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Circadian Clock Genes: A Review on Biological Time

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ABSTRACT:

Nearly all organisms exhibit time dependent behavior and physiology across a 24 hour day known as circadian rhythms. Circadian rhythms (CR) are a series of endogenous autonomous oscillators generated by the molecular circadian clock. Circadian rhythms disruption is a common issue in modern society, and researches about people with jet lag or shift works have revealed that CR disruption can cause cognitive impairment, psychiatric illness, metabolic syndrome, dysplasia, and cancer. Cyclic gene expression determines highly tissue specific functional activity regulating such processes as metabolic state, endocrine activity and neural excitability.

Keywords: Circadian, Genetics, Clock, Genes, Circadian rhythms etc

INTRODUCTION

Across a spectrum of living organisms, ranging from cyanobacteria to humans, it has been observed that biological functions follow a pattern of circadian rhythmicity.[1]

Circadian rhythm is the term used to describe the physiological and behavioral twenty four hour cycle that most organisms experience. This, of course, includes the sleep/wake cycle, but includes many other factors, which vary as well (e.g., hormonal levels, eating, and drinking).[2]

The existence of an "endogenous" timekeeper that operates autonomously without any direct light signal was recognized early on and termed the "circadian clock"(CC). The CC acts as a biological oscillator or pacemaker with an approximately 24 h cycle, synchronized with solar day and night.[3]

It is clear that these "clock genes" produce 24 hrs. rhythms in individual cells through interlocked transcription–translation feedback loops. However, the organization of these individual molecular oscillators into a "biological clock" controlling behavior and physiology remains to be fully elucidated.

Circadian time keeping allows appropriate temporal regulation of an organism's internal metabolism to anticipate and respond to recurrent daily changes in the environment.[5] Daily and seasonal changes of light and temperature influence also human physiology, metabolism, and behavior (sleep/wakefulness alternation and fasting/feeding time) in health and disease.[6]

The circadian clock is a temporal Programme found in organisms from all phyla. It is an adaptation to earth's rotation, conferring a 24-h structure on processes at all levels—from gene expression to behaviour. Circadian clocks are autonomous, producing circa- 24-h rhythms even in the absence of daily environmental signals (zeitgebers). The mammalian circadian programme shows both top-down and bottom-up organisation. The ability to generate daily rhythms is a cellular quality. These cellular clocks form networks that build up the circadian programme in tissues, organs, and the entire organism. The top-down organisation in mammals is rooted in a nucleus located above the optic chiasm (the suprachiasmatic nucleus, SCN). This pacemaker receives photic information via the retina, synchronises its own neuronal cellular clocks, and transduces the 'internal day' to a network of peripheral clocks.[7]

The circadian clock runs with a period that is not exactly 24 h. Therefore, the clock needs to be reset periodically to ensure synchronization of the organism's physiology to the environmental light-dark cycle. Many aspects of physiology and behavior are governed by the clock. It acts on neural and endocrine pathways to regulate individual circadian rhythms.[8]

Early photosynthetic cyanobacteria acquired internal "circadian" time-keeping mechanisms entrained by recurring environmental cues, notably day and night. As the earth's rotation slowed to 24-hour days, cyanobacteria and eukaryotic life continued to evolve in the setting of natural light–dark periods modified north or south of the equator by the seasons.

Nearly all light-sensitive organisms, including unicellular eukaryotes, fungi, plants, and animals, developed biological clocks. These clocks were composed of different molecules that used somewhat similar strategies of interlocked transcription/translation feedback loops to generate cycles with a period slightly less or more than 24 hours (*circadian* means about 24 hours).[9]

THE CLOCK IN OUR BRAIN: THE SUPRACHIASMATIC NUCLEI

The brain area that appears to play a central role in circadian rhythms is the **suprachiasmatic nucleus (SCN)** in the hypothalamus.[2] The SCN and other sites within the central nervous system (CNS) play a major role in controlling circadian rhythms, acting both directly through neural communication via the autonomic nervous system on target organs and, indirectly, through the release of circulating systemic factors.[6]

It is known that light input has two distinct effects on SCN neuronal rhythms:

(1) Altering the absolute phase relationship between SCN rhythmic activity and locomotor activity onset.

(2) Reorganizing the relative phases of constituent oscillators within the SCN.[4]

SCN's "circadian clock" genes can control behavior, feeding, and reproduction through neurotransmitters and hormones. Circadian rhythms are synchronized by the SCN. This "master clock" relies on the functional role of the core clock proteins, Clock and Bmal1, which enables clock gene expression. As mentioned before, these circadian clock genes are all expressed in animal and human placenta, and their expression shows a potential circadian rhythm.[10]

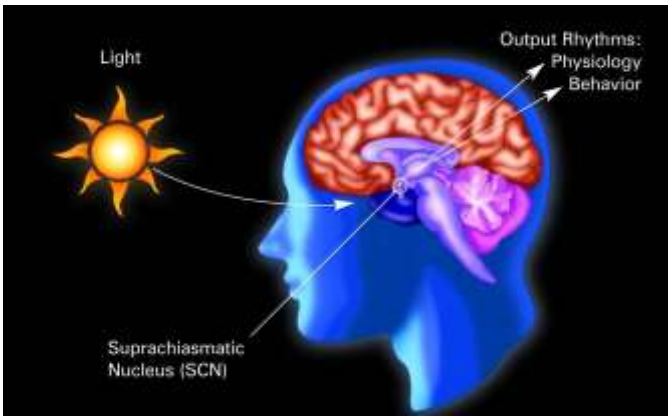

Figure 1. An area near the central amygdala is circled and labeled the suprachiasmatic nucleus (SCN). There is also two arrows from the SCN that point to physiology and Behavior which is listed under Output rhythms. An image of the sun is labeled light and has an arrow pointing through the eyes and to the SCN.[11]

In humans and other mammals the primary body clock is located in the suprachiasmatic nuclei, a cluster of around 10 000 neurones located on either side of the midline above the optic chiasma, about 3 cm behind the eyes. If these nuclei are destroyed, either experimentally in animals or as a result of disease in humans—for example, compression by expanding pituitary tumours—the ability to express any overt circadian rhythms is destroyed. The temporal pro- gramme of behaviour and physiology is scrambled.

