ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2013 Vol. 56, Št. 1: 29–43

Circadian rhythms and their role in living organisms

Cirkadiani ritmi in njihova vloga v živih organizmih

Rok Košir

Center za funkcijsko genomiko in biočipe, Zaloška cesta 4, 1000 Ljubljana
DiaGenomi d.o.o., Češnjica 3, 1261 Ljubljana-Dobrunje
correspondence: rok.kosir@mf.uni-lj.si

Abstract: Numerous physiological processes in organisms as diverse as bacteria and man are regulated by a small molecular clock termed the circadian clock. It is present in virtually all cells of the body and enables various physiological processes to occur at specific times of the day and with a period of about 24 hours. It was not until recent years that the role of the circadian clock has become evident for normal physiology of humans as well as other mammals. Disruption of the normal circadian rhythms can lead to a number of metabolic disorders characteristic of modern lifestyle including diabetes, obesity and cancer. It is the aim of this review to give the reader a general overview of what circadian rhythms are, how they look at the molecular level and why they can influence various metabolic processes in the way they do.

Keywords: Biological rhythms, circadian rhythms, chronobiology, circadian clock

Izvleček: Številne fiziološke procese v raznolikih organizmih uravnava majhna molekularna ura, ki jo imenujemo cirkadiana ura. Nahaja se v skoraj vseh celicah telesa in omogoča, da različni procesi v telesu potekajo ob določenih delih dneva ter da se le ti ponovijo v periodi 24 ur. Šele v zadnjih nekaj letih je pomen cirkadiane ure postal jasen tudi za pravilno homeostazo telesa, tako človeka, kakor tudi drugih sesalcev. Motnje normalnega cirkadianega ritma lahko vodijo v razvoj metabolnih motenj, kot sta diabetes in prekomerna telesna teža, značilnih za sodoben način življenja. Namen preglednega članka je bralcu predstaviti osnove cirkadianih ritmov, njihove lastnosti na molekularnem nivoju ter njihovo prepletenost s procesi metabolizma.

Ključne besede: Biološki ritmi, cirkadiani ritmi, kronobiologija, cirkadiana ura

Biological rhythms

What would life on Earth look like if there were no biological rhythms? This might seem like an irrelevant question since obviously biological rhythms are not that important, or are they? If you look at various processes occurring in living organisms it becomes evident that biological rhythms are an integral part of life. We are all aware of our heart beat, yet we almost never truly comprehend it as a biological rhythm, despite the fact that the absence or perturbation of its rhythm is used in everyday medical practice to distinguish between life and death or illness and health. This is one simple example among many that shows how biological rhythms are not only

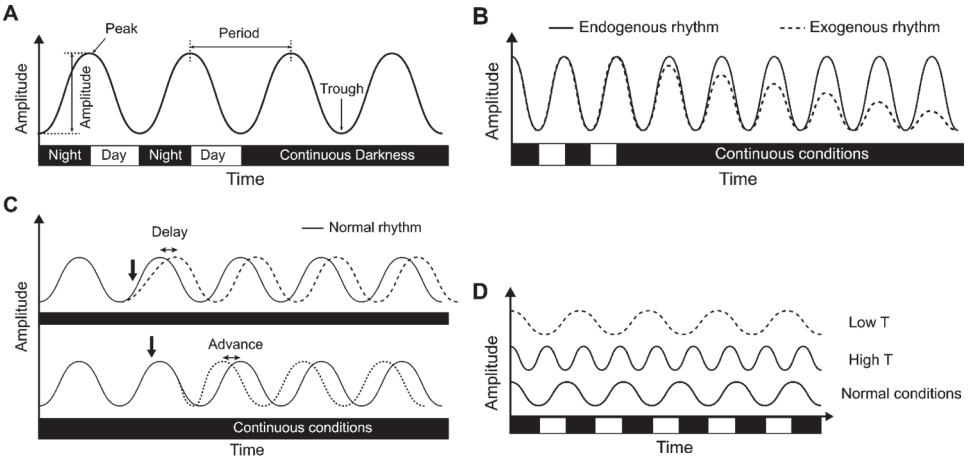

Figure 1: Characteristics of biological and circadian rhythms. A – An example of a circadian rhythm of hormone concentration in blood is shown. The difference between the maximum (peak) and minimum (trough) concentration is the *amplitude*. *Period* equals the interval between two time points. B – Endogenous rhythms persist with no dampening in constant conditions such as complete darkness. Contrary, exogenous rhythms dampen when put in constant conditions. C – Resetting of the circadian rhythm. Exposure to light (black arrow) can shift the rhythm either back (delay) or ahead (advance) depending when during the cycle it is presented. Normal rhythm is depicted by a full gray line. D – temperature compensation. If circadian clocks were not temperature compensated they would run faster at higher temperatures (high T, period < 24h) and slower in lower temperatures (low T, period > 24h) compared to normal conditions.

