to the light-dark cycle, marking the evolutionary significance of the circadian rhythm [4]. These studies on cyanobacterial circadian rhythms offer profound insights into investigating the biological process, thereby enhancing the knowledge of the circadian rhythm by providing a general molecular model of the complex system.

Even organisms of different kingdoms exhibit significant distinctions in most biological and physiological aspects, they all perform a similar fundamental feedback loop in their circadian rhythm, while the molecular mechanism underlying the circadian cycle varies dramatically. Due to the highly conservative properties of the circadian genome, for example, the molecular circadian clock of *Drosophila* species demonstrates a high level of homology in their molecular components featuring the core genes of clock (CLK), period (PER), cycle (CYC), and timeless (TIM), it might infer that absent of compositional orthologs in the circadian rhythm support independent and analogous evolution among the species [5]. For example, in the kaiABC gene cluster of the cyanobacteria, circadian regulation is achieved by the phosphorylation of kaiC, whereas the animal clock is regulated via transcription factors, indicating a convergence in the evolutionary pathway of the circadian rhythm. The essay aims to discuss and analyse the hypothetical evolutionary pathways in organisms, via comparisons of the molecular circadian rhythm across kingdoms and with the unicellular ancestors. By analysing and tracing back component genes of the circadian rhythm, a pathway of the circadian clock's evolution might as well be established.

2. Circadian Rhythms in Phylogenetic Kingdoms

2.1. Circadian Rhythms in Animals

2.1.1. Emergence of Circadian Clock in Basal Animals

Throughout the studies and explorations on the rudimentary circadian clocks of prokaryotes, prior researchers have established a fundamental overview of the complex molecular mechanism underlying the trait, and animals are where the clock is profoundly studied. Evolution of multicellularity in animals' origin in Porifera, or sponges, these primordial forms of animals have been present in the ocean before the Ediacaran-Cambrian boundary 542 million years ago, making them the most ancient type of animals [6]. Despite their ancient origin and their rudimentary structure, Porifera still displays complex circadian regulating systems. In the molecular analysis of Porifera species *Amphimedon* Queensland Ica, researchers identified diurnal and nocturnal entrained gene expressions and revealed degrees of similarity compared to the molecular clocks of other animals but lacking specific circadian components [7]. While studying a species of cnidarian, *Acropora millepora*, researchers have pointed out two light-dependent cryptochrome genes, AmCry1 and AmCry2, demonstrated peaks in their expression during the day and night respectively and showed control over the spawning event of *A. Millepora* [8]. In addition, the findings support the evolutionary origin of cryptochromes under the blue light of the Precambrian Ocean to elude from the high-level ultraviolet light near the surface. These investigations on the circadian clock of basal animal forms suggest that

the emergence of the circadian rhythm is prior to the earliest evolution of animals. Despite the circadian regulations discovered in Porifera and cnidaria species being different from that of high-level vertebrates, these disparities could be categorised as the evolution of animals adapting to distinctive environmental conditions.

2.1.2. Circadian Clock in *Drosophila*

From the studies in basal animal circadian clocks, the circadian rhythm of *Drosophila*, the most extensively studied molecular mechanisms underlying the circadian rhythm, exhibits various degrees of similarities. The earliest research on the *Drosophila* circadian clock can be traced back to 1970 when scientists first identified the 24-hour cycle in the locomotor activity of *Drosophila melanogaster*. Following the discovery is the publications of numerous comprehensive studies on the circadian clock of insect, as studying the underlying mechanisms provides insights into studying the human circadian clock. Subsequent studies on the *Drosophila* circadian clock reveal an intricate interwinding of critical proteins and transcription factors interacting with the genetic expression of the circadian clock. One of the key components of the circadian clock is the period (*per*) gene, involved in a negative feedback mechanism in which the PER protein accumulates rhythmically and inhibits the expression of *per* gene. Another critical part of the circadian clock is the timeless (*tim*) gene, and the protein it codes forms a complex with PER to enter the nucleus, inhibiting the transcription of *tim* and *per* genes, which completes a negative feedback loop [9]. By comparing the circadian clock among the insect class, the core clock genes, featuring PER, TIM, CLK, CYC, and CRY, are highly conserved in the evolution of insects due to the intense selective pressure of maintaining their functions [10]. However, discrepancies are also observed among the clocks of many species within the class, with the example being *D. melanogaster* having two paralogs of the PER gene. In contrast, other insects, such as the mosquito species *Aedes aegypti*, have one paralog. The researchers proposed that the ancestral insect species processes a single paralog of CRY and PER, and the genes duplicate into isoforms in species of insects. Similar evolutionary pathways can also be inferred in the evolution of animals in general, as the earliest circadian genes have been conserved but diverged into paralogues or isoforms of a similar circadian system.

