MOLECULAR BASIS OF BIOLOGICAL CLOCKS AND CIRCADIAN RHYTHMS

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ABSTRACT

Circadian rhythm is the mechanism of the diurnal cycle and the response of the body towards the intensity of light and temperature on daily activities, excluding the effect of environmental stimuli. The circadian rhythm has a direct impact on the life of all living organisms, which might be diurnal or biennial. Still, all of these organisms exhibit molecular or metabolic processes that function under the effect of light, temperature, day and night and even seasons. Different model organisms have been used for studying the circadian functioning or system by various researchers, for example; within fungi, *Neurospora* was used, in prokaryotes, *Cyanobacteria* were used while among insects *Drosophila* and in mammals, mouse was taken as a study model. Later on, plants were also studied to explain the circadian rhythms under the effect of light and temperature, and the researchers chose the light-harvesting complex as the primary source of their study. Along with the external factors like light and temperature, the intrinsic study of genes and proteins is also well stated. In these studies, different genes showed their presence by generating the proteins that will increase or decrease according to temperature and provide the information of day and night timing after every 24 hours. This showed the molecular basis of the biological clock. The malfunctioning of the body due to metabolic changes leads to various disorders such as diabetes, obesity, hypertension, the cardiovascular disease which can arise due to lack of REM-sleep, workload, travel stress, jet lag and sometimes can even be fatal. This review intends to highlight the mechanism of the biological clock, the effect of the external environment on biological clock and genetics of circadian rhythm. Moreover, it will also discuss the association of metabolic disorders with circadian rhythms and assessment of the different model organisms to understand the circadian rhythm.

KEYWORDS: Circadian, light, temperature, biological clock, model, genes, malfunctioning

INTRODUCTION

As various chemical processes occur within the body of a living organism, whether it be *Neurospora, Drosophila*, mouse and humans, every organism has a particular life span and all the various developmental changes occur within the lifetime of an individual. There are various sub-cycles which work within a time period such as circannual rhythms, menstrual cycles, semilunar cycles and daily circadian cycles. During seasonal changes, i.e. twice a year, our biological clock gives indications of circadian rhythms, and this is regarded as Chronobiology [1].

Almost every living species present on this planet is bounded by the diurnal cycle whether it is cyanobacteria, plants or humans. The changes which they face are in the form of temperature and intensity of light. Moreover, these changes alter the working of the cell and lead to the generation of periodicity within a time period of 24 hours, and this is known as Molecular clock or Biological clock [2]. According to previous researches, it has been observed that in the day time

or during working hours there is an increase in body temperature and specific proteins are generated that activates the metabolism of the body to do work, and even different body functions like excretion, digestion etc. [3]. On the other hand, some proteins are generated at night, which maintain the temperature homeostasis. The mechanism of the diurnal cycle and the response of the body towards the intensity of light and temperature and our daily activities such as waking and sleeping without the effect of environmental stimuli are known as Circadian rhythm [4].Various aspects that are considered as a topic of research regarding biological clocks are the mechanism of the biological clock in the body of an organism, effect of external conditions like light and temperature on it, genetic changes occurring due to circadian rhythms or the genes involved in this process and metabolic diseases that are occurring due to malfunctioning of biological clocks and its effect on different species. [5].

This review intends to highlight the mechanism of the biological clock, the effect of the external environment on biological clock and genetics of circadian rhythm. Moreover, it will also discuss the association of metabolic disorders with circadian rhythms and assessment of the different model organisms to understand the circadian rhythm.

MECHANISM OF BIOLOGICAL CLOCK

The mechanism of biological clocks usually depends on the intensity of light and temperature during day time. When the temperature starts increasing the PER protein (Period Circadian Regulator) is produced and its starts accumulating in the cytoplasm of the cell, when its concentration reaches to the maximum point it starts travelling towards the nucleus and reaches to the maximum point at night. In the nucleus, the PER protein which starts degrading and is removed completely until mRNA produce it the next day and when the quantity of PER protein is maximum in the nucleus it stops the production of mRNA by deactivating the transcription factor and this process repeats within every 24 hours [6]. Studies proved the relationship between the physiological processes, circadian rhythms and certain diseases and metabolic homeostasis acts as an essential element in regulating the metabolic rate in the adipose tissue and adipose tissue is the central primary organ which helps in maintaining the energy homeostasis of the body [7]. The white adipose tissue which is made up of proteins, acts as a reserve of energy and provide it to the rest of the organs whereas brown adipose tissue which is made up of lipids and work as thermoregulator, i.e. maintain the temperature of the body. Hypothalamus secretes a hormone during night time, i.e. Growth hormone which is helpful in the growth of the young children and any kind of irregularity in sleep can affect the secretion from hypothalamus which will directly affect the growth of the children [8].