Slika 1: Lastnosti bioloških in cirkadianih ritmov. A – primer prikazuje cirkadiani ritem koncentracije hormona v krvi. Razlika med največjo koncentracijo (vrh) in najnižjo koncentracijo (dno) je enaka amplitudi. Perioda je enaka intervalu med dvema točkama. B – endogeni ritmi ohranjajo amplitudo tudi v primeru konstantnih pogojev, kot je popolna tema. V nasprotju pa začne amplituda pri eksogenih ritmih v konstantnih pogojih počasi upadati. C – ponastavitev cirkadianega ritma. Svetlobni pulz (črna puščica) lahko povzroči premik faze cirkadianega ritma nazaj (zamuda) ali naprej (napredovanje) v odvistnosti od tega kdaj v fazi cikla je bil pulz prisoten. Normalni ritem je prikazan s polno sivo črto. D – Temperaturna kompenzacija cirkadianih ritmov omogoča, da le-ti v primeru visokih temperature ne potekajo hitreje (perioda < 24h) in v primeru nizkih temperature počasneje (perioda > 24 h), kot pri normalni temperaturi.

important for the survival of an organism itself but also for the survival of species and the ecosystem in general.

Biological rhythms are defined as biological events or functions that reoccur in a repeated order and with a repeated interval (period) between occurrences (Aschoff 1981) and can be divided into three classes based on the duration of the phase (Fig. 1A). While the majority of efforts in both early studies as well as in recent years have been focused on circadian rhythms with a period of about 24 h, rhythms having longer or shorter periods are also important. Infradian rhythms are rhythms with periods longer than 28 hours. A well-known example is the menstrual cycle in women. In male subjects on the other hand the presence of such rhythms is still controversial. Very few studies have been conducted on man, mainly due to the lack of a distinct marker, such as monthly bleeding in women. The results although statistically significant have indicated several variables to have an infradian period, among them body weight, grip strength, estrogen and testosterone production, sexual activity and mood. The small number of subjects on which the studies have been performed necessitates additional research to confirm published results (Koukkari and Sothern 2006). Ultradian rhythms on the other end have periods shorter than 20 hours as shown in the example of the heart beat

Table 1: Examples of biological rhythms. The table shows examples of different biological rhythms belonging to three classes (ultradian, circadian and infradian), defined by the length of the period (based on Koukkari and Sothern (2006)).

Tabela 1: Primeri bioloških ritmov. Tabela prikazuje primere različnih bioloških ritmov iz vseh treh razredov (ultradiani, cirkadiani in infradiani), ki jih definira dolžina periode. Povzeto po Koukkari in Sothern (2006).

Time	Period	Variable	Organism	Source
Seconds	< 1s	EEG activity (delta frequency)	Human (*Homo sapiens*)	(Kripke 1972)
	< 1s	ECG (depolarization of heart ventricles)	Human (*Homo sapiens*)	(Koukkari and Sothern 2006)
Minutes	2–4 min	Leaflet movement	Telegraph plant (*Desmodium gyrans*)	(Koukkari et al. 1985)
	15 min	Cortisol secretion	Horse (*Equus caballus*)	(Drake and Evans 1978)
	30 min	Transpiration	Ota (*Avena sativa*)	(Johnsson 1973)
	90–100 min	REM-NREM sleep	Human (*Homo sapiens*)	(Aserinsky and Kleitman 1953)
Hours	4 h	Enzyme activity	Euglena (*Euglena gracilis*)	(Balzer et al. 1989)
	12 h	Amylase activity	Alfalfa (*Medicago sativa*)	(Henson et al. 1986)
Day	24 h	Body temperature	Human (*Homo sapiens*)	(Aschoff et al. 1972)
	24 h	Sleep-wakefulness	Human (*Homo sapiens*)	(Kleitman 1963)
	24 h	Leaf movements	Alibizzia (*Alibizzia julibrissin*)	(Koukkari et al. 1974)
	24 h	Activity	Mouse (*Mus musculus*)	(Decoursey 1960)
Week	7 days	Oviposition (egg laying)	Spring Tail (*Folsomia candida*)	(Chiba et al. 1973)
	7 days	Organ transplant	Human (*Homo sapiens*)	(DeVecchi et al. 1981)
	7 days	Imbibition of seeds	Bean (*Phaseolus vulgaris*)	(Spruyt et al. 1987)
Month	27–34 days	Menstrual cycle	Human (*Homo sapiens*)	(Presser 1974)
	6 months	Ulcer perforation	Human (*Homo sapiens*)	(Svanes et al. 1998)
Year	1 year	Seed germination	Pole bean *(Phaseolus vulgaris)*	(Spruyt et al. 1988)
	1 year	Migration	Willow warbler (and others) (*Phylloscopus trochilus*)	(Gwinner 1977)
	1 year	Hibernation	Golden-mantled ground squirrel (*Citellus lateralis*)	(Pengelley and Fisher 1963)
	1 year	Gonadal weight	Purple sea urchin (*Strongylocentrotus purpuratus*)	(Halberg et al. 1987)
	8–10 years	Population	Ruffed Grouse (*Bonasa umbellus*)	(Gullion 1982)
	100–200 years	Flowering	Chinese bamboo (*Phyllostachys bamusoides*)	(Janzen 1976)

above. In humans several ultradian rhythms are known both in males and females. Among them is the cycling of the human brain between REM and non-REM sleep (Kishi et al. 2011), regulation of body temperature (Lindsley et al. 1999), hormone release (Ho et al. 1988, Saad et al. 1998, Simon and Brandenberger 2002) and bowel action (Moore 1992). Some examples of different biological rhythms are shown in Table 1. Another important aspect of biological rhythms is whether they are endogenous or exogenous. Exogenous rhythms are simply responses of the organism to external cyclic stimuli, whereas endogenous rhythms are a product of the organism itself and

are self-sustained (Fig. 1B) (Aschoff 1981). This review is intended to introduce the basic principles of circadian rhythms, their molecular structure and their role in normal physiology.