Most aspects of physiology and behaviour are governed by a central clock mechanism in the hypothalamus. The clock acts on neural and endocrine pathways to regulate individual circadian rhythms so that internal state varies predictably over 24 hours. This enables adaption to daily and seasonal environment and enhances efficiency by separating anabolic and catabolic processes in time.[12] Mice mutant in the *Per2* gene show abolished rhythmic expression of the AVP gene in the SCN, demonstrating circadian control of AVP gene expression in the SCN.

HISTORICAL OVERVIEW OF CHRONOBIOLOGY

Researchers began studying biological rhythms approximately 50 years ago.[13] Early on, scientists knew that plants and unicellular organisms had cellular clocks and that cell– cell communication was not required for rhythm generation. However, there was a presumption that the clock in complex mammals was located in the brain.[9]

Although no single experiment serves as the defining event from which to date the beginning of modern research in chronobiology, studies conducted in the 1950s on circadian rhythmicity in fruit flies by Colin Pittendrigh and in humans by Jürgen Aschoff can be considered its foundation. The area of sleep research, which also is subsumed under the field of chronobiology, evolved some-what independently, with the identification of various sleep stages by Nathaniel Kleitman around the same time. The legacies of these pioneers continue today with the advancement of the fields they founded. The roots of the study of biological rhythms, however, reach back even further, to the 1700s and the work of the French scientist de Mairan, who published a monograph describing the daily leaf movements of a plant. De Mairan observed that the daily raising and lowering of the leaves continued even when the plant was placed in an interior room and thus was not exposed to sunlight. This finding suggested that the movements represented something more than a simple response to the sun and were controlled by an internal clock.[13]

The modern era of "clock genetics" began in 1971 with the discovery by Ron Konopka and Seymour Benzer of the *period* (*per*) locus in *Drosophila*. At the time, even the existence of single-gene mutations affecting a process as complex as circadian rhythms was met with great skepticism. We have lived

through decades when it was hard to clone a gene— now; we can manipulate genes at will. We have complete genome sequences for most of the important model organisms.[14]

EXPERIMENTS AND EVIDENCES

Scientists learn about circadian rhythms by studying humans or by using organisms with similar biological clock genes, including fruit flies and mice. Researchers doing these experiments can control the subject's environment by altering light and dark periods. Then they look for changes in gene activity or other molecular signals. This research helps us understand how biological clocks work and keep time. Scientists also study organisms with irregular circadian rhythms to identify which genetic components of biological clocks may be broken.[15]

1. Since the 1940s, many circadian rhythms have been described in the digestive system of rodents and man. Rhythms in some gastrointestinal tissues, such as the esophagus, show an extremely high amplitude variation in DNA synthesis2 and cyclin expression. Other rhythms in the gut, such as the cycling of carbohydrase activity, show low amplitude but robustly reproducible variation.[9]

2. Kleitman (1963) was the first to study human circadian rhythms in human subjects shielded from periodic environmental cues. In 1938, he studied two subjects living on non-24-hour sleep/wake, light/dark, and meal schedules while living deep within Kentucky's Mammoth Cave, shielded from the influence of the Earth's 24-hour day. Measurement of the daily rhythm of body temperature in one of the subjects revealed the circadian temperature rhythm to be endogenously generated, persisting for a month with a near-24- hour period despite imposition of a 28-hour rest/activity schedule. That first underground cave study of human circadian rhythms, in the longest known cave on Earth, was far ahead of its time. The fact that a physiological rhythm could oscillate *not only* in the absence of periodic changes in the environment, *but also* at a period different from that of behavioral cyclicity established the endogenous and physiologic nature of human circadian rhythms for the first time.[16]

3. More than two decades later, in a paper on human circadian rhythms that was presented at the first CSHL symposium on circadian rhythmicity, Lobban reported on a series of field studies in which subjects lived on non-24-hour schedules during the continuous light of summer within the Arctic circle. [16]

4. Subsequently, Jürgen Aschoff and Rütger Wever attempted to determine the average period of the human circadian system by conducting a series of month-long studies of human subjects living in underground bunkers in Germany, beginning in 1960 and continuing for more than 25 years. In contrast to the finding from Kleitman's first cave study, they reported that under such conditions, human subjects exhibited rhythms of body temperature, urine volume, and sleep/wake with an average period of about 25 hours. Their studies of humans exposed to a self-selected, periodic light/dark cycle suggested that—unlike that of other mammals—the period of the activity rhythm in humans was highly variable, ranging from 13 to 65 hours (median 25.2 hours) and that the circadian period of the body temperature cycle averaged nearly 25 hours. [16]

5. Similar studies were performed in France which yielded similar reports from The Cave Studies. England, the United States, and other similar laboratory studies of humans shielded from external time cues conducted in Baltimore, Gainesville, Florida, and New York.[16]