2.1.3. Circadian Rhythm in Mammals

The circadian clock in vertebrates exhibited homology to the essential circadian genes discovered in both basal animal forms, including Porifera and *Drosophila*, and it is involved in many physiological events in mammals. The core component of a mammalian circadian clock features the activator CLOCK, which forms a dimeric complex with BMAL1. CLOCK-BMAL1 complex further binds with essential rhythmic genes of PER and CRY [11]. While studying the mice model, researchers discovered that the level of proteins PER and CRY accumulates in the evening as the CLOCK-BMAL1 complex initiates the *per* and *cry* gene in the daytime, and the cycle repeats daily [12]. Despite the molecular circadian rhythm present in most somatic cells, the paramount evolution of circadian rhythm in mammals is the suprachiasmatic nucleus (SCN), playing a significant role in unifying the circadian rhythm of the organism. The bilateral structure located at the anterior hypothalamus, characterized by a small cluster of intricate networks of circadian neurons, has the function of the master clock in the body, synchronizing and controlling the individual circadian clocks of the somatic cells and their metabolic events. The SCN can be subdivided into several types of neurons, such as GABAergic and neuropeptide-containing neurons, each with its endogenous circadian cycle and interacting with other neurons in producing coherent oscillations through the neuronal network. The SCN also receives external inputs from the retina via the retinohypothalamic tract, providing information about the day-night cycle, along with inputs from other parts of the brain, such as intergeniculate leaflet, aiding the synchronization of the clock with other organs. In mammal circadian regulation, SCN can regulate the key hormones melatonin and cortisol, allowing the animal to perform the regular sleep-wake cycle. Melatonin aggregates during the nighttime as the sunlight stimulus inhibit the secretion of the hormone, and cortisol regulates the blood sugar level and metabolism. Combining the circadian clock and hormonal regulation ensures that animals are alert

and active during specific periods of the day and lowers metabolic activities in resting hours. Overall, the unique adaptation of the circadian rhythm shown in mammals has become a successful mechanism which enhances survival fitness.

2.2. Circadian Rhythm in Fungi

Fungi, as well as animals, developed their unique circadian rhythms, facilitating their capability of anticipating periodic environmental changes and thriving in a diversity of ecological conditions. Phylogenetic studies of fungi indicate it has a closer relationship with animals than plants as it is categorised under the large eukaryotic group opisthokont along with animals. In the model species studied, *Neurospora crassa*, researchers have identified the cluster of genes, white-collar 1 (wc-1), white-collar 2 (wc-2), and frequency (frq), governing the endogenous circadian clock of fungi by performing a feedback loop. The loop entails bindings of WC-1 and WC-2 proteins, the result of the transcription and translation of wc-1 and wc-2, to the promoter region of frq. Then the transcription and translation of frq genes into FRQ proteins inhibit the activity of the frq-promoting transcription factors WC-1 and WC-2 [13]. The cycle unveiled dynamic interplays of phase-specific phosphorylation events in *N.crassa*, as the phosphorylation of FRQ protein by the enzyme casein kinase I (CK1) peaked during the late night, and phosphorylation of WC-1 and WC-2 peaked at day [14]. However, the FRQ/WC oscillator in *N.crassa* is not the only circadian oscillator since strands with dysfunctional FRQ/WC oscillators still exhibit rhythmic behaviour. For example, the Δ frq strand has null frq alleles carrying out a varying conidiospore-development rhythm in the constant dark environment for 12 to 35 hours [15]. VIVID, a photoreceptor protein that controls the light sensitiveness of *N.crassa* via physically binding to the white-collar complex (from the binding of WC-1 and WC-2), VIVID regulates the complex white-collar functions, thereby manipulating the light responses in circadian rhythms [16]. These intricate interplays of proteins optimise the selective fitness of fungi, for example, in controlling reproductive events like sporulation or mediating the growth rate of the organisms, further enabling fungi to become well-adapted in various ecological niches.

