

Review Article

The Circadian Timing System: A Recent Addition in the Physiological Mechanisms Underlying Pathological and Aging Processes

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ABSTRACT: Experimental findings and clinical observations have strengthened the association between physio-pathologic aspects of several diseases, as well as aging process, with the occurrence and control of circadian rhythms. The circadian system is composed by a principal pacemaker in the suprachiasmatic nucleus (SCN) which is in coordination with a number of peripheral circadian oscillators. Many pathological entities such as metabolic syndrome, cancer and cardiovascular events are strongly connected with a disruptive condition of the circadian cycle. Inadequate circadian physiology can be elicited by genetic defects (mutations in clock genes or circadian control genes) or physiological deficiencies (desynchronization between SCN and peripheral oscillators). In this review, we focus on the most recent experimental findings regarding molecular defects in the molecular circadian clock and the altered coordination in the circadian system that are related with clinical conditions such as metabolic diseases, cancer predisposition and physiological deficiencies associated to jet-lag and shiftwork schedules. Implications in the aging process will be also reviewed.

Key words: molecular clock, circadian physiology, peripheral oscillator, uncoupling, jet-lag, metabolic diseases

An appropriate physiological performance involves the integration of metabolic networks that can measure and adapt to the progression of time. The functional unit formed in this way constitutes a timing system with the ability to anticipate environmental fluctuations, allowing physiological activities to adjust in a timely manner to endogenous and ecological demands. Biological rhythmicity is pervasive in all types of cells and organisms. Depending on the period of oscillation, the rhythms are classified as ultradian (less than 24 h, e.g., ionic fluxes in membranes), infradian (more than 24 h, e.g., tidal and annual events), and circadian (approximately 24 h) [1]. The best characterized rhythms and the ones that will be discussed in this review are the circadian rhythms.

Circadian rhythms are endogenous fluctuations that are commanded by a pacemaker entity which is synchronized by environmental cues to allow daily fluctuations in biochemical, physiological, and behavioral

activities. Although circadian rhythmicity is present in all phyla, the different molecular mechanisms are used to measure time in blue-green cyanobacteria, sordariomycetes such as *Neurospora crassa*, invertebrates such as *Drosophila melanogaster*, and mammals [2]. In particular, the timing system in mammals is formed by a set of coordinated peripheral oscillators under the control of one principal pacemaker located in the ventral hypothalamus and known as the suprachiasmatic nucleus (SCN). The SCN is formed by approximately 15,000 neurons and a high proportion of glial cells. It is entrained by photic stimuli of the daily light-dark cycle. However, other synchronizers different from light (non-photoc) can also entrain the circadian timing system. Among these synchronizers are the feeding time, access to exercise devices, and social interactions.

The underlying cellular mechanism used by the timing system to estimate the intervals of ~24 h is called the molecular clock. In each cell with the ability to

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compute the passing of time, this complex is formed by a set of interconnected loops of transcriptional and translational activities, and will be reviewed in more detail in a subsequent section of this review.

Harmonious communication between the SCN and the rest of the peripheral oscillators is necessary to assure healthy physiology. This interconnection is accomplished by bidirectional signals of a neural and humoral nature, and it underlies what is called a “coupled state” [3]. Indeed, as discussed below, uncoupling between the principal and the secondary circadian oscillators is frequently the source of pathologies that range from the jet-lag symptoms associated with trans-meridian trips to the biochemical alterations accompanying obesity, diabetes, and the metabolic syndrome.

It is well documented that circadian rhythms become functional during ontogeny, and clock-genes reach diurnal rhythmicity during different post-embryonic developmental periods [4]. At the same time, circadian physiology becomes disturbed during aging. For example, it has been reported that old organisms show changes in their circadian rhythms such as reduction in amplitude, earlier presentation of the phase, shortening of the natural free-running period (*tau*), and an impaired ability to adapt to abrupt phase shifts (jet travel or night work). This topic will be also treated in this review.

This review highlights advances in understanding the molecular and physiological coupling between circadian and metabolic networks, and its relevance to pathological processes and aging.