6. Benzer's (1973) bold foray into the genetics of brain and behavior in *Drosophila* remains one of the most remarkable achievements in biology—by unraveling complex systems using systematic screens focused on a judicious array of related phenotypes.[14]

7. The initial identification of a biological clock in the retina began with the demonstration, in *Xenopus*, that the activity of the rate-limiting enzyme for melatonin synthesis, aryl alkylamine *N*-acetyltransferase (AA-NAT), was governed by a circadian rhythm. Subsequent work showed that AA-NAT was located in photoreceptor cells and that an isolated layer of photoreceptors was capable of maintaining a circadian rhythm of melatonin production. More recent studies on *Xenopus* retina have identified multiple clock components, all of which are located exclusively in the photoreceptors.[17]

8. Transcriptional assays using cell culture systems have been applied to study the interaction of clock components in vitro. PER1 might be a negative regulator only for the expression of its own gene. Two-hybrid studies in yeast have shown that each of the three PER proteins can interact with itself, with the other two PERs, as well as with each of the two CRY proteins. Coimmunoprecipitation has demonstrated association of PER proteins with each other and with CRY proteins in SCN tissue. Subcellular localization of the clock components as well as the timely synchronized presence of specific components will determine which complexes of clock proteins are functionally relevant in vivo.[8]

FACTORS AFFECTING CIRCADIAN RHYTHM

Natural factors within the body produce circadian rhythms. However, signals from the environment also affect them. The main cue influencing circadian rhythms is daylight. This light can turn on or turn off genes that control the molecular structure of biological clocks. Changing the light-dark cycles can speed up, slow down, or reset biological clocks as well as circadian rhythms.[15]

1. **Circadian phase dependant resetting of light:** The most important functional property of circadian oscillators is phase-dependent resetting; i.e., the magnitude and direction of phase resetting are dependent on the circadian phase at which a synchronizing stimulus occurs. On the

basis of winfree's phase-amplitude resetting model of circadian rhythms in *Drosophila*, Winfree predicted that a critical stimulus of intermediate strength, when administered at a critical circadian phase during the subjective night (the phase at which phase delays transition to phase advances), would drive the circadian oscillatory system to its singularity, characterized by a marked reduction in amplitude such that phase cannot be determined. Winfree demonstrated the property of critical resetting in *Drosophila*, in which the circadian amplitude of the eclosion rhythm approached zero in response to a carefully titrated light pulse.[16]

2. **Dose-dependent Resetting to Light:** Resetting responses to light can be enhanced by increasing the duration or intensity of the stimulus. In humans, it has been demonstrated that phase resetting of the circadian system exhibits a saturating nonlinear dose response curve to different levels of illuminance.[16]

3. **Wavelength Sensitivity of Circadian Responses to Light:** The wavelength sensitivity of a circadian system is dependent on the underlying photoreceptors that provide input to the circadian pacemaker. The human visual image forming system consists of rods and cones that mediate night vision and color vision, respectively. Some blind individuals, with complete loss of conscious visual perception, show intact photic circadian entrainment and melatonin suppression in response to light suggesting that the classical visual photopigments are not required for circadian photoreception.[16]

CHRONODISRUPTORS

Different chronodisruptors have been identified such as shift work, feeding time, long days, and stress. Many adverse effects of disrupted circadian rhythmicity may, in fact, be linked to disturbances in the sleep-wake cycle.[13] Inappropriate timing of food intake and high-fat feeding also lead to disruptions of the temporal coordination of metabolism and physiology and subsequently promote its pathogenesis.[5]

CIRCADIAN CLOCK GENES

Clock genes are components of the circadian clock comparable to the cogwheels of a mechanical watch. They interact with each other in an intricate manner generating oscillations of gene expression.[18] The discovery of circadian rhythm genes rests largely (indeed, almost exclusively) on the use of forward genetic screens to isolate circadian mutants, which in turn were used to identify the causative gene. Some of the important genes are as follows:

Timeless (tim)

The circadian gene Timeless (Tim) was discovered in Drosophila melanogaster and is retained in mammals, but at present its role in mammalian circadian clock function is not clear. TIM and its partner TIMELESS interacting protein (TIPIN) work together with components of the DNA replication system to regulate DNA replication processes under both normal and stress conditions.[6]

Three alleles of TIM have been reported recently (TIM was found to exist a decade ago), TIM a temperature sensitive, long period (26-30 hr) allele, TIM, which was found as a suppressor of PER, Tim, which is known to generate rhythms with periods of around 33hr. Characterization of these TIM mutations showed that the Drosophila Pacemaker can support very long period rhythms that are remarkably stable. It was proved that the PER and TIM are physically associated during the interval of prolonged protein accumulation and innunocytochemical analysis showed that TIM persist in photoreceptor cell nuclei for an extended time. Thus, the TIM mutation prolongs the duration of nuclear localization of the PER/TIM complex and affects light independent degradation of TIM.TIM independent regulatory activity of PER was also suggested by in-vitro cell culture studies. In absence of TIM, PER alone could repress CLOCK/CYCLE mediated transcription of PER in the cultured Drosophila S2 cells. [19]

Cryptochrome (Cry)

The *Cry1* null mutation causes a relatively subtle 1-h shortening of period, while the *Cry2* null mutation causes a 1-h lengthening of period whereas, the *Cry1/Cry2* double knockout has a profound phenotype with a complete loss of circadian rhythms in constant darkness.[14] Mice mutant in *Cry1*/ *Cry2* retain the ability to have *Per* gene expression induced by light, suggesting that the *Cry* genes are dispensable for light-induced phase shifts.[8]