Circadian rhythms

Although the first mention of daily rhythms dates all the way back to 4th century BC, when Androsthenes, a historian of Alexander the Great, described diurnal movements of leaves of several trees, the French astronomer Jean Jacques Ortous de Mairan, is regarded as the discoverer of circadian rhythms. In 1729 he was the first to describe the daily opening and closing of leaves of the mimosa plant (*Mimosa pudica*) even when put in complete darkness (Devlin 2002). However it was not until the 1950s that the field of circadian biology began to develop with studies on fruit flies and humans done by Colin Pittendrigh and Jürgen Aschoff respectively (Vitaterna et al. 2001).

As mentioned circadian rhythms are biological rhythms with a period of about 24 hours, which is implied by the term circadian derived from the Latin *circa*, meaning "around or approximately ", and *diem*, meaning "day". In order for a biological rhythm to be classified as circadian four criteria need to be meet (Vitaterna et al. 2001). First the biological process or function needs to repeat itself with a period of approximately 24 hours. Secondly, the rhythm has to have a characteristic of an endogenous cycle, meaning that it has to continue with a period of close to 24h even in constant conditions devoid of any external time-giving cues (Fig. 1B). Thirdly, the rhythm needs to maintain its period over a range of different temperatures, called temperature compensation. Temperature compensation is important because without it the clock would run faster at higher temperatures compared to lower temperatures due to higher thermal energy of molecular processes (Fig. 1D). Lastly, the rhythm has to have the ability to adapt to changes in the environment and synchronize itself to new conditions (Fig. 1C). This process called entrainment is achieved with the help of external time cues (Zeitgebers), the main one being the light-dark cycle produced by Earth's rotation around its axis (Vitaterna et al. 2001). Pittendrigh discovered that animals will respond differently to light depending on the phase of the cycle they are at (Pittendrigh 1960). For instance, if animals are exposed to light in the early part of their normal night, they will respond with

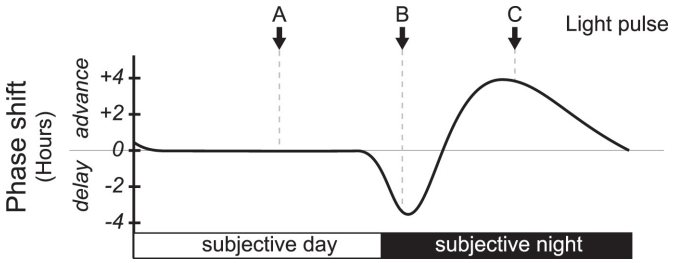

Figure 2: Phase response curve (PRC). A phase response curve shows in what way (advance or delay) the phase of a circadian function (e.g. locomotor activity) will respond, when an external stimuli is given at different times of the circadian cycle. The x-axis represent the time of day, the y-axis shows the amount of phase shift in hours. Light pulse A – (subjective day) won't have any effect on the phase of the circadian function; light pulse B – (beginning of subjective night) will induce a phase delay in the circadian function (also see Fig. 1C); light pulse C – (end of subjective night) will induce a phase advance in the circadian function (also see Fig. 1C).

Slika 2: Krivulja faznega odziva (KFO). Krivulja faznega odziva nam pove v katero smer (zamuda ali napredovanje) se bo premaknila faza cirkadianega procesa (npr. lokomotorna aktivnost), kot posledica odgovora na zunanji dražljajev (svetloba), ki ga dajemo ob različnih časih dneva. X os predstavlja čas dneva, y os predstavlja velikost zamika faze naprej ali nazaj v urah. Svetlobni signal A – (subjektivni dan) ne bo imel vpliva na fazo cirkadianega procesa; svetlobni signal B – (pričetek noči) bo povzročil zamik faze cirkadianega procesa (glej tudi Sl. 1C); svetobni signal C – (konec noči) bo povzročil napredovanje faze cirkadianega procesa (glej tudi Sl. 1C).

Table 2: Examples of circadian rhythms. Some examples of circadian rhythms present in a variety of organisms ranging from bacteria to humans.
Tabela 2: Primeri cirkadianih ritmov. Nekateri primeri cirkadianih ritmov prisotni pri različnih organizmih od bakterij do človeka.