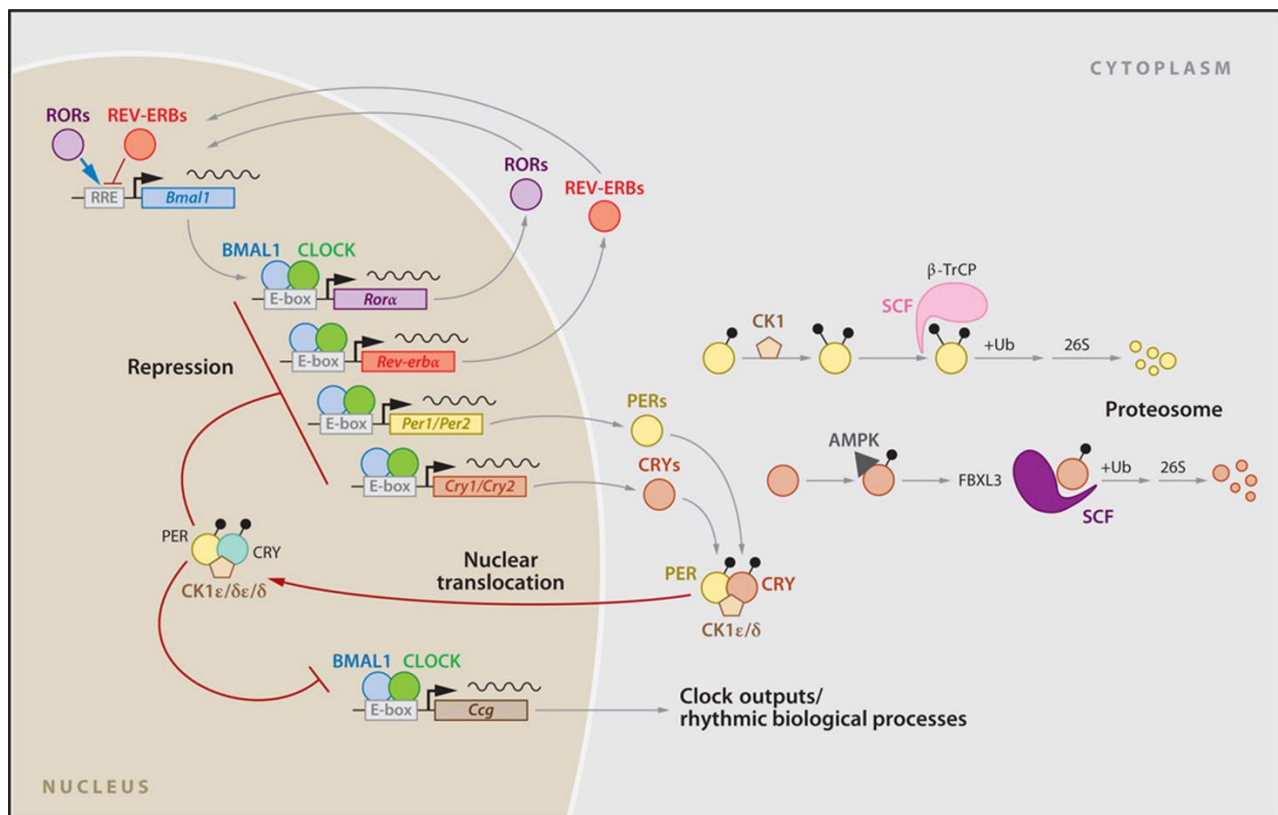


Figure 1. Molecular mechanism of the circadian clock in mammals. Different feedback loops induces both the transcription and the repression of the clock genes. Positive feedback loop is integrated by CLOCK and BMAL1, while the negative feedback loop is integrated by PER1/PER2 and CRY1/CRY2. Regulation of the clock proteins is given by post-translational mechanisms as phosphorylation by the kinases casein kinase 1 ϵ/δ (CK1 ϵ/δ), Adenosine Monophosphate-Activated Protein Kinase (AMPK) and by SCF (Skp1-Cullin-F-box protein) E3 ubiquitin ligase complexes involving β -TrCP and FBXL3. Ub (ubiquitination), 26S (26S proteasome complex), RRE (retinoic response element), RORs (retinoic orphan receptors), Ccg (clock controlled genes). Taken from [8].

Molecular clock

Function

Biological rhythms represent a basic feature of life, occurring as periodic oscillations at different levels of organization from genes to systems. As a consequence, most physiological and biochemical functions are periodic. This periodicity is given by the rotational movement of the Earth approximately every 24 h, resulting in the day/night cycle. For that reason, these rhythms are denominated circadian (from the Latin *circa*, meaning "around" or "approximately", and *diem* or *dies*, meaning "day"). Circadian physiology establishes an internal temporal order to optimize biological functions during specific phases of the day. For example, in diurnal animals the day is considered the activity phase and the animal's physiology is mainly catabolic to obtain energy, while at night it is largely anabolic. In mammals, the

circadian timing is established by a clock located in the hypothalamic SCN which, by means of humoral and neural signals, synchronizes the rhythmicity of the subordinated oscillators throughout the organism. These peripheral oscillators regulate local physiological and behavioral rhythms, which are reflected – for example - in defined times of hormone secretion during the day. A set of specialized genes, known as clock genes, is the substrate of the molecular mechanisms of the circadian clock [5] (Figure 1). Clock genes exert their function across a network of interacting transcriptional-translational feedback loops that are phylogenetically conserved from microorganisms to vertebrates [6]. Therefore, the timing system seems to be constituted by a hierarchical, multi-oscillatory entity that confers precise phase control and stability on the daily activities of each cell, tissue, and organ [7].