EFFECTS OF EXTERNAL CONDITIONS ON BIOLOGICAL CLOCK

During research it has been found that neurons found in *Drosophila* have a direct impact from temperature fluctuations as when these neurons are exposed to low temperature, they get excited and are responsible for the induction of sleep On the other hand when these neurons are exposed to high temperature, they become active and this process is quite similar in humans as the genes *Drosophila* have similarity to the genes of humans. The response towards temperature is much more active in the organisms having neurons in the form of impulses that excite the neural clock [9].

Likewise, the light also affects the biological clock. It has been hypothesised that the diurnal organisms, apart from human beings, have a well-established mechanism to understand the time-patterns. The super chiasmatic nucleus present

in the hypothalamus is the centre of the circadian system. Also, on the retina, the ganglion cells are present that are highly photosensitive, and on absorbing light, they transmit to the hypothalamus to activate the super chiasmatic nucleus. Pigment melanopsin is also responsible for the response to the light along with rod and cone cells [10].

GENETICS OF CIRCADIAN RHYTHMS

The genes that are responsible for the expression of circadian rhythms shows intrinsic behaviour and are functional in the form of the transcriptional or translational feedback loop. This loop has a forward and a backward regulation. An activated transcription factor exposes the genes that have been downregulated until now. During day time, the negative arm gets translocated to the nucleus. It reduces the activation of the positive arm, and once the negative arm of the loop is degraded completely, the positive arm becomes active and starts functioning during the night. The loop itself is self-perpetuating and activates a gene which expresses with an oscillatory period of 24 hours [11].

Transcriptional activators are: BMAL1 and CLOCK

Genes are: PER1, PER2, PER3, and Cryptochrome (CRY1 and CRY2)

Different genes can regulate circadian rhythm/periodicity. These genes include the “kaput” gene, which is implicated in the circadian cycle of locomotion; Clock, which is a cycle gene; two plant cryptochrome genes: Cry1 and Cry2. These genes combine and interact with each other to make a transcription/translation feedback loop at the molecular level for the circadian rhythmicity. During the day time, Clock and Bmal1 gene help in the transcription of Per and Cry genes that help in protein translocation to the nucleus due to which the gene expression increases. At night, when the level of Per and Cry protein increases, they inhibit Clock and Bmal1 genes so that the transcription is stopped and they get degraded [12].

METABOLIC DISORDERS OF CIRCADIAN RHYTHMS

The circadian dysfunctioning is implicated with several other non-communicable diseases that may be fatal if not diagnosed at an early stage. It includes obesity, type 2 diabetes mellitus (T2DM), cardiovascular diseases, cancer and mood disorders. All of these disorders are a result of disturbed sleep, restlessness, depression, workload, work shifts, et cetera. People who work at nights are susceptible to these metabolic syndromes and usually have hypertension and T2DM. Circadian rhythms also help in maintaining energy homeostasis and metabolism. Consuming a balanced diet helps in maintaining the periodicity of the circadian clock genes in the liver and initiate the metabolism of the body to function properly. Dysregulation of the circadian rhythm can also lead to insomnia, delayed sleep phase disorder, advanced sleep phase disorder, jet-lag, work-shift disorder and narcolepsy. The plants also show some disorders due to malfunctioning of circadian rhythms. For example, when the plants resistant to pathogens show disturbance in circadian rhythms become easily affected as the stomatal opening and closing is improper due to which pathogens can enter very easily and make them infected by affecting their immune system [13].