Domain	Process	Organism	Source
Archea	Oxygen-dependent metabolism	*Halobacterium salinarum*	(Whitehead et al. 2009)
Bacteria	Cyclic surface variations during growth	*Pseudomonas putida*	(Soriano et al. 2010)
	N.D.	*Thermosynechococcus elongatus*	(Onai et al. 2004)
	Rhythms of nitrogen fixation	*Synechococcus sp.* RF-1	
Fungi	Growth patterns	*Neurospora crassa*	(Pittendrigh et al. 1959)
Plants	Several physiological processes	*Chlamydomonas reinhardtii*	(Mittag et al. 2005),
	Leaf movement rhythm, germination, growth, enzyme activity, stomatal movement and gas exchange, photosynthetic activity, flower opening, and fragrance emission	*Mimosa pudica, Arabidopsis thaliana,* bean *(Phaseolus vulgaris),* chestnut *(Castanea sativa) ,* pea *(Pisum sativum),* soybean *(Glycine max),* tail (*Brassica rapa),* tomato *(Solanum lycopersicum),* poplar (*Populus spp*.)*, papaya (*Carica papaya*)*, grape (*Vitis vinifera*)*	(McClung 2013), (McClung 2011)
Animals	Time of Eclosion, foraging and mating activities	fruit fly (*Drosophila melanogaster*)	(Panda et al. 2002)
	visit flowers to collect pollen and nectar in a rhythmic manner	honeybee *(Apis mellifera)*	(Moore et al. 1998)
	Timing of their mating flights	ant (*Camponotus compressus)*	(Sharma et al. 2004)
	Timing of migratory flights	Monarch butterflies *(Danaus plexippus)*	(Froy et al. 2003)
	Preparation for hibernation	Golden-mantled ground squirrel (*Callospermophilus lateralis*)	(Dunlap et al. 2004)
	Diving timing	loggerhead turtle *(Caretta caretta)*	(Oishi et al. 2010)
	Locomotor activity	Japanese grass lizard (*Takydromus tachydromoides*)	(Oishi et al. 2010)
	Body temperature and locomotor activity	*Iguana iguana*	(Oishi et al. 2010)
	Diurnal rhythms in hypothalamic/ pituitary AVT synthesis and secretion	*Oncorhynchus mykiss* (rainbow trout)	(Rodriguez-Illamola et al. 2011)
	Body temperature, blood pressure, metabolism, hormone synthesis etc.	Mouse (*Mus musculus*)	(Green et al. 2008; Tzameli 2012)
	Body temperature, blood pressure, metabolism, hormone synthesis etc.	Human (*Homo sapiens sapiens*)	(Green et al. 2008; Tzameli 2012)

* – clock genes have been found by genome wide analysis however functional assesments of the clock are still missing (McClung 2013).

a phase delay, whereas they will respond with a phase advance when they are exposed to light in the later part of their normal night (Fig. 1C, Fig. 2). The exact way an animal will respond to a zeitgeber at a specific time can be studied with the help of phase response curves. A phase response curve is constructed by determining whether a phase advance or delay of a certain circadian

variable (e.g. locomotoric activity) is produced when the same zeitgeber is given at different times of the circadian cycle (Fig. 2) (Pittendrigh 1960, Golombek and Rosenstein 2010).

The importance of entrainment may not seem obvious at first, but simple mathematics shows how quickly a species can come out of synch with the day-night cycle if the phase of the rhythm changes by just a fraction. Let's assume a mouse's endogenous period would be a mere 10 minutes longer tha˝n 24 h. With no entrainment to external conditions, it would take only 6 days for the mouse to be 1h in advance of the normal day night cycle and in just a matter of 2 months it would become a diurnal instead of a nocturnal animal. This would have a significant negative impact on the fitness of an individual that would substantially reduce its success of survival and reproduction. For this reason if a mouse's active night period is too long and extends into morning hours, the light will trigger a phase advance (Fig. 2). As a consequence the active period will begin sooner in the coming day and also end before the morning, entraining the internal mouse clock to the environmental conditions.

In spite of these four restrictions a large fraction of today's organisms, ranging from bacteria to humans, display a clear circadian rhythm in various physiological and behavioral processes (some are listed in Table 2). Due to its almost ubiquitous presence, the circadian rhythm clearly has an evolutionary advantage. Anticipation of daily changes in the environment by an organism rather than just reacting to them seems to be one of the main ones (Ramsey et al. 2007). At least two studies in cyanobacteria and *D. melanogaster* have shown that wild-type strains are more successful in survival compared to their mutant ones when grown in the same test tube (Johnson et al. 1998, Klarsfeld and Rouyer 1998).

Genetics of the clock

Despite the discovery of the double helix in 1953 and the development of various genetic and molecular biology techniques thereafter, the first two decades of circadian rhythm research were devoted mainly to understanding the basic principles (Pittendrigh et al. 1959, Pittendrigh 1960) including resetting of the rhythm by light pulses (Bruce et al. 1960), construction of phase response curves (Aschoff 1965), temperature compensation (Zimmerman et al. 1968) etc. It was not until 1971 that the era of clock genetics began, when Ron Konopka and Seymour Benzer first described the existence of the *period* (*per*) locus in *Drosophilla melanogaster*. Using genetic screens of mutated fruit flies they discovered 3 mutants which significantly changed their 24h rhythm of both eclosion and locomotor activity: long period (28h rhythm), short period (19h rhythm) and arrhythmic (no rhythm) (Konopka and Benzer 1971). By the beginning of the 21th century, similar genetic screens were used in various model organisms to discover other clock related genes including: *per* and timeless (*tim*) in *D. melanogaster*; white collar 1 and 2 (*wc1* and *wc2*) and frequency (*frq*) in *N. crassa;* timing of crab (*TOC1*) in *A. thaliana* and *Clock, Bmal1*, *Per1, Per2, Cry1* and *Cry2* in mice (Takahashi 2004, Zhang and Kay 2010). Regardless of the fact that different organisms use different sets of genes the basic molecular mechanism behind all circadian clocks seems to be the same and can be described by a simple transcription-translation feedback loop (Roenneberg and Merrow 2002). While the transcription-translation feedback loop remains at the core of the circadian clock, the use of novel high-throughput technologies in the last decade showed that the clock is not a simple loop but is composed of multiple networks operating on different levels (Zhang and Kay 2010). It is not within the scope of this review to present any details about the molecular components of circadian clocks in various organisms. However, since the basic principle of how molecular clocks work is similar in all species, we will take a closer look at the molecular clock of mammals (Fig. 3).