2.3. Circadian Rhythm in Plants

Plants are in another evolutionary lineage than animals and fungi, which is believed to have arisen from a strand of green algae under the larger group of Archaeplastida that has adapted to survive terrestrial life. Similarly, plants are evolved to be highly dependent on the periodic light-dark cycles in many physiological processes such as flowering, the opening of stomata, and hormone signalling, and its circadian clock is often categorised as 'green clocks', referring to its green algae ancestry. One central area of research in plant circadian rhythm is on a molecular basis. The major genetic component of the circadian feedback loop is known as the Timing of CAB expression 1 (TOC1), which interacts with other genes, such as the Circadian Clock associated 1 (CCA1), and late elongated Hypocotyl (LHY), constructing expression control and feedback cycle [17]. CCA1 and LHY are the transcription factors activating the expression of TOC1, in analogy to CLK and CYC in animals. As the level of TOC1 accumulates within the cell, the expressions of CCA1 and TOC1 are repressed, completing the feedback cycle [18]. Proteins other than the core feedback loop of the circadian rhythm also contribute to the physiology of plants. GI interacts with the protein ZTL, a protein that undergoes blue light-dependent degradation, and functions as an F-box protein that targets TOC1 for degradation [18]. In particular, the flowering process of plants has presented strong correlations with the regulation of genes GIGANTEA (GI) [19]. The study showed that the overexpression of GI in *Arabidopsis* prolonged the circadian period and delayed the onset of flowering in long-day conditions, and the integration of GI in the plant's circadian clock promotes plants' survival fitness by adjusting the plant more precisely responds to external environmental changes such as the light intensity and temperature cycles. Furthermore, GI is regulated by the quality and duration of the light, with blue light being a potential inducer of its expression. Further studies revealed the interaction of GI modulating the level of clock genes LHY and CCA1, as well as influencing the expression of a

specific flowering time gene *CONSTANS* (*CO*), establishing a connection between the photoperiodic flowering process and the endogenous circadian clock. In a study of the model species *Arabidopsis thaliana*, the regulation of photosynthesis and defending pathogens are regulated by the interactions of the circadian clock genes and other vital genes, with the circadian clock disruption leading to hindered growth, reduced photosynthesis, and easy contamination of pathogens. Plants, despite having a diverging evolutionary origin, still have adapted their ways of anticipating periodic environmental changes, which are vital in plant physiology.

3. Evolutionary History of the Circadian Rhythm

3.1. Selective Advantages of the Circadian Clock

The study of the evolution of the circadian rhythm in all organisms necessitates the investigation of the emergence of the circadian clock in eukaryotes, as modern complex multicellular organisms have ancestry in these protists. Evidence indicates that the circadian clock emerges due to natural selection since it gives organisms the ability to respond to the periodic day-night cycle in the primordial earth [20]. Furthermore, circadian clocks help separate antagonistic physiological processes, such as feeding and digesting may conflict with repair and resting and compartmentalize them in different phases of the day. This is achieved by the circadian clock signalling internal cues, which synchronise with external cues (day-night cycle) to avoid conflict in incompatible processes. The pursuit of energy efficiency has always been vital in which the organism that best utilises the limited resources gains a higher selective advantage. The emergence of the circadian clock ensures organisms get control of the occurrence of events, for example, restricting energy-intensive events to the most efficient or necessary time of the day. Similarly, organisms might decrease their metabolic activities at night, saving them for the efficient hours of the day.