DIFFERENT SPECIES AS MODEL ORGANISM FOR CIRCADIAN RHYTHM

a. Cyanobacteria- Within non-eukaryotes transcriptional/translation feedback loop was first observed in the Cyanobacteria *Synechococcus*. It was identified by Kondo et al. in 1994. *Synechococcus elongates*

expresses three genes, namely, Kai A, Kai B and Kai C, which help in controlling the gene expression, cell division timing and arrangement of chromosome pattern. It has two classes: class 1 gene and class 2 gene. Class 1 gene comprises of Kai B and C, which express at the peak at dusk, whereas Class 2 gene comprises of promoter purF which express at the peak during dawn. Cell-cycle periodicity does not influence the circadian oscillation. However, the cell cycle maintains its periodicity of 24 hours independently and also helps in maintaining the superhelical structure of chromosome [14].

b. *Neurospora*– In the culture of *Neurospora* development changes which occur in aerial mycelia are studied. It has been observed that mycelia develop when incubated from late night to morning whereas no development is seen when incubated on other times. It was identified by Pittendrigh et al. in 1959, and due to the conidia formation, it was taken as a research organism as it helped in understanding the clock at the molecular level. The culturing of conidia is done on a solid agar-based media and is then transferred to the constant light region and then to constant darkness, and this is done so that the clock of hyphae of *Neurospora* is synchronised in order to show proper growth [15].

c. *Drosophila* – an insect circadian system model that is very helpful due to its close resemblance to a human's nervous system. Moreover, its rhythmicity is observed in lateral neurons of its brain. *Drosophila pseudoobscura* is one of the species used as the insect model In this model, we can analyze the circadian rhythm at the genetic and molecular level. We can also study the maintenance of circadian rhythms, their synchronization with the environment and can also study the behaviour changes [16].

d. **Mouse**- In this mammalian model for the circadian rhythms, Per1 gene and CLOCKS transcription activators are studied by the molecular genetics and classical genetics. In a model named 'Knockout', two strains of mice; one laboratory mouse and the other wild mouse was taken and it was established that the laboratory mice contained NPAS 2 homolog but doesn't contain clock protein in the super chiasmatic nucleus. In contrast, the wild mouse contained clock protein due to which behaviour changes were seen in both the traits. Clock protein has a greater impact on the oscillations of the brain as compared to NPAS 2 due to which wild mice can easily express the gene expression [17].

e. Plants- After animal's rhythmic process in plants is also studied along with its clock genes, behaviour and clock response is seen in photoperiodism during flowering. During the initial research on the biological clock, dissection of photoreceptors of the light complex was done to study the circadian rhythms. During the study, it was seen that the expression of the light-harvesting complex genes is the maximum during the day. Research has been done on all the organisms that are usually active during the day like plants and fungi, but the effect on gene expression during the night is yet to be observed [18].

Table 1: Circadian Clock Genes-Roles, Products and Regulation [2], [14], [15], [17], [18]

SYSTEM	**GENE**	CLOCK ROLE	PROTEIN PRODUCT(S)	REGULATION	PHENOTYPE OF MUTANTS
Synechococcus	Kai A	Positive element	No structural motifs identified	CR-RNA peaks CT 9-12; no protein data	Long period (30 hours, 33 hours)