Cryptochrome-1, or Cry1, has major importance in the maintenance of circadian rhythmicity. Cry1 is known to regulate DNA damage repair, cell proliferation, and several biological processes. The role of Cry1 in maintaining normal testis is important for development and function. By contrast, the Cry2 gene is only shown to be involved in the reproduction of diapausing animals through the seasons. [10]

PER Genes

The cellular location and rhythmic expression of *Period 1* (*Per1*) circadian clock gene were examined in the retina of a *Per1*::GFP transgenic mouse.[17] Mutations in the *Per1* and *Per2* genes show that they are required for normal photic resetting of the mammalian clock. The fact that *Per* genes can respond to common signaling pathways, it is very likely that clock-controlled pathways will feed back directly or indirectly to the clock. [8] *Per2* gene causes a 1.5-h period shortening and eventual loss of rhythms in the majority of mice in constant darkness. Knockouts of *Per1* and *Per3* fall into the subtle (0-1 hour period changes) category at best whereas the *Per1/Per2* double-knockout has strong effects, causing complete loss of rhythms in constant darkness.[14]

Per1, Per2, and Per3 are a class of circadian clock genes, which act as transcriptional repressors, forming a core component of the circadian clock. They are the three members of the period circadian protein homolog. Per1 is associated with cell proliferation and apoptosis and is involved in the initiation and progression of several cancers such as head-neck carcinoma, prostatic cancer, colorectal cancer, breast cancer, and endometrial cancer. Per2 is also associated with cellular proliferation and differentiation, playing an important role in the development of breast cancer, milk duct, and maintaining the polarity and morphology of the mammary epithelium.[10] Period (*Per*) genes are involved in the regulation of the circadian clock and are thought to modulate several brain functions.*Per2Brdm1* mutant mice display alterations in the glutamatergic system.[1] It seems that PER2 unifies several characteristics necessary for a function to serve as an integrator. Its promoter seems to be responsive to a plethora of transcriptional activators, the protein can be finely tuned in its stability and localization by posttranscriptional modifications, and it seems to interact with various proteins to mediate specific functions.[20] One important function of morning and evening oscillators is to adapt to seasonal changes in day length. It is possible that PER3-P415A/H417R affects the evening oscillators, resulting in maladaptation of activity rhythms to short photoperiods during the winter months. The sleep reducing effects of mutant PER3 are light dependent. PER3 serves as a node linking the regulatory processes of circadian rhythms with those of mood. PER3 may participate in modulating these processes to adapt to the short photoperiods in winter. [21] The clock gene Per2 may play a role in the regulation of long-term potentiation and in the recall of some forms of learned behaviour.[22]

BHLHE41

BHLHE41 is part of the transcription factor family that is regulated by the mammalian molecular clock. *BHLHE41* influences other molecules such as BMAL/CLOCK. The CLOCK and BMAL1 proteins form a heterodimer and through E-box elements in role in both the clock machinery and sleep homeostatic mechanisms. *BHLHE41* binds to class B E-box elements (CACGTG) as a homodimer and to repress the transcription of target genes in an HDAC-dependent manner. Thus, although the role of clock genes such as *BHLHE41* is well established in controlling the diurnal rhythm of gene expression in many organs, they may also play a fundamental role in determining the duration of sleep that an individual needs. Future research may elucidate possible mechanisms of the gene variants' role in sleep length as well as reveal target molecules that enhance sleep homeostasis and improve neurobehavioral responses to sleep deprivation.[23]

Circadian Locomotor Output Cycles Kaput (CLOCK)

Clock gene expression regulated exclusively by transcriptional processes would run into equilibrium and no oscillation of gene expression would be observed. Transport of clock proteins from the cytoplasm into the Nucleus as well as post transcriptional processes are additional levels of regulation of the clock mechanism for generating oscillations of approximately 24 h.[18]

Clock Mutation results in Loss of Behavioral Rhythms. *Clock* mouse was identified in an ENU mutagenesis screen. Light/Dark cues are sufficient to maintain activity rhythms in *Clock* mice. Free running behavior rhythms are lost D-element binding protein (DBP) is a liver-enriched transcription factor that is expressed with a circadian period. It has been shown that this gene is regulated by CLOCK/BMAL1, indicating that clock genes can regulate rhythmic transcription of clock output regulators. Sleep homeostasis is also altered in *Clock* mutant mice, and it remains to be seen whether other components of the circadian clock can alter sleeping behavior.[8] Animal model studies, particularly in the setting of obesity, diabetes, atherosclerosis, or other metabolism syndrome conditions by Clock genes, have led to an increase in recognition that a multitude of rhythmic functions, such as reproductive tissues in mammals, are controlled by molecular clockwork.[10] Characterization of genotypic variants of CLOCK genes could lead to a better definition of endophenotypes in mood disorders.[26]