3.2. Evolution and Ancestor of Plant Circadian Clock

In the marine picoeukaryote species *Ostreococcus tauri*, a species of green algae, researchers have identified a circadian oscillator, which they have compared with other green algae and plants in determining the evolutionary emergence of the clock [21]. Phylogenetic studies revealed that the origin of terrestrial plants took place around 475 million years ago during the Ordovician period, they evolved from a group of green algae which developed special adaptations to terrestrial life, such as specialized cells for gas exchange and a cuticle for preventing desiccation [22]. A similar circadian gene regulation cycle is identified in the green algae in which the two master clock gene *TOC1* and *CCA1*, homologues to the protein in the plant, is conserved, but lacking other plant proteins such as *GI*, which evolved later in plants as a means of better adapting the terrestrial life. The discovery indicates that the circadian rhythm is conserved before the emergence of plants. By applying the luciferase reporter strategy to *O. tauri*, researchers demonstrate that the rudimentary circadian rhythm in *O. tauri* has a similar genetic sequence as the circadian clock in plants. However, the two transcription factors showed degrees of discrepancies in the mechanism of advanced plant species like *Arabidopsis*. Although the *TOC1* in *Ostreococcus* showed a peak at dusk, similar to the homolog version of the gene in plants, *CCA1* in *Ostreococcus* displayed a different pattern than their *Arabidopsis*, which its expression begins much earlier than the expression in *Arabidopsis*. Another species of green algae, *Chlamydomonas reinhardtii*, is another model species widely studied for circadian rhythm; in which researchers identified a circadian rhythm that persists independently of daily environmental oscillation, which as well organises the temporal order for biological processes [23]. In further studies of the green algae, researchers present a consistency toward the “escaping from light” hypothesis, the primordial life beings avoiding the intense and harmful solar radiation. The study then revealed the circadian rhythm of *C. reinhardtii*, demonstrating an ultraviolet (UV) - sensing mechanism occurring at phases in which UV intensity is relatively lower [24]. Moreover, in comparisons made between 3 distant algae of *Chlamydomonas*, *Ostreococcus*, and *Cyanidioschyzon*, researchers have only identified the conservation of the *CCA1*-like transcription factors among the

three algae lineages, suggesting the selective pressure from their unique ecological niches influencing the diversification of the circadian clocks. In summary, the comparisons between the green algae and the circadian rhythm of plants indicated a conserved evolutionary pathway of the ‘Green Clocks’.