	Kai B	Unknown	No structural motifs identified	CR-RNA peaks CT 9-12; no protein data	Short period (21 hours, 22 hours)
	Kai C	Negative element	ATP and GTP binding sites	CR-RNA peaks CT 9-12; no protein data	14 alleles, long, short
Neurospora	frq	Negative element	Two proteins of single open reading frame via temperature-responsive translational control; rhythmically phosphorylated	CR – RNA peaks CT 4, induced by light: protein peak CT 8-12	Long period (24 hours, 28 hours) A short period (16 hours, 19 hours)
	wc-1	Positive element; required to activate frq transcription	Transcription factor: Zn finger DNA binding domain, GLN rich activation domain, PAS domain with WC - 2	Induced by light: constant expression in dark	Null mutant and photo blind
	wc-2	Positive element; required to activate frq transcription	Transcription factor: Zn finger DNA binding domain, acidic rich activation domain, PAS domain with WC - 1	Not induced by light: protein always present in dark.	Null mutant and photo blind
Drosophila	per	Negative element	PAS domain mediate interaction with negative element TIM : rhythmically phosphoryalted	CR-RNA peaks CT 14 :protein peak CT 19	Long period allele show loss of temperature Short period allele several ARR
	tim	Negative element	No PAS domains: interacte with PER with: phosphorylated	CR-RNA peaks CT 14 :protein peak CT 19	Long period length short period and ARR allele
	dbt	Facilitating element	Sequence homolog of casein kinase: required for development: regulate accumulation of PER	Constitutive	Long period length short period and ARR allele
	Clk(Jrk)	Positive element	Transcription factor: bHLH DNA binding domain, GLN rich activation domain PAS domain mediate heterodimerization with CYC: molecular mammalian CLOCK	CR-RNA unimodal peaking at CA.CT 23 or bimodal peak near dusk	Null mutant ARR, low PER and TIM expression: no light induced
	cyc	Positive element	Transcription factor: bHLH DNA binding domain, GLN rich activation domain PAS	Constitutive	Null mutant ARR, low PER and TIM expression: no

			domain mediate heterodimerization with CYC: molecular mammalian BMAL1/ MOP3		light induced
Mouse	Per 1	Negative element (putative)	PER protein interact with the negative element, TIM – a molecular relative of insect 'per'.	mRNA expression with peak around CT 4 in SCN, CT 10 in retina induced by light	No mutants available
	Per 2	Negative element (putative)	PER protein interact with negative element, TIM – molecular relative of insect 'per'.	mRNA expression with peak around CT 8 in SCN, CT 14 in retina induced by light	No mutants available
	Per 3	Negative element (putative)	PAS domain interact with other mammalian proteins, PER protein interact with negative element, TIM – molecular relative of insect 'per'.	mRNA expression with peak around CT 6 in SCN, CT 10 -14 in retina induced by light	No mutants available
	tim	Facilitating element (Negative?)	Clear sequence homolog of insect TIM	Not rhythmically or weakly expressed	No mutants available
	Clock	Positive element	Transcription factor: bHLH DNA binding domain, GLN rich activation domain PAS domain mediate heterodimerization with CYC: molecular mammalian BMAL1/ MOP3	Not expressed	1 allele with long period length reduce light induction of PER 1
	bmal1/ mop 3	Positive element	Transcription factor:bHLH DNA binding domain, GLN rich activation domain PAS domain mediate heterodimerization with CYC: molecular mammalian CLOCK	Rhythmically expressed in rats may not express in mice	No mutants available

CONCLUSION

Biological clocks are primary timing devices present in organisms that have specific molecules which interact with the cells throughout the body and are present in almost every tissue and organ of living organisms. Researchers have found similarity in the genes among the fruit fly, fungi, mice, humans and other living organisms. These genes form a clock, and the brain acts as a master clock which controls several other cycles viz menstrual cycle, circadian cycle, semilunar and even the hormones secreted by the endocrine system. The hypothalamus of the brain receives direct signals from the retina of the eye in the super chiasmatic nucleus, which help in analysing the circadian rhythms which change with the change in day and night. The cells of the body generate specific proteins to perform specific functions. The quantity of protein generation is high during day time as the temperature is high due to which the metabolic rate of the body is quite high. In contrast, at night, the temperature decreases, which decrease the metabolic rate of the body and thus, the protein generation by the cells is reduced. The circadian cycle affects the hormone release, eating habit, digestion, body temperature and other metabolic function and if any malfunctioning is observed in the circadian rhythm of the body, chronic health problems can be faced. Moreover, thus it can be concluded that the circadian clock plays a crucial role in maintaining the physiological processes undergoing in the living organisms and the further future studies can reveal the role of `unrecognised proteins of the circadian clock.

REFERENCE

[1] D. A. Paranjpe and V. K. Sharma, "Evolution of temporal order in living organisms," *Journal of Circadian Rhythms*, vol. 3. Ubiquity Press, p. 7, 04-May-2005, doi: 10.1186/1740-3391-3-7.

[2] Y. Xu, T. Mori, and C. H. Johnson, "Circadian clock-protein expression in cyanobacteria: rhythms and phase setting," *EMBO J.*, vol. 19, no. 13, pp. 3349–3357, 2000, doi: 10.1093/emboj/19.13.3349.