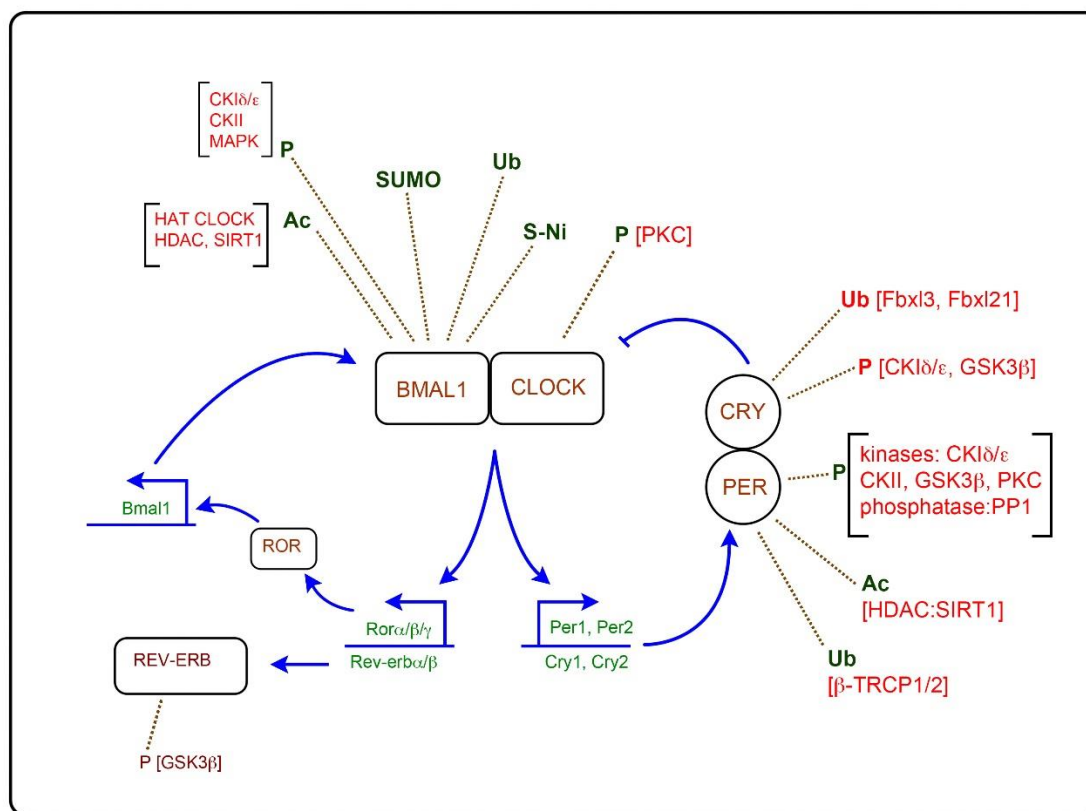


Figure 2. Post-translational regulation of clock proteins. Fine-tuning regulation of clock proteins is involved by a variety of post-translational modifications as phosphorylation (P), ubiquitination (Ub), acetylation (Ac), SUMOylation (SUMO) and S-nitrosylation (S-Ni). GSK3β (glycogen synthase kinase 3 β), β-TRCP1/2 (β-transducin repeat containing protein 1/2), HDAC (histone deacetylases), HAT (histone acetyltransferases), SIRT1 (sirtuin 1), CKIδ/ε (casein kinase 1δ/ε), CKII (casein kinase II), PKC (protein kinase C), PP1 (protein phosphatase I), MAPK (mitogen activated protein kinase), Fbxl (F-box and leucine-rich repeat protein). Taken from [11].