3.3. Evolution and Ancestor of Animal and Fungi Circadian Clock

Differ from the evolution of the ‘green clocks’ animal (metazoan) and fungi kingdom origins from another diverse clade of protist, the Opisthokonts. An innovation in the evolution of animal and fungi is the acquisition of multicellularity that have already evolved several times independently in the phylogenetic tree, with an example of the evolution of high-level plants from green algae. The adaptation of multicellularity in animals is also an independent occasion that enhances the selective fitness of the organisms, leading to the flourishing of complex multicellular organisms. Despite the overall similarity in the transcriptional-translational feedback loop in kingdoms of fungi, plants, and animals, phylogenetic analysis reveals the independent evolution of the circadian rhythm. In research on the first emergence of animals, researchers have noticed a free-living eukaryote, Choanoflagellate, resembles significant similarities to the choanocyte of the sponges, the most basic form of metazoans [25]. In gene research of a model Choanoflagellate species, *Monosiga brevicollis*, researchers discovered several genes previously identified to be metazoan-specific, including roles of cell adhesion, transcription, and signalling. One major evolution that contributed to the emergence of multicellularity in metazoan is the evolution of adhesion proteins cadherin and integrin, which facilitate the evolution of complex tissue and organs, and the gene coding for the metazoan adhesion protein is found in *M. brevicollis* sequence, suggesting early choanoflagellates might apply the adhesion protein in recruiting similar unit in forming multicellular tissues [26]. Although the studies on the circadian rhythm of choanoflagellate, the closest unicellular organisms relating to animals, are scarce, researchers do indicate clusters of conserved clock genes in the genome choanoflagellates that are homologs to the animal molecular clock. In cnidarians, a more derived animal group than Porifera already possesses the transcription-translation feedback loops discovered in bilaterians, in which the CLOCK and BMAL1 genes are conserved while the PER and CRY diverge significantly, as a possible result of its unique adaptation to the environment. Furthermore, the core components of the circadian cycle are also conserved in bilaterian, indicating fewer changes in the circadian rhythm throughout the phylogenetic tree of animals. In the case of the multicellularity of fungi, the advantages of multicellular structures, such as hyphae and fruiting body, contributed to the selective success and fitness, promoting the adaptation of fungi to a diversity of ecological niches due to its enhanced nutrient acquisition and reproduction ability. In the analysis of gene divergences, researchers revealed the divergence of fungi from other forms of life around 1.5 billion years ago following the colonization of the land approximately 500 million years ago during the Cambrian era [27]. Similarly, the circadian rhythm in early fungi species serves the function of anticipating and adjusting to the preceding periodic environmental changes. Analogous to the evolution of animals’ circadian clock having similarities with the unicellular eukaryotes choanoflagellates, the circadian clock of fungi is found to share significant similarities with two species of amoebae, *Capsaspora* and *Acanthamoeba* [28]. In circadian proteins found in the two amoebae species, including FRQ, LARP, ARNT, and HSF (Heat shock factor), researchers have identified similarities that might draw a connection between the amoeba and early fungi species. Due to their evolutionary homology, the fungi circadian rhythm resembles significant similarities to the animal circadian clock, which might infer that the emergence of circadian arose in before the divergence of animal and fungi kingdoms [29].

3.4. Genetic Transferring in Circadian Rhythm Evolution

As a result of the importance of time managing ability of the circadian clock, it is believed to emergent at least three times in the evolutionary tree: the *kaiABC* gene cluster of the cyanobacteria, the green clocks in green algae and the higher plants, and the emergence of fungi and animal circadian clocks. Convergent evolution depicts phenomena of similar mechanisms, physiological, morphological, and behavioral aspects, evolved in organisms that are believed to be distantly related

in evolution. In the case of the circadian rhythm, kingdoms of organisms all exhibit such physiological processes in which the properties of the clocks, including entrainment, phase responses, and temperature compensation, are conserved in organisms, as well as the transcription-translation feedback loop. Closer genetic studies on this vital circadian gene and proteins examine them to be too distinctive to evolve from a common ancestor of unicellular organisms. Despite their seemingly independent evolution, the similarities shared in the transcriptional-translational feedback loop might not entirely be a coincidence. In the early stages of evolution, a well-studied genetic movement, horizontal gene transfer (HGT), led to genetic materials transfer among distantly related organisms via three major mechanisms of transduction, transformation, and conjugation, allowing phylogenetically distant species to acquire vital traits. More importantly, in the genetic analysis of 24 bacteria and archaeal genomes, researchers identify the percentage of HGT genes varies from 1.56% to 14.47%, and among these transferred, there might be genes coding the circadian rhythm due to the significant advantages in boosting the selective fitness [30]. Furthermore, studies revealed that HGT is not limited to genetic transfer among prokaryotes but also from prokaryotes to eukaryotes, casting a dramatic impact on evolution. For example, the kingdom of plants arose from a group of green algae that have adapted to survive terrestrially, which they gained the ability to synthesise abscisic acid, a vital plant hormone leading to desiccation tolerance [31]. Furthermore, recent studies have shown HGT in the mutualistic symbiosis of plants, fungi, and bacteria, which provides insights into the ecological interaction leading to the co-evolution of unique traits [32]. Similarly, the early animal species perform HGT with bacteria in extending and acquiring biochemical pathways that promote the wide adaptation of organisms in various ecological roles [33]. On the origin of eukaryotic cells, the Russian scientist Konstantin Marschke's noticed similarities in prokaryotes and eukaryotic organelles such as mitochondria and plastids, which he proposed the double-membrane organelles are prokaryotes that are engulfed by the larger cell, named endosymbiosis. In addition, another compelling theory suggests eukaryotic cells first originated in the symbiotic partnership between archaea and bacteria that eventually became one cell in which the bacteria oxidises hydrogen to generate energy in the form of adenosine triphosphate (ATP), whereas the archaea provide the shelter and the constant supply of hydrogen [34]. These engulfed organelles not only perform extending biochemical pathways featuring energy generation but also are able to perform genetic transfer through the process of endosymbiotic gene transfer (EGT), where the genes from the plastid or mitochondria integrate to be part of the host genome similar to HGT. Comparative genome studies revealed the existence of genes that are homologous to the genes that are originally present in organelles but transferred to the host genome [35]. For example, in the study of plant EGT, researchers identify EGT as promoting adaptations to different environments, especially the transferring of sulfur metabolism genes from the chloroplast to the nucleus allowing the plant to survive sulfur-deficient soils [35]. HGT and EGT provide another perspective in answering the question of circadian rhythm evolution, as circadian genes, like any other selective advantageous genes, could transfer at specific times of the evolution, explaining the general similarities among the circadian clocks believed to evolve convergently. However, further phylogenetical studies have to be made to fully understand the effects of gene transferring in circadian clock evolution