[3] K. Eckel-Mahan and P. Sassone-Corsi, "Metabolism and the circadian clock converge," *Physiol. Rev.*, vol. 93, no. 1, pp. 107–135, Jan. 2013, doi: 10.1152/physrev.00016.2012.

[4] R. L. Sack *et al.*, "Circadian rhythm sleep disorders: Part I, basic principles, shift work and jet lag disorders: An American Academy of Sleep Medicine review," *Sleep*, vol. 30, no. 11, pp. 1460–1483, 2007, doi: 10.1093/sleep/30.11.1460.

[5] E. D. Buhr and J. S. Takahashi, "Molecular Components of the Mammalian Circadian Clock," in *Handbook of experimental pharmacology*, no. 217, Howard Hughes Medical Institute, 2013, pp. 3–27.

[6] H. Okamura, K. Yagita, and S. Yamaguchi, "Molecular Mechanism of the Regulation of the Biological Clock in Mammals," Springer, Boston, MA, 2002, pp. 381–384.

[7] J. Richards and M. L. Gumz, "Mechanism of the circadian clock in physiology," *Am. J. Physiol. Integr. Comp. Physiol.*, vol. 304, no. 12, pp. R1053–R1064, Jun. 2013, doi: 10.1152/ajpregu.00066.2013.

[8] C. Dubowy and A. Sehgal, "Circadian rhythms and sleep in Drosophila melanogaster," *Genetics*, vol. 205, no. 4, pp. 1373–1397, Apr. 2017, doi: 10.1534/genetics.115.185157.

[9] V. Sheeba, M. Kaneko, V. K. Sharma, and T. C. Holmes, "The Drosophila circadian pacemaker circuit: Pas de Deux or Tarantella?," *Critical Reviews in Biochemistry and Molecular Biology*, vol. 43, no. 1. NIH Public Access, pp. 37–61, Jan-2008, doi: 10.1080/10409230701829128.

[10] T. S. Andreani, T. Q. Itoh, E. Yildirim, D. S. Hwangbo, and R. Allada, "Genetics of circadian rhythms," *Sleep Medicine Clinics*, vol. 10, no. 4. W.B. Saunders, pp. 413–421, 01-Dec-2015, doi: 10.1016/j.jsmc.2015.08.007.

[11] A. Jagannath, L. Taylor, Z. Wakaf, S. R. Vasudevan, and R. G. Foster, "The genetics of circadian rhythms, sleep and health," *Hum. Mol. Genet.*, vol. 26, no. R2, pp. R128–R138, 2017, doi: 10.1093/hmg/ddx240.

[12] M. D. Li, C. M. Li, and Z. Wang, "The role of circadian clocks in metabolic disease.," *The Yale journal of biology and medicine*, vol. 85, no. 3. Yale Journal of Biology and Medicine, pp. 387–401, 2012.

[13] S. E. Cohen and S. S. Golden, "Circadian Rhythms in Cyanobacteria," *Microbiol. Mol. Biol. Rev.*, vol. 79, no. 4, pp. 373–385, Dec. 2015, doi: 10.1128/mmbr.00036-15.

[14] P. L. Lakin-Thomas, D. Bell-Pedersen, and S. Brody, "The genetics of circadian rhythms in Neurospora," in *Advances in Genetics*, vol. 74, Academic Press Inc., 2011, pp. 55–103.

[15] O. Tataroglu and P. Emery, "Studying circadian rhythms in Drosophila melanogaster," *Methods*, vol. 68, no. 1, pp. 140–150, Jun. 2014, doi: 10.1016/j.ymeth.2014.01.001.

[16] P. L. Oliver *et al.*, "Disrupted circadian rhythms in a mouse model of schizophrenia," *Curr. Biol.*, vol. 22, no. 4, pp. 314–319, Feb. 2012, doi: 10.1016/j.cub.2011.12.051.

[17] C. R. McClung, "Plant circadian rhythms," *Plant Cell*, vol. 18, no. 4. American Society of Plant Biologists, pp. 792–803, Apr-2006, doi: 10.1105/tpc.106.040980.