Neuromedin gene

It is well-documented that NMS/NMU plays critical roles in the CNS of mammals. Neuromedin U (NMU) and its structurally-related peptide, neuromedin S (NMS), are reported to regulate many physiological processes and their actions are mediated by two NMU receptors (NMUR1, NMUR2) in mammals. However, the information regarding NMU, NMS, and their receptors is limited in birds. *cNMU* and *cNMUR1* are widely expressed in chicken tissues with abundant expression noted in the gastrointestinal tract, as detected by quantitative real-time PCR, whereas *cNMUR2* expression is mainly restricted to the brain and anterior pituitary, and *cNMS* is widely expressed in chicken tissues. Neuromedin U (NMU) is a brain-gut peptide originally isolated from porcine spinal cord, which was named based on its ability to stimulate uterine smooth muscle contraction. Subsequently, NMU peptide was also identified in other vertebrate species, including rats, dogs, humans, frogs, rabbits, guinea pigs, and chickens. In pigs, two forms of NMU peptides, NMU-8 (8 amino acids) and its N-terminally extended form, NMU-25 (25 amino acids), have been identified. Both NMU-8 and NMU-25 display similar biological activities. NMU is reported to regulate many physiological processes in the CNS and peripheral tissues, such as food intake, energy expenditure, body weight, stress response, thermoregulation, nociception, blood pressure, immune response, and smooth muscle contraction. In mammals, the biological actions of NMU and NMS peptides are mediated by two NMU receptors, namely NMUR1 and NMUR2. In mammals, *NMUR1* has been reported to be mainly expressed in the peripheral tissues, while *NMUR2* is mainly expressed in the CNS including the hypothalamus, hippocampus and spinal cord.

In non-mammalian vertebrates, the information regarding the structure, expression and functionality of NMU, NMS and their receptors is limited, although these genes are predicted to exist in some non-mammalian vertebrate species. *NMU* and *NMS*, *NMUR1* exists in all vertebrate species examined. In the study, it was found that *cNMU* consists of 10 exons, similar to that of human *NMU.* In a study, the tissue expression of *NMS, NMU, NMUR1* and *NMUR2* was examined in chickens. It was found that *cNMUR1* is widely expressed in chicken tissues, with a high expression level noted in the GI tract and adrenal gland. In contrast, *cNMUR2* expression is mainly restricted to the CNS with the highest expression level noted in the hypothalamus.[27]

Nonessential Clock Genes

Circadian genes that are involved in the clock mechanism but are not essential for the generation of circadian rhythms are known as Nonessential Clock genes. This second "nonessential category" is exemplified by *Rev-erbα*. This gene is activated by the CLOCK-BMAL1 complex and is in turn regulated by PER/CRY–mediated repression. REV-ERBα then feeds back on *Bmal1* (and *Clock*) to drive cycles of transcription that are out of phase with *Per* and *Cry*. This pathway forms a second feedback loop in the circadian network that has been labeled as a "positive feedback" loop which we now know is the result of 2 inhibitory steps. Null mutations of *Rev-erbα* abolish the feedback effects of this pathway on *Bmal1*, and transcription of *Bmal1* is constitutively elevated. However, circadian rhythm generation persists in the absence of REV-ERBα, and there are only subtle effects on the period and stability of circadian rhythms in the behavior of knockout mice. This specific example illustrates the quandary that exists when we define clock components: REV-ERBα is clearly part of the native circadian oscillator network, yet it is not essential for the generation of circadian oscillations.[14]

CIRCADIAN RHYTHMS IN HEALTH AND DISEASE

The relationship between the circadian clock and health can only be understood by unravelling entrainment characteristics and mechanisms at all levels of the circadian network, i.e., not only how the SCN synchronises to the environment but also how all tissues and organs perform as part of an orchestrated, daily programme.[7]

Circadian rhythms can influence sleep-wake cycles, hormone release, eating habits and digestion, body temperature, and other important bodily functions. Biological clocks that run fast or slow can result in disrupted or abnormal circadian rhythms. Irregular rhythms have been linked to various chronic health conditions, such as sleep disorders, obesity, diabetes, depression, bipolar disorder, and seasonal affective disorder.[15]

Circadian Rhythms and Reproduction

Several circadian clock genes such as Clock, Bmal1, Per2, and Cry1 are expressed in human and animal full-term placenta tissue, suggesting a potential circadian rhythm. As demonstrated in prior research, the relationships between the placenta and fetal circadian signals are complex and are essential for the development of a successful pregnancy.[10] The reproductive control of various species is connected to circadian rhythm via light and the length of days. Many aspects of reproductive biology are regulated by the circadian rhythm. This includes the estrus cycle, levels of luteinizing hormone (LH), ovulation, production and maturation of sperm, fertilization, insemination, and embryo implantation. Disruption of a single clock gene, in this case, specifically Bmal1, is enough to disrupt the reproductive cycle. Studies have shown that there is an increased risk to pregnancy in female night-shiftworkers. Exposure to artificial light at night has caused great concern as it leads to chronodisruption which harms the human biological clock, and causes possible negative effects in human pregnancy.

Studies investigating flight attendants demonstrated that miscarriages have an association with their sleep patterns during work, suggesting, disruptions of circadian rhythm may actuate a miscarriage. They found that implantation of the embryo in the uterus is negatively impacted by circadian disruption; only impairing the uterus does not explain the negative pregnancy outcome during the maternal disruption of the circadian cycle. Circadian clock genes could be harnessed and redirected; they could be used as a potential cure for female infertility. In addition, repeated shifting of the light/day cycle can reduce the number of pregnancies and implantation sites in animal models. Many of the body's processes and physiology function follow a natural circadian rhythm on a 24-hour day-night cycle. However, a female's fertility can be affected by the amount of light in modern society, shift work, or crossing time zones. There is expression of Per2 associated with circadian rhythm at embryonic day 16 (after fertilization), which is approximately equivalent to the end of the first trimester in humans.