4. Conclusion

In the article, we have established an evolutionary pathway of the circadian rhythm from the earliest emergence in prokaryotes to the complex mechanisms observed in multicellular kingdoms of animals, plants, and fungi, in which the evolution of the circadian clock is believed to emerge independently from their unicellular ancestors, indicating universal convergent evolutions in the circadian clocks. The such evolutionary pattern is contributed by the significant selective advantages the clock has on the organisms possessing it, including the benefit of synchronizing metabolic events according to the periodic change of the environment, separating antagonistic physiological processes, and energy saving by carrying out physiological events at most efficient hours of the day. Its

importance conserved the genes coding for the circadian rhythm throughout the evolution. In comparative genetic studies of vital components of the circadian rhythm, it is inferred that the evolution of plant circadian rhythm diverges from the rhythm found in animals and fungi, in which the plant belongs to the lineage of the green clock. In contrast, animals and fungi belong to the diverse eukaryotic group Opisthokont. The circadian rhythm of the two groups emerges independently, in which the plant shares homologous circadian genes with its green algae ancestor and fungi, and animal circadian clocks resemble similarities to the amoeba species and choanoflagellates under the larger group of eukaryotes. These studies also confirmed the existence of circadian clocks prior to the emergence of multicellularity of evolution. Despite the different genes coding circadian proteins among these phylogenetically distant organisms, a similar transcription-translation feedback loop is identified in them, performing the daily regulation of the circadian rhythm. The resembled similarities in the daily rhythm might not entirely be a serendipitous coincidence in the evolution as the transformation of genetic materials can occur laterally as well as vertically from the parents to the offspring. Especially in HGT, the process allows the exchange of genetic information among prokaryotes and eukaryotes, promoting the diversification of the genomes. Researchers have already identified vital genes passing from prokaryotes to eukaryotes that give the organisms the ability to perform extending metabolic pathways, allowing the adaptations to environmental changes. Similarly, some genes of the circadian rhythm could as well be exchanged in the very early stages of the evolution leading to the overall alike transcriptional-translational feedback loop found in many circadian rhythms of distant species. Moreover, the emergence of a eukaryotic circadian clock can also be linked to the circadian clock of preexisting prokaryotes contributed by the event of EGT, where the genetic information is transferred in the mutualistic symbiotic relationship among the engulfed cell and the host. Overall, the evolution of the circadian is convergent due to its significant selective advantages, which the organisms lacking the circadian rhythm are ultimately eliminated in the history of the evolution. However, the question of whether the intricate circadian rhythm is fully independent and evolved or does gene transfer played a role in the overall similarities of the feedback loop still requires further studies to examine.

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