The transcription-translation feedback loop of mammals is composed of a positive, represented by *Clock* and *Bmal1* and a negative limb, represented by *Per1*, *Per2*, *Cry1* and *Cry2*. During the day, CLOCK and BMAL1 proteins form a heterodimer that acts as a transcriptional factor, binding to E-box promoter regions of various genes, including *Pers* and *Crys*, and activating their transcription. The resulting PER and CRY proteins heterodimerize and translocate back to the nucleus where they inhibit the transcriptional activity of the CLOCK/

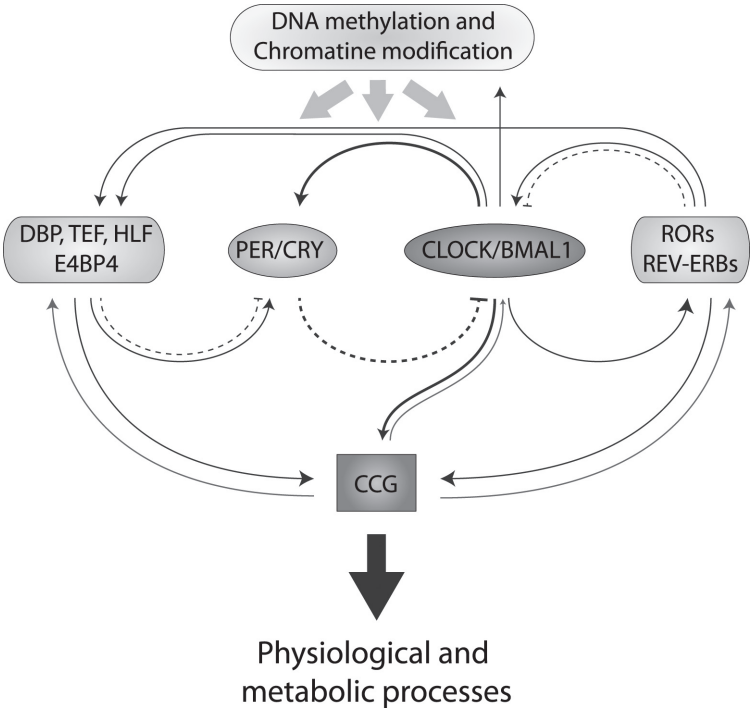

Figure 3: Molecular organization of circadian rhythms. Although different model organisms have different clock components the overall architecture of the transcriptional translational feedback loop is similar. The CLOCK/BMAL1 heterodimer presents the positive limb and activates transcription of various core clock and clock output genes. PER and CRY proteins represent the negative limb that inhibits CLOCK/BMAL1 transcriptional activation. PER and CRY degradation leads to a new round of CLOCK/BMAL1 initiated activation. Various other loops (D-box and RRE) can influence the core clock mechanism. In addition DNA methylation and chromatin modifications influence various components of the clock mechanism. Clock controlled genes (CCG) regulate circadian physiological processes and can also feedback information to the core clock mechanism.

Slika 3: Molekularna osnova cirkadianih ritmov. Kljub razlikam v sestavi genov in proteinov, ki tvorijo molekularno osnovo cirkadianih ur pri različnih organizmih, pa je arhitektura transkripcijsko translacijske povratne zanke pri vseh podobna. Heterodimer CLOCK/BMAL1 aktivira izražanje genov centralne cirkadiane ure in output genov. Proteina PER in CRY predstavljata negativno povratno zanko, ki inhibira transkripcijsko aktivnost heterodimera CLOCK/BMAL1. Proteolitska razgradnja PER in CRY proteinov povzroči ponovno aktivacijo transkripcije preko heterodimera CLOCK/BMAL1. Poleg opisanih, obstajajo še druge zanke, kot sta D-box in E-box zanka, ki lahko vplivajo na mehanizem centralne ure. Mehanizem centralne cirkadiane ure je podvržen tudi regulaciji preko DNA metilacije in modifikacije kromatina. CCG (*Clock controled genes*): geni, ki jih uravnava cirkadiana ura, omogočajo cirkadiano izražanje fizioloških procesov, hkrati pa lahko tudi posredujejo informacije nazaj k cirkadiani uri.

BMAL1 heterodimer. During the night however, the PER/CRY heterodimer is degraded enabling a new round of transcription by the CLOCK/BMAL1 dimer to start. This whole process takes about 24h to complete (Ko and Takahashi 2006). In addition to the core loop other loops exist that interact with the core loop. One such is the REV-response element (RRE) loop, which is composed of proteins belonging to the nuclear receptor family of transcriptional factors. By binding to the RRE element in promoter regions of *Bmal1* proteins such as *Rorα, Rorβ* and *Rorγ* or *Rev-erbα* and *Rev-erbβ* activate or repress its transcription respectively (Preitner et al. 2002). Similar to the RRE, the D-

box loop represents the third feedback loop and is generated by transcription factors D-box binding protein (DBP), thyrotroph embryonic factor (TEF) and hepatic leukemia factor (HLF) as activators and E4 promoter-binding protein 4 (E4BP4) as a repressor (Fig. 3)(Takahashi et al. 2008). These additional loops are important because they provide (1) robustness of the clock, (2) enable the clock to receive entrainment signals from various sources and (3) provide several different clock output ways (Zhang and Kay 2010).