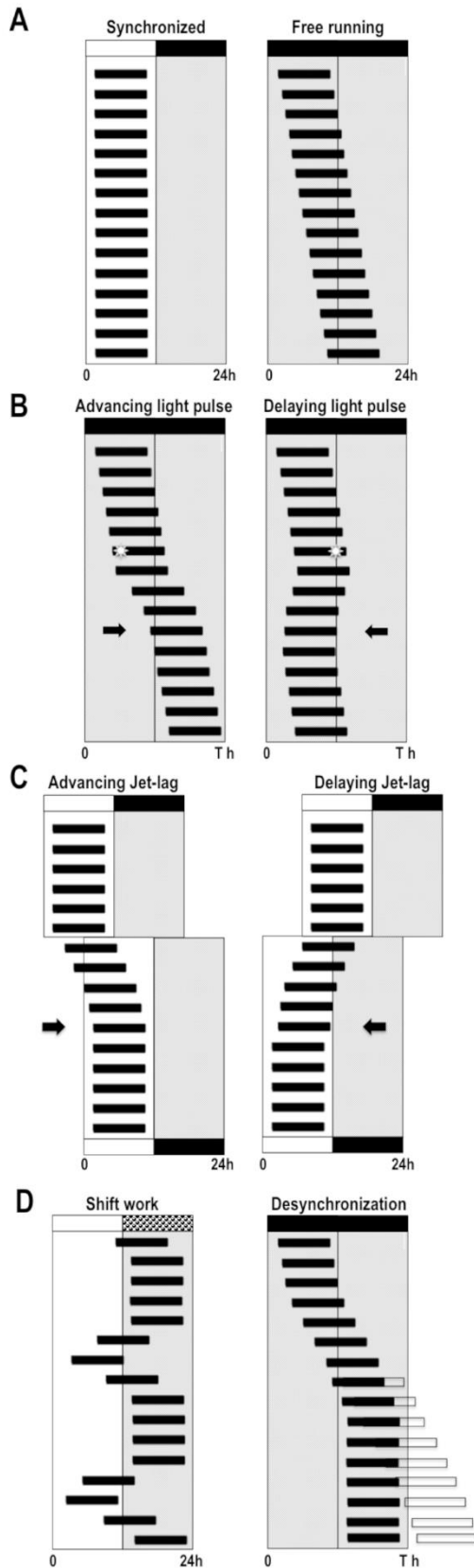


Figure 3. Schematic actograms of a behavioral rhythm in a diurnal organism under different lighting conditions, and their effects in the expression of the locomotor activity rhythm. The black blocks represent the bouts of locomotor activity; the clear and the shaded areas represent the light or dark conditions, respectively. Actograms representing a synchronized rhythm (A, left panel) under a 12 h light and 12 h dark (L:D) cycle and under constant dark condition (A, right panel), situation in which the endogenous circadian period is evident. The administration of light pulses at early and late subjective night, produce phase delays and advances of the activity rhythm, respectively (B). Typically, the phase advances require several cycles of transients before reaching the new steady state. Abrupt changes in the phase of the L:D cycle or social timing cues, produce a transitory desynchronization of the activity rhythm, as occurs in the jet lag or the shift work, requiring several cycles to acquire the new steady state (C and D). Under particular conditions, as continuous advancing jet-lag situation, produce a disassociation of the behavioral rhythm in two components one entrained to the L:D cycle and the second in relative coordination (D, right panel).

Components

The core of the molecular clock consists of a set of “clock genes” that are grouped in 3 different feedback loops (Figure 1): 1) a positive feedback loop composed of the constitutive gene *clock* and the gene *baml1* whose translational products heterodimerize in the cell nucleus and can then recognize specialized promoter regions known as the E-box of other clock genes such as *per1*, *per2*, *cry1*, *cry2*, *rev-erba*, and *rora*, facilitating their transcription (most of the genes under circadian regulation contain promoters with E-boxes); 2) a negative feedback loop formed by *per1/per2* and *cry1/cry2*, whose corresponding proteins form an heterodimer upon a phosphorylation by casein kinase 1 epsilon/delta (CK1 ϵ/δ). The heterodimer enters the nucleus to inhibit its own transcription, as well as transcription of the other clock genes; 3) an accessory feedback loop of proteins encoded by *rev-erba* and *rora*, which enter nucleus where they act on the retinoic response elements (RRE), either as a transcriptional inhibitor (REV-ERB α) or activator (ROR α) of BMAL1 transcription [8].

Expression of clock proteins from the positive loop is in antiphase in relation to that of proteins from the negative loop. All of them are subject to fine regulatory processes, individually and as a group. In the end, a coordinated, harmonic function emerges to ensure the correct function of the circadian physiology.

Regulation

Clock proteins are transcription factors with a limited half-life that allows their daily oscillation. Their syntheses is the result of transcriptional and translational activities, whereas their degradation is performed by the proteasomal machinery. Besides the temporal aspect, clock proteins also have distinct spatial distributions and their transit into and exit from the nuclear compartment are important features.

Clock proteins are regulated at different levels to orchestrate the processes of dimerization, nuclear import/export, activity, covalent modifications, and proteolytic degradation. Two sets of modulatory processes are particularly important for the proper function of the clock proteins: post-transcriptional modifications (PTMs) and epigenetic events.