The development of oscillations in vivo may be due to the tissue's autonomous programming, which could occur in isolation without signals from elsewhere in the embryo or from the mother. It is well known that the relationships between maternal and fetal circadian signals are complex and essential for a successful pregnancy. Circadian rhythms implement necessary functions for proper reproduction, sparking interest in evaluating whether there is a link between Clock genetic variants, and the impact of idiopathic recurrent spontaneous abortion (IRSA). Clock plays a role in the male reproductive system, and can be related to further studies that seek validation of the relationship between the role of circadian clock genes and spermatogenesis.[10] Men have biological clocks that affect their hormone levels, fertility, and the genetic quality of their sperm. The effects of andropause and advanced paternal age on fertility and offspring are still under investigation and further research is needed to fully characterize the associated risks and to treat the underlying abnormalities.[28]

Hormone Regulation

Melatonin

Circadian rhythm and melatonin affect fetal and maternal health and human reproductive success. The use of shift workers and electric lighting has disrupted this cycle and disturbed the optimal levels of melatonin in the blood as well. The role of melatonin is seen not only in the labor and delivery mechanisms but also in ovulation and early pregnancy. The mother maintains a normal, light/dark, and sleep/wake cycle, it helps to stabilize and maintain the fetus's circadian clock. Melatonin has many effects on the body from inducing a state of sleep to regulating circadian rhythms. Since the embryo cannot produce its own melatonin until after birth, it is reliant on melatonin from the mother.[10]

Estrogen

Estrogen, as an ovarian hormone, is associated with suppressed food intake and produces important anti-obesity and antidiabetic effects in female animals. Estrogen has been shown to differentially regulate the expression of Per1 and Per2 genes between central and peripheral clocks and between reproductive and nonreproductive tissues in female animals.[10]

Cortisol

Cortisol also plays an important role in reproduction. Cortisol is a steroid hormone, our body's main stress hormone. The studies suggest that extensive maternal use of cocaine during pregnancy may constitute constant stress, which results in increased maternal and fetal cortisol secretion and prolonged exposure to elevated cortisol levels. Thus, further research regarding these factors is warranted to know more about the involvement of circadian regulation performed by cortisol in the reproductive process.[10]

Sleep Disorders

In humans, some pathological conditions are characterized by the misalignment of the sleep episode relatively to the light-dark cycle. Studies of the biology of clock genes in circadian rhythm disorders (mainly delayed sleep phase disorder and advance sleep phase disorder, DSPD, ASPD) have contributed to understanding their etiology as well as to underline their complexity. Additionally, other sleep disorders have been reported to involve changes in clock genes expression. Various studies have revealed the genes responsible for various Sleep Disorders viz. *CLOCK* gene, the T allele of this T3111C polymorphism, *PER1* gene, *PER2* gene etc. The involvement of PER2 polymorphisms in circadian disorders seems to involve changes in *PER2* protein phosphorylation levels. Shift-work sleep disorder (SWSD) has been classified as a circadian and sleep disorder (AASM, 2005) and is most likely the result of misalignment between clocks of different tissues, as well as between circadian clocks and the light/dark cycle.

Another example of sleep pathology changing the normal sleep/wake pattern is sleep apnea, where hypoxic episode and awakenings during sleep generate very shallow sleep and drastic been shown to be affected in sleep apnea patients.[30]

Clock genes and other psychiatric Disorders

Circadian disorders with sleep symptoms are commonly seen in patients with neurodegenerative diseases. For instance, Parkinson's disease (PD) patients are disrupted for the cortisol and melatonin rhythms. Alzheimer's disease (AD) patients are long recognized for SCN neuronal loss, of that VIP neuron is a prominent case Patients with Huntington's disease (HD) have sleep symptoms including advanced sleep phase, insomnia and reduced REM sleep.[29] Links have been reported between the molecular components of the circadian system and psychiatric disorders such as schizophrenia, Alzheimer's disease, major depressive disorder, bipolar disorder, anxiety disorder, drug addiction and alcoholism. Bipolar disorder has been associated with reduced circadian amplitude in clock genes expression (e.g. *BMAL1, REV-ERBα*, *DBP*) and reduced phosphorylated levels of GSK3β in fibroblasts from patients. Also, a possible mechanism for normal circadian changes in mood as well as for the link between clock genes and mood disorders was revealed in recent work showing that the regulation of monoamine oxidase A (a key enzyme in dopamine metabolism) was under the control of the clock proteins BMAL1, NPAS2 and PER2. Repeated administration of cocaine alters the expression of many clock genes in rat and mouse brains. For example, a single or a repeated exposure to cocaine differentially increases the expression of different clock genes (*Per1*, *Per2*, *Per3*, *Clock*) in the hippocampus and the striatum. Exposure to drug of abuse appears to entrain circadian oscillators in the body independently of the signals from the master SCN clock.[30]

Sustaining efficient circadian activities are likely key to prevent age-related disorders, including neurodegenerative diseases.[29] Since CLOCK genes play a central role in the generation and control of circadian rhythms; it has been hypothesized that mutations in CLOCK genes could lead to disturbances in molecular feedback loops which on a behavioral level could appear as mood disorders.[26]

Clocks genes and metabolism

Considerable evidence points towards the involvement of molecular clock components in the regulation of metabolism, as well as the responsiveness of the clock to metabolic challenges. Increased metabolic dysfunctions are associated with circadian disorganization, for example in shiftworkers or in animals with a deficient circadian clock. Indeed, lipid metabolism seems to be impaired when eating is redistributed during night shifts, as higher LDL cholesterol and higher triacylglycerol have been reported with night food intake in humans. In the liver, the effect of the circadian molecular machinery on lipid metabolism was shown to result from the circadian regulation of key lipid-metabolism players such as low-density lipoprotein receptor and polypeptide-1.[30]