Regardless of the complexity of the loops mentioned above the circadian rhythm in cells is also controlled by other means. In mammals posttranslational modifications (PTM) play an important role by modulating protein half-life and their subcellular location. All of the core clock proteins in mammals (CLOCK, BMAL1, PERs and CRYs) are known to be modified by one or several modifications including phosphorylation (all), acetylation (BMAL1, PER2), ubiquitination (all) and sumoylation (BMAL1) (Bellet and Sassone-Corsi 2010). PTM are also important for epigenetic control of the clock next to DNA methylation and miRNA. Several studies have shown that chromatin remodeling is involved in expression of circadian genes as well as that chromatin modifications appear to follow a circadian pattern at different clock controlled genes (CCG) (Curtis et al. 2004, Doi et al. 2006, Bellet and Sassone-Corsi 2010).

It is evident that the control regulation of circadian clocks in cells is a complex process involving different levels of regulation ranging from transcriptional control all the way to epigenetic modifications. Likewise, because of the interaction between different molecular loops that feed information into the core circadian loop, the clock is well integrated with other physiological processes and vice versa. The exact interplay between the clock and cell physiology and metabolism is still a matter of research, but much has been learned in recent years.

Interplay between circadian rhythms and metabolism?

In multicellular organisms such as mammals light cannot reach every cell in the body and therefore cannot synchronize the clock in these cells directly. For this reason the circadian system evolved a hierarchical structure in which a master clock residing in the suprachiasmatic nuclei (SCN) of the hypothalamus synchronizes peripheral clocks in various tissues such as liver, adipose tissue, heart, intestine and adrenal gland (Fig.4).

The SCN receives light signals from the retina through the retinohypotalamic tract and thereby synchronizes its internal clock to the outside world (Reppert and Weaver 2002). It is responsible for driving various behavior rhythms (e.g. locomotor activity) as well as synchronizing circadian clocks in peripheral tissues, with the help of neural and humoral signals, to maintain proper phase relationships and prevent clocks in these tissues from dampening out (Dickmeis 2009). While the SCN is primarily entrained by light, peripheral tissues can in addition to SCN signals, also be entrained to various other stimuli among which feeding is the dominant zeitgeber (Damiola et al. 2000). There has been a lot of debate in recent years of whether the SCN can also be entrained by temperature fluctuations or not. While some publications have shown this to be true (Ruby et al. 1999, Herzog and Huckfeldt 2003) other have proven the opposite (Buhr et al. 2010). What has been show by all is the fact that a single SCN neuron can be affected by temperature fluctuations, however for the SCN as a whole this has not yet been proven and is still a matter of further research.

It was not until only recently that the influence of circadian clocks on metabolism became evident in mammals. With the use of DNA microarrays it was shown that between 5 % and 20 % of all transcripts in a particular tissue have circadian profiles of expression. Different tissues showed only limited overlap between rhythmic genes, suggesting that the expression is regulated in a tissue specific manner (Akhtar et al. 2002, Durgan et al. 2006, Zvonic et al. 2006, Kosir et al. 2012). Among genes shown to have rhythmic expression were transcripts involved in gluconeogenesis, glycolysis, lipid and cholesterol metabolism, steroid hormone synthesis and xenobiotic metabolism (Green et al. 2008, Acimovic et al. 2011, Zmrzljak and Rozman 2012, Kosir et al. 2013). It has also been discovered that different hormones regulating metabolism in mammals including glucagon, insulin, leptin, adiponectin and corticosterone

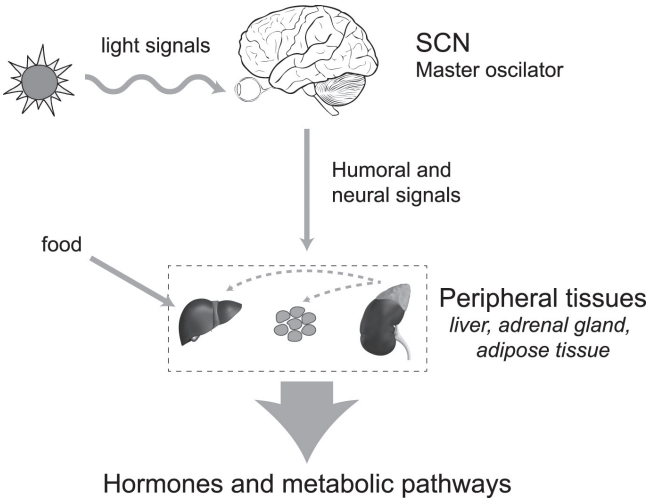

Figure 4: Anatomical organization of circadian rhythms in mammals. In mammals the circadian system is composed of a master oscillator located in the suprachiasmatic nucleus and is synchronized to the outside world by light pulses that reach it from the retina through the retinohypotalamic tract. The SCN controls peripheral clocks in tissues through various humoral and neural signals. In addition to SCN signals, food can also entrain some peripheral tissues especially liver. Adrenal glands excrete glucocorticoids in a circadian fashion that can also influence expression of genes in other tissues.