PTMs confer complexity and fine-tune the metabolic control of the molecular circadian clock, allowing precise regulation of the whole physiological processes. In this context, the subcellular localization and stability of clock proteins depend on PTMs, primarily phosphorylation, acetylation, ubiquitination, sumoylation and s-nitrosylation (Figure 2). For example, depending on the extent of phosphorylation by CK1 ϵ/δ , PER proteins can

be ubiquitinated and degraded by the proteasome [9; 10], or they can be subjected to subcellular relocation [9] between the nuclear and the cytoplasmic compartments. Another kinase involved in the clock machinery is the glycogen synthase kinase-3 (GSK-3), which can phosphorylate PERs and CRYs [11], promoting their entry into the nucleus, and their retention in that organelle.

Epigenetic events related to the control of the molecular clock are diverse. Ten years ago, it was shown that chromatin remodeling is crucial in regulating the expression of the major clock components, as well as in regulating the clock-controlled genes (ccgs) [12]. Genes encoding circadian clock proteins are regulated by epigenetic mechanisms, such as histone phosphorylation, acetylation, and methylation, which have been shown to follow circadian rhythmicity [13]. For example, light pulses are able to modify chromatin remodeling by promoting a rapid phosphorylation on serine 10 of histone 3 (H3-S10) in the SCN [14], and subsequent observations indicated that histone modifications at ccg promoters occur in a circadian manner [15]. Interestingly, it was reported that CLOCK has an intrinsic histone acetyltransferase activity (HAT), reinforcing the idea of a link between the molecular circadian clock and epigenetic control [16]. CLOCK can acetylate non-histone proteins as well as its partner BMAL1 and the glucocorticoid receptor [17, 18], supporting the notion that CLOCK is involved in establishing functional connections to a variety of metabolic pathways that impact the cell cycle and metabolic pathways [19].

Desynchronization of the circadian system

A remarkable feature of circadian oscillators is their ability to be entrained to cyclic environmental cues (also called *zeitgebers* or *synchronizing cues*), enabling organisms to anticipate and adaptively respond to daily fluctuations in a particular environment. Synchronization is the adjustment of the endogenous period τ of the circadian system so that it equals the period T of the zeitgeber, reaching a stable phase relationship between them [20].

The timing system is composed of a network of endogenous circadian oscillators in many peripheral tissues that are coordinated by the master clock located in the SCN. At the same time, these oscillators are synchronized with the environment, keeping most biological rhythms internally coupled and synchronized to external periodic cues, in such a way that internal variables occur in an appropriate time sequence and according to the time of day.

The circadian system is particularly sensitive to the environmental light-dark (LD) cycle; synchronization is achieved by the chronic effect of the LD cycle on the

endogenous period, and the acute effects or phase-resetting property of light. It is well known that the magnitude and direction of light-elicited phase shifts depend on the timing of the stimulus (Figure 3); this has been characterized in the phase response curve (PRC). In mammals, the administration of brief light pulses during early night induces phase delays, while light induces phase advances during late night but only small effect during subjective day [21]. In diurnal and nocturnal rodents, the PCR to light exhibits close similarities at both behavioral and molecular levels, indicating that the mechanisms of photic entrainment are phylogenetically highly conserved [22].

The retina perceives photic stimuli by a subset of retinal ganglion cells (RGC) that contain the photopigment melanopsin [23]. The neuronal projections of RGC impinge directly onto the SCN, by the retino-hypothalamic tract (RHT). Exposure to light pulses during the night induces the release of glutamate and pituitary adenylate cyclase-activating polypeptide (PACAP) from the RHT terminals, activation of N-methyl-D-aspartate receptors (NMDAR), and calcium influx within the SCN cells that can modify the expression of components of the molecular clock [24, 25]. It has been demonstrated that the phase-shifting effects of light on the locomotor-activity rhythm are restricted to the night time, producing a fast-resetting in the ventral SCN and a slow-resetting in the dorsal SCN [26], accompanied by a differential induction of the *per1* and *per2* genes [review in 27].

In particular situations, the function of the circadian system becomes dissociated from astronomical time; this is known as desynchronization. Altered circadian organization has been implicated as a cause of various health problems, such as cardiovascular and gastrointestinal diseases, metabolic disorders, cognitive deficits, sleep disorders, reduced fertility, alterations in immune response, and increased cancer risk [review in 28]. Desynchronization may result from both external and internal factors that disrupt the phase relationship between the endogenous timing system and external cues.

External factors include shift work and jet lag, where the phase relationship between the zeitgebers and the circadian system is disrupted, and a certain number of transitory cycles are required to establish a new, stable phase relationship with the synchronizing cue (Figure 3). Shift work is a chronic situation in which subjects are exposed repeatedly to conflicting synchronizing cues (e.g., light, physical activity, social interaction, among others); usually shift workers are undergoing a desynchronization or resynchronization of the circadian timing system and the LD cycle, since they are subjected to repeated changes in the time of day when they are awake or asleep. Jet lag is produced by a single abrupt

shift of environmental timing cues, as occurs in intercontinental travels, forcing the circadian system to resynchronize to a different LD schedule [28-30]. In both cases, the resynchronization takes place gradually over several cycles, due to the transitory desynchronization of SCN neuronal populations [31, 32] and differential rates of re-entrainment among the peripheral oscillators [33-35]. Recent studies suggest that glucocorticoids participate as a key hormonal pathway involved in the rate of resynchronization [35].

Internal factors that have been associated with desynchronization are those that produce the inability to process the photic information, such as blindness, genetic sleep disorders, and aging. In the advanced sleep-phase syndrome (ASPS), the times of sleep onset and waking occur abnormally early; this syndrome is more prevalent in the elderly, and has been associated with a mutation in two core components of the molecular clock, *per2* and *casein kinase 1 δ* . The delayed sleep-phase syndrome (DSPS) is characterized by abnormally late sleep onset and wake times, and this condition is associated with a mutation in *per3* [30, 36].

Elderly subjects commonly exhibit significant changes in circadian organization which are evident at various levels: the overt rhythmicity of different variables becomes imprecise or fragmented, and there are alterations in the function of the central pacemaker, particularly the network properties of the SCN and peripheral oscillators [37, 38]. In addition, the phase resetting of the circadian system by light is affected by aging due to impaired light perception and defects in the canonical clockwork of the SCN.

The sensitivity of the circadian system to light in old rodents and primates is markedly reduced; this occurs within the SCN itself, since no changes were observed in the RHT projections [39-41]. Several studies demonstrated that aging produces an impairment of the photic re-synchronization at different levels; at the behavioral level, old animals required a greater number of transitory cycles than younger mice [42]. At the SCN level, old animals show reduced phase responses of key proteins to light, including the expression of c-Fos, an indirect marker of neuronal activity [39-41], and of some core components of the molecular clock such as of *per1* [43, 44]. There is also an increase with age in the phase dispersion among the SCN neurons and the cycles required for re-synchronization [45, 46]. In addition, the phase shifting ability of glutamate, NMDA, and histamine, neurotransmitters that reset the circadian clock in a similar way to light, is markedly decreased in old mice [47]. Considering peripheral oscillators, it has been found that the liver, esophagus, and thymus of old rats displayed slower re-synchronization [45, 46].

Thus, aging is associated with an altered re-synchronization of the SCN and peripheral tissues that reflect a marked reduction in the amplitude and functional output of the circadian hypothalamic pacemaker.

Molecular alterations associated with clock-genes

Recent years have witnessed the recognition of modified phenotypes, pathological processes, and clinical reports involving the malfunction of clock genes and the timing

system. From experimental models involving manipulation of gene expression to medical descriptions of human patients presenting altered alleles in clock genes, the prevalent notion is that circadian timing influences many physiological activities. In addition, it can be the substrate of pathological manifestations when the organism cannot correctly adapt to a variety of environmental stresses.

Table 1. Metabolic and pathological impact of mutations in clock genes: Positive limb of the molecular clock (BMAL1 and CLOCK)

Clock Protein	Model and type of analysis	Action or Phenotype Effect	Reference
BMAL1	Mice BMAL1 ^{-/-}	Hypoglycemia, Gluconeogenesis abolished	Rudic et al., 2004 [63]
CLOCK	Mice CLOCK ^{mut}	Hypoglycemia, Protection to develop diabetes under high-fat feeding.	Rudic et al., 2004 [63]
BMAL1	Mice BMAL1 ^{-/-}	Premature aging with increased sensitivity to oxidative stress	Khapre et al., 2011 [64]
BMAL1	Mice BMAL1 ^{-/-}	Lifespan reduced, progeria-associated alterations	Kondratov et al., 2006 [65]
BMAL1	Mice and human cell lines, BMAL1 ^{-/-} , overexpression and silencing	Alterations in adipose tissue differentiation and lipogenesis	Shimba et al., 2005 [66]
CLOCK	Mice CLOCK ^{-/-}	Lifespan reduced with development of cataracts and dermatitis	Dubrovsky et al., 2010 [67]
CLOCK	Mice CLOCK ^{-/-}	Normal rhythms of locomotor activity, but with altered responses to light	DeBruyne et al., 2006 [68]

The experimental approaches to characterize the impact of mutations in clock genes were: a) single, double and triple knockout mouse models and, b) punctual mutations. Different animal models were used as male mice, female mice, C57BL/6J mice and humans. Abbreviations: BMAL1 (Brain and Muscle Arnt-Like protein-1) and CLOCK (Circadian Locomotor Output Cycles Kaput).

Table 2. Metabolic and pathological impact of mutations in clock genes: Negative limb of the molecular clock (PER and CRY) and in the Casein kinase I epsilon (CK1ε).

Clock protein	Experimental Model	Action or Phenotype Effect	Reference
PER1	Per1 ^{-/-} mice	Short period (~1h) in locomotor activity; delaying the expression of clock genes (Per1 and Per2) in peripheral tissues	Cermakian et al., 2001 [69]
	Per1 ^{Brd} mice	Impaired glucocorticoid rhythm; smaller body mass; different food and water intake; increased glucose metabolism	Dallmann et al., 2006 [70]
	Per1 ^{-/-} middle-aged female mice	Lower reproductive rate	Pilorz & Steinlechner, 2008 [71]
	C57BL/6J Per1 ^{-/-} mice	Failure in circadian rhythmicity in adult SCN slices in vitro	Pendergast et al., 2009 [72]
PER2	hPer2 S662G mutation	Familial advanced sleep phase syndrome (FASPS)	Toh et al., 2001 [73]
	mPer2 ^{mut} mice	Sleep disorders; phase advance of motor activity onset	Kopp et al., 2002 [74]
	Per2 ^{Brd} mice	Higher food and water intake; altered glycemia	Dallmann et al., 2006 [70]
	Per21 ^{-/-} middle-aged female mice	Lower reproductive rate	Pilorz & Steinlechner., 2008 [71]
Per3	mPer3 ^{-/-}	Shortening of the circadian period (0.5 h)	Shearman et al., 2000 [75]
	Per3 ^{tm1Drw} mice	Obesity on a high fat diet	Dallmann & Weaver, 2010 [76]
PER1-3	mPer1/2/3 ^{tm1Drw} male mice	Higher body mass on a high fat diet	Dallmann & Weaver, 2010. [76]
CRY1	Cry1 ^{-/-} mice	Accelerated free-running periodicity of locomotor activity in DD	Van der Horst et al., 1999 [77]
CRY2	Cry2 ^{-/-} mice	Delayed free-running periodicity of locomotor activity in DD	Van der Horst et al., 1999 [77]
Cry1-2	Cry1 ^{-/-} /Cry2 ^{-/-} mice	Complete loss of free-running rhythmicity in DD	Van der Horst et al., 1999 [77]
	CK1ε ^{-/-} mice	Circadian period lengthened	Meng et al., 2008 [78]
CK1ε	CK1ε ^{tau/tau} mice	Shortened circadian rhythm in vivo and in vitro; accelerated 24 h- oscillations in peripheral tissues	Meng et al., 2008 [78]

The experimental approaches to characterize the impact of mutations in clock genes were: a) single, double and triple knockout mouse models and, b) punctual mutations. Different animal models were used as male mice, female mice, C57BL/6J mice and humans. Abbreviations: Per (period genes), Cry (cryptochrome genes), CK1ε (casein kinase I epsilon).

Table 1 and Table 2 summarize the most representative reports showing the pathophysiologic effects associated with genetic disruption of clock genes. Table 1 shows examples of molecular alterations in the

positive loop of the molecular clock, namely BMAL1 and CLOCK, whereas Table 2 summarizes modifications in the elements of the negative loop, CRY and PER. It can be seen that health problems including sleep disorders,

propensity to neoplastic events, and metabolic alterations such as diabetes, obesity, and metabolic syndrome are among the maladies associated with malfunctions of the circadian physiology. However, as already mentioned, environmental circumstances can also influence the timing system, and in some instances like trans-meridian travels and nocturnal work sessions, they act as triggers of physical disorders.

Ontogeny and aging of the circadian system

Ontogeny

Ontogeny is the functional maturation, especially during embryonic stages, of an organisms' homeostasis, behavior, reproductive systems, and the wane of these systems by aging; during ontogenetic development in mammals, environmental and maternal signals are involved on fetal to early postnatal periods [48, 49].

The ontogeny of the mammalian circadian system and the mechanisms of entrainment have been studied mainly in rodents. The two processes imply maturation as well as adaptation. The SCN of rats is formed, on embryonic days (E) 14 through E17, from a specialized zone of the ventral diencephalic germinal epithelium [50, 51]. Even though the rhythmic fluctuations of the clock genes are detected between E19 and P (postnatal day) 3, the body temperature daily rhythm is not evident during the first days of life. It has been proposed that the phase of the circadian clock is coordinated with that of the mother [51]. The photic and non-photoc cues coming from the mother are part of the entrainment process, but they differ temporally. Synchronization of peripheral oscillators takes place at different times from that of SCN entrainment, and it occurs later in development [51].