REV-ERBα is an essential regulator of metabolic reactions as it is involved in lipid metabolism, adipogenesis and energy balance. Data showed that the expression of PPARα is regulated by CLOCK-BMAL1 and that this regulation is directly involved in lipid metabolism. Additional support to the involvement of nuclear receptors in bridging circadian rhythms and metabolism come from studies in which the disruption of the function of coactivators – e.g. PGC-1α or PGC- 1β – or corepressors – e.g. NCoR/HDAC3 – of nuclear receptors was shown to alter behavioural circadian rhythms and metabolic

processes.[30] The REV-ERB/ROR proteins are well-known for their role in metabolism and also participate in the core loop, highlighting the intimate connection between circadian rhythm and metabolic transcription.[5]

Lipid metabolism is linked to circadian clock in the cases of REV-ERB and ROR families. They are important for regulating lipogenesis, lipid storage and adipocyte differentiation in a rhythmic manner.[29] In humans, variations of the *PER2* gene are associated with the regulation of alcohol consumption. Collectively, these data establish glutamate as a link between the dysfunction of the circadian clock gene *Per2* and enhanced alcohol consumption. an association between alcoholic patients and genetic variations in the human *PER2* gene was found. These findings support the view that a hyperglutamatergic state can be involved in several aspects of alcohol dependence.[1]

Clocks genes and cardiac function

Circadian oscillation is evident in cardiovascular functions such as heart rate and blood pressure. Furthermore, circadian oscillation is also associated with the frequency of onset of cardiovascular diseases.[31]

Clock components have been linked to the proper functioning of the cardiovascular system either directly or via their effects on metabolism. Initial evidence were provided by the observations of an increased risk of cardiovascular events (e.g. stroke, sudden cardiac death, myocardial infarction, ventricular arrhythmias) at specific clock times, notably between

6 AM and noon. Moreover, individuals submitted to circadian misalignment are more at risk of developing cardiovascular problems. Accordingly, shift work has recently been observed to increase the probability of developing atherosclerosis and myocardial infarction at a younger age and to exacerbate the negative effects of high blood pressure on coronary heart diseases. On the whole, these data show that appropriate circadian alignment is necessary to maximize cardiovascular health.[30] There is also evidence to indicate that functional peripheral circadian oscillators are important in the tissue function such as in the cardiovascular system.[32] The link between circadian clock and cardiac health seems to be multifactorial. Among the factors, many cardiovascular components and mediators have been found to be clock-regulated. Notably, the circadian rhythm in platelet activity has been reported to depend on the core-clock component CLOCK, whereas the expression of plasminogen activator inhibitor-1, a major contributor to fibrinolysis, also seems to be clockregulated Moreover, the cardiac autonomic response, more precisely the sympathovagal balance during sleep, depends on the variable number tandem repeats polymorphism in the *PER3* gene. Therefore, the molecular clock appears highly relevant to cardiovascular physiology.[30]

Clocks genes and cancer

One of the most striking associations between circadian rhythms and health relates to cancer. Epidemiological studies have showed an increased incidence of cancer in shift-workers or individuals chronically submitted to jet lag. Moreover, increased light levels at night have been observed to co-distribute with breast cancer in women. The expression of clock genes in cancer patients undergoing surgery have been reported to be changed. Some tumor show altered *PER* gene expression likely due to changes in promoter sequence. The association between clock genes and cancer resides not only in the fact that a good circadian synchronization and intact circadian machinery are important for cancer prevention but also in the observation that the occurrence of cancer generates a circadian dysfunction, which itself can exacerbate cancer state.

Accordingly, circadian rhythms disruption was shown to increase tumor growth. The cycle of cell division of normal and cancer cells has been reported to be under circadian control as clock controlled genes are involved in the cell cycle and in cell proliferation. CKIε and PER2 appear to be particularly important for cell cycle and cell survival in tumor cells. Also, mutation of *Clock* decrease cell growth and proliferation in fibroblasts, while the proliferation of hepatocytes from *Bmal1* KO mice is slowed down compared to wild-type counterparts. Among clock components, *Per* genes appear central for their involvement in cancer mechanisms.

A genetic variation in the *PER3* gene was proposed as a marker of breast cancer in young women. *PER* genes have been reported to act as tumor suppressors, given that *Per1* and *Per2* are potential modulator of DNA damage. Recent studies showed that on one side *Per1* is involved in the suppression of cancer cells proliferation via TNF-α while on the other hand that *Per2* is crucial to prevent tumorigenesis. Additionally, mediators of the immune response have been shown to influence clock gene expression. Notably, inteleukin-6 was shown to induce *PER1* expression in human hepatoma cells, and tumor necrosis factor-α suppresses the expression of several clock genes in the liver and alters locomotor activity rhythms.[30] Loss of PER2, a core circadian component, is linked to cancer predisposing. The animals are sensitive to γ irradiation later developed salivary gland hyperplasia, teratoma and malignant lymphomas. A recent finding of targeting BMAL1 and CLOCK for acute myeloid leukemia (AML) therapy indicates further the pro-cancerous option of clock components.[29]

There exists evidence to suggest that the molecular clock may significantly affect tissue function and interact with the regulation of the cell cycle and tumorigenesis. Cancerous cells may also demonstrate abnormalities in circadian clock genes.[32]