Slika 4: Anatomska struktura cirkadianih ritmov pri sesalcih. Pri sesalcih je cirkadiani sistem zgrajen iz glavne cirkadiane ure, ki se nahaja v suprahiazmatičnem jedru (SCN) v hipotalamusu, ter perifernih cirkadianih ur, ki se nahajajo v različnih organih. SCN se vsakodnevno sinhronizira z zunanjimi pogoji svetlobe in teme, preko retine in retinohipotalamičnega trakta. V nadaljevanju SCN preko živčnih ali hormonskih poti sinhronizira periferne cirkadiane ure. Periferne cirkadiane ure se lahko sinhronizirajo tudi z drugimi signali, neodvisno od SCN, kot je npr. hrana. Nadledvična žleza izloča tudi glukokortikoide, ki prav tako vpliva na izražanje genov v nekaterih perifernih tkivih. Koncentracija glukokortikoidov, kot je kortizol ali kortikosteron je v plazmi cirkadiana.

show circadian oscillations (Froy 2011). These examples clearly show a direct influence of the circadian clock on various metabolic processes, but there are several key metabolic factors which can also influence the core clock mechanism. We previously already mentioned REV-ERBa and RORa, that regulate the expression of *Bmal1* but are also important in adipocyte differentiation and regulation of lipogenesis respectively (Froy 2011). PPARa, another member of the nuclear receptor family, is important in lipid and glucose metabolism. It shows circadian rhythmicity but also activates the transcription of *Bmal1*, indicating yet another feedback loop of the clock (Canaple et al. 2006). Other molecules such as AMPK (AMP-activated protein kinase), PGC-1a (PPARg co-activator 1a) and SIRT1 (sirtuin I) have also been implicated in the regulation of clock genes either directly through transcription (PGC-1a) or indirectly through phosphorylation (AMPK) and deacetylation (SIRT1) (Canto and Auwerx 2009).

The importance of an intact circadian clock for normal homeostasis and metabolism has been well established and it has been show that disruption of circadian rhythms may lead to development of various forms of metabolic syndrome (Green et al. 2008, Froy 2011, Naik et al. 2013). The most compelling evidence comes from mouse models. Here both obesity and metabolic syndrome have been discovered in mice carrying mutations in core clock genes. For example *Bmal1* knock-out mice are completely arrhythmic and have disruptions in rhythmic levels of glucose and triglycerides. To see whether these disruptions are a consequence of the loss of rhythmicity of the SCN or of peripheral oscillators, *Bmal1* liver and pancreas specific knock-out mice we generated. Despite normal locomotor rhythm both tissue specific knock-outs

Table 3: Mouse experimental models. Examples of metabolic defects in mice with mutations or gene knock-outs of clock genes. Based on Froy (2011) and Sahar and Sassone-Corsi (2012).
Tabela 3: Eksperimentalni mišji modeli. Primeri metabolnih motenj, ki se pojavijo pri miših z mutacijami ali izbitimi geni cirkadiane ure. Povzeto po Froy (2011) ter Sahar in Sassone-Corsi (2012).

Gene mutated or knocked-out	Metabolic Consequence
Clock	Hyperlipidemia, hyperleptinemia, hypoinsulinemic and hyperglycemia
Bmal 1	Abolished oscillations in plasma glucose and triglycerides impaired gluconeogenesis, hyperleptinemia, glucose intolerance, and dyslipidemia
Per 1	Increased urinary sodium excretion
Per 2	Altered lipid metabolism, lower body weight
Cry 1 and *Cry 2*	Hyperglycemia Salt-sensitive hypertension
Reverba	Increased serum triglycerides
Rora	Reduced plasma triglycerides and HDL Enhanced atherosclerosis
Pgc-1a	Increased sensitivity to insulin Altered thermogenesis
Nocturnin	Resistant to diet-induced obesity Altered lipid metabolism

displayed disturbances in blood glucose levels (Sahar and Sassone-Corsi 2012). Several other mouse models with mutations or deletions of core clock genes have been generated that display perturbation to normal metabolism (Tab. 3). In addition to mouse genetic models epidemiological studies on humans have identified a correlation between shift work and metabolic disorders. Humans that were active and eating during normal night were shown to have decreased leptin (adipose tissue specific hormone that promotes satiety) levels and increased insulin and glucose levels. Leptin levels were also found to be reduced in healthy patients that were subjected to only 4 hours of sleep in six consecutive nights (Spiegel et al. 2004). It is interesting to note that in the same time period that we have seen an increase in metabolic diseases and obesity we have also seen a decrease in the quality and duration of sleep.

Low quality and duration of sleep and disruptions of the normal circadian rhythm can also be related to another problem facing modern societies: light pollution. Light pollution is defined as artificial light (usually over illuminated streets, buildings, commercial ads etc.) present during the otherwise dark night. The effects of light pollution on various animal species have been well established unfortunately less research has been done on human subjects. Nevertheless a study done in Israel compared the level of artificial light at night and occurrence of breast cancer in 147 communities and discovered that women living in areas with high night light had a greater chance for developing breast cancer (Kloog et al. 2011). Several studies have shown that the production of the night hormone melatonin, by the pineal gland, is abruptly terminated when individuals are exposed to light during the night faze. Since melatonin is known for helping to regulate the body's biologic clock, it might be an important link between the disrupted circadian clock of the body and light pollution (Chepesiuk 2009).

Conclusion

The presence of circadian rhythms in almost all organisms ranging from bacteria and unicellular eukaryotes to multicellular organisms including humans clearly shows their importance and evolutionary advantage. While a lot has been learned in the six decades of circadian rhythm research it is only in the last few years that we began to appreciate their importance in human health. The alarming increase in the rate of hypertension, obesity, metabolic syndrome and cancer worldwide, especially in developed and developing countries, could well be related to a disrupted

circadian rhythm caused by lifestyle changes. For this reason much research is needed to completely understand the intricate relationships between the circadian clock and metabolism as well as the circadian clock and cancer to eventually be able to reset the inner clock and prevent metabolic or cancer disorders from developing.

Povzetek

Cirkadiani ritmi so biološki ritmi, ki se ponavljajo s periodo okoli 24h in predstavljajo pomembno evolucijsko adaptacijo organizmov na ciklične spremembe v okolju, ki so posledica vrtenja Zemlje okoli svoje osi. Najdemo jih v skorajda vseh organizmih od bakterij pa vse do ljudi, kjer uravnavajo številne fiziološke in metabolne procese. Začetki obširnejših raziskav cirkadianih ritmov segajo v 50. leta 20. stoletja, ko sta Colin Pittendrigh in Jürgen Aschoff predvsem z opazovanjem sprememb obnašanja živali razkrila osnovne značilnosti cirkadianih ritmov in njihove lastnosti. Moderna doba raziskav cirkadianih ritmov, ki je vključevala tudi molekularne osnove ritma, pa se je pričela šele v 70 letih 20. stoletja. V tem času sta Ron Konopka in Seymour Benzer odkrila prve mutante lokusa *period* pri vinski mušici (*D. melanogaster*), ki so povzročile spremenjen cirkadiani ritem lokomotorne aktivnosti mušic. Kmalu so z uporabo različnih modelnih organizmov kot so *N. crassa, D. melanogaster, M. musculus* in še nekaterih drugih odkrili, da je osnovni molekularni mehanizem cirkadiane ure pri vseh organizmih zelo podoben. Osnova ritma je transkirpcijsko translacijska povratna zanka, ki je npr. pri sesalcih sestavljena iz aktivatorjev, kot sta *Clock* in *Bmal1,* ter represorjev, kot so družina genov period in kriptokrom. Proteina CLOCK in BMAL1 v heterodimeru delujeta kot transkripcijska faktorja, saj aktivirata izražanje represorjev kot tudi številnih drugih genov uravnavanih s cirkadiano uro. Proteini PER in CRY pa delujejo tako, da preprečijo traskripcijsko aktivnost heterodimera CLOCK/BMAL1 in ustavijo transkripcijo tako sebe kot drugih genov. Po določenem času se proteini PER in CRY razgradijo in tako omogočijo, da se aktivacija transkripcije s CLOCK/BMAL1 ponovno prične. Celoten cikel traja približno 24 ur da se ponovi. Predvsem pri višjih organizmih, kot so sesalci in človek, v zadnjih nekaj letih prihaja vedno bolj do izraza prepletenost cirkadiane ure in različnih fizioloških procesov ter metabolizma. Postalo je jasno, da lahko porušen cirkadiani ritem povzroči nastanek različnih metabolnih motenj, kot sta diabetes in prekomerna teža. Nadaljne raziskave bodo pripomogle k boljšemu razumevanju prepleta med cirkadiano uro in metabolizmom ter morda v prihodnosti omogočile izdelavo režima, s katerim bomo vzpostavili normalno delovanje, sicer porušene cirkadiane ure pri številnih bolnikih, in tako pripomogli k njihovemu zdravljenju.

Acknowledgement

This study was supported by the Slovenian Research Agency program, project J7-4053 and by funds from Diagenomi, d.o.o.

References

Acimovic, J., Kosir, R., Kastelec, D., Perse, M., Majdic, G., Rozman, D., Kosmelj, K.Golicnik, M., 2011. Circadian rhythm of cholesterol synthesis in mouse liver: a statistical analysis of the post-squalene metabolites in wild-type and Crem-knock-out mice. Biochem Biophys Res Commun, 408 (4), 635–641.

Akhtar, R. A., Reddy, A. B., Maywood, E. S., Clayton, J. D., King, V. M., Smith, A. G., Gant, T. W., Hastings, M. H.Kyriacou, C. P., 2002. Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. Curr Biol, 12 (7), 540–550.

Aschoff, J., 1965. Response curves in circadian periodicity. Amsterdam, North-Holland.

Aschoff, J., 1981. A survey on biological rhythms. New York, Plenum Press.

Aschoff, J., Giedke, H., Pöppel, E.Wever, R., 1972. The influence of sleep-interruption and of sleep-deprivation on circadian rhythms in human performance. London, English University Press.

Aserinsky, E.Kleitman, N., 1953. Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. Science, 118 (3062), 273–274.

Balzer, I., Neuhaus-Steinmetz, U., Quentin, E., Wüllen, v. M.Hardeland, R., 1989. Concomitance of circadian and circa-4-hour ultradian rhythms in Euglena gracilis. Journal of interdisciplinary cycle research, 20, 10.

Bellet, M. M.Sassone-Corsi, P., 2010. Mammalian circadian clock and metabolism – the epigenetic link. J Cell Sci, 123 (Pt 22), 3837–3848.

Bruce, V. G., Weight, F.Pittendrigh, C. S., 1960. Resetting the sporulation rhythm in Pilobolus with short light flashes of high intensity. Science, 131 (3402), 728–730.

Buhr, E. D., Yoo, S. H.Takahashi, J. S., 2010. Temperature as a universal resetting cue for mammalian circadian oscillators. Science, 330 (6002), 379–385.

Canaple, L., Rambaud, J., Dkhissi-Benyahya, O., Rayet, B., Tan, N. S., Michalik, L., Delaunay, F., Wahli, W.Laudet, V., 2006. Reciprocal regulation of brain and muscle Arnt-like protein 1 and peroxisome proliferator-activated receptor alpha defines a novel