Using tissues cultured with a PER1-luciferase reporter (PER1:LUC expression) the maturation of peripheral oscillators was analyzed during the postnatal development of rat pups. Peripheral oscillators matured at different rates, with a strong maternal influence dictating the phase of the pup's liver rhythm. The bioluminescence rhythms in the liver were affected by the nursing time, and became stabilized on P25 with a phase corresponding to late night. In contrast, the lung rhythms reached a stable phase at night on P10 [52].

The circadian physiology during fetal life is dependent on the periodic maternal entrainment provided by a variety of endocrine and metabolic signals [53]. In rat and human, the SCN is histologically detected by mid gestation, and it shows changes in metabolic activity from day to night; however, innervation by the retino-hypothalamic tract in human is completed in utero, whereas in rodents this process occurs after birth [53].

Circadian profiles of SCN rhythmicity in the rat develop before birth. Several mRNAs and clock proteins were assessed at E19 by immunohistochemistry, but they did not show daily fluctuations. At P3, rhythms in mRNAs for PER1, PER2, CRY1, and BMAL1, but not in CLOCK, were detected in the SCN. The rhythms matured gradually, but they did not reach complete rhythmicity until P10 [50]. Light sensitivity and activation of *per* genes in rat SCN develop in sequence, *per1* preceding *per2* gene expression [54].

Non-photoc maternal entrainment cues seem to be dominant, without being the generator of fetal rhythms intrinsically. Fetal SCN and possibly peripheral clocks are entrained by rhythmically delivered maternal signals, including: signaling by dopamine through induction of c-fos, melatonin, periodic feeding, maternal behavior, stress signals [49], however there is still necessary research to gain more insight into the mechanisms of ontogeny of circadian rhythms in mammals.

Aging

Aging is a complex biological process that is defined as an age-dependent or age-progressive abatement in intrinsic physiological functions, and it is associated with an increase in mortality rate and a decrease in reproductive success. Aging is not controlled exclusively by genetics, but rather by the interaction of environmental cues and genetic activity [55, 56].

In humans, the changes in circadian properties associated with aging are: 1) the amplitude of the circadian rhythms is reduced, 2) there is a phase advance in the circadian rhythms dependent on the SCN, and 3) there is a disruption of nocturnal sleep. However, the natural free-running period (τ) does not change, and the ability to tolerate abrupt phase shifts is similar to younger people [57].

Free radicals and oxidative stress have been implicated in the biology of aging [56]. A progressive loss of circadian photoreception with aging has been reported, due to a reduction in pupil area and an increase in crystalline lens light absorption [58].

Aging is characterized by decreases in mitochondrial functions associated with an accumulation of mitochondrial DNA mutations, and reactive oxygen species (ROS) are identified as being responsible for some of these age-related changes. It has been postulated that pro-oxidant reactions associated with higher ROS levels could be responsible for the circadian desynchrony and metabolic dysregulation that characterize aging and age-related pathologies.

Longevity is impacted also by nutritional physiology [59]. SIRT1 (silent repressor of transcription) is a NAD⁺-dependent deacetylase that activates the transcription of

BMAL1 and CLOCK in the SCN and other peripheral oscillators. However, in aged mice, the levels of SIRT1 are reduced, resulting in circadian abnormalities and lower activity in comparison to younger animals. Interestingly, some of the circadian changes associated with a senescent SCN are reversed by an overexpression of SIRT1 [60].

Melatonin is a natural antioxidant with significant anti-aging properties. Circulating melatonin decreases with age, and its diurnal rhythm is altered with phase advance in the elderly versus young female humans [61].

Feeding regimens affect circadian rhythms; they can also attenuate aging and increase longevity. Caloric restriction (CR), which limits the daily calorie intake, and intermittent fasting (IF) influence the entrainment of the SCN and extend the life span of diverse species by a mechanism that is still unclear. SIRT1 has been suggested to mediate the induced effects of CR, but the underlying mechanisms of IF are still unknown [62]. Currently, chrono-nutrition is a novel dietary lifestyle strategy used to counteract the deleterious actions of oxidative stress on physiological systems during aging [56].

Concluding remarks

Based in the knowledge generated in the last twenty years, it is now well accepted that circadian rhythms, along with the temporal adjustments needed to deal with the daily environmental changes, are part of the normal status that characterizes the physiological response in most living beings on our planet. The interdependence of timing and energetic metabolism seems inextricable; hence, circadian physiology covers all aspects of functional adaptations, including developmental and aging-related processes. Examples of pathologies associated with altered functioning of the timing system are ever more numerous. The intimate links between the circadian networks of the molecular clock and the metabolic networks are now considered as a potential substrate to test novel therapies and medical approaches that might prevent or improve diverse health abnormalities. Indeed, we foresee in the near future an increase in the interest of both the scientific community and the general public regarding the inextricable interdependence among circadian rhythmicity, pathological processes, and aging.

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