Clock genes and ageing

Aging is a major risk factor for many human pathologies, including cancer, diabetes, cardiovascular disorders and neurodegenerative diseases.[29] The molecular machinery of circadian clocks is also relevant to ageing and particularly to the age-related decrement in circadian amplitude, memory and cognitive function. It is well known that aged humans experience reduced amplitude in circadian rhythms of activity, core body temperature, melatonin

secretion and EEG circadian hallmarks. Moreover, recent literature supports the involvement of the central circadian clock in age-dependent changes in neuronal function. For example, data suggest that distinct neuronal populations of the SCN are implicated in different functional modifications in human dementia, as activity and temperature rhythms In rats, the temporal relationship between the main circadian clock and peripheral oscillators appears modified with age, and resetting of internal circadian clocks following phase shifts are modified in aged rats such that the synchrony between clocks is reduced whereas in primates, age was shown to affect clock gene expression in pineal gland. Mice mutant for the core clock component BMAL1 exhibit many signs of premature ageing in combination with a reduced lifespan. The relationship between clock genes and neuronal or cellular health throughout ageing could be mediated by the control of the length of chromosome extremities (telomere), which could virtually indicate a role for circadian regulation of ageing in every cells of the organism, as suggested by a recent study. Overall, these results suggest that a proper functioning of the circadian system and its underlying molecular components is protective against age-related degeneration.[30]

Prior studies have indicated that the age-related decrease of melatonin is a consequence of functional changes linked to this system, which controls the sleep/wake cycle.[10] with seminal successes in biomedical researches; the improved medical conditions markedly lengthened human lifespan however also led to emerging threats as known the age-associated complexities. The wide range of age-associated diseases, including neurodegenerative diseases, cardiovascular disorder, type-2 diabetes, and higher cancer incidences, are driven by the causes of time, genetic and environmental situations that remain difficult to dissect for major effector(s) in individuals. Declined circadian rhythmicity in endocrine rhythm, phase alignment and sleep are commonly seen with aging. Mice deficient for Bmal1 are suffered from a series of conditions related to aging. e.g., sarcopenia (with both reduction in muscle fiber size and quantity), cataracts, cornea inflammation, osteoporosis, premature hair loss, and failed to form adequate visceral and subcutaneous adipose storage.[29]

Circadian rhythms and arthritis

Expressions of the clock genes, Bmal1 and Dbp, are disturbed in spleen cells by arthritis induction. When arthritis is induced, Cry12/2Cry22/2 mice develop maximal exacerbation of joint swelling, and upregulation of essential mediators of arthritis, including TNF-a, IL-1b and IL-6, and matrix metalloproteinase-3. The biological clock and arthritis influence each other, and this interplay can influence human health and disease. Important feature of RA would be a circadian manifestation of the disease such as morning stiffness, which is included in the diagnostic criteria of RA. Such circadian characteristic is also seen in the proinflammatory cytokines and disease-specific markers important in RA: IL-1b, IL-6, and TNF-α are all elevated in sera of rheumatoid patients reaching the peak levels in early morning, and secretion of the IgA and IgM types of the rheumatoid factor also exhibits a rhythmic pattern with a peak at morning core clock machinery regulating circadian time exists in virtually all cells of the body, and controls thousands of clock-controlled genes. The circadian rhythm plays an important role in the pathogenesis of arthritis: especially, a novel regulatory cross talk between circadian clock gene *Cry* and proinflammatory cytokine TNF-α is demonstrated.[33] The symptoms of rheumatoid arthritis are worst when awaking from night time, while those of osteoarthritis are worst later in the day. The morbid and mortal events of myocardial infarction are greatest during the initial hours of daytime.[34]

Circadian Rhythm and Obesity

Obesity is a multifactorial disease caused by the interaction of genetic and environmental factors related to lifestyle aspects. It has been shown that reduced sleep is associated with increased body mass index (BMI). Negative consequences of obesity have been linked to a variety of chronic diseases, including type 2 diabetes, hypertension, cardiovascular pathologies, and different forms of cancer Sleep disturbances can alter brain functions involved in the control of appetite, which can generate overeating in the current obesogenic environment. Studies suggested that more hours of sleep might act as a protective factor against the genetic predisposition to develop obesity from the CLOCK polymorphism.[35]

Significance of Circadian Rhythms:

- Migration, hibernation, fattening, and fur growth are all adaptations to winter, while the annual rut of large animals and the summer population explosion of smaller ones are all cued, months in advance, by the change in day length.[12]
- The nocturnal peak of melatonin secretion by the pineal gland, which is tightly controlled by the suprachiasmatic nuclei, provides an internal endocrine calendar.[12]
- A veritable circadian expression of functional clock components may be observed in human peripheral blood mononuclear cells (PBMCs) and, therefore, plays certain roles in controlling the immune circadian physiology.[48]
- Circadian clock is thought to be advantageous in synchronising physiological and biochemical pathways, allowing the organism to anticipate daily changes, thus ensuring better adaptation to the environment.[1]
- A better understanding of the cellular and biochemical mechanisms of "gonadal" aging is highly important in order to determine safe, effective ways to delay this process and, in effect, "rewind" the Reproductive biological clock.[28]
- The vast majority of parasites are transmitted by vectors such as birds and arboreal insects, which have their own circadian rhythms, thus creating a tripartite circadian interaction: Vector, parasite, and host. Hence, Circadian Rhythm plays a role in the Transmission of Parasites and Fungi. [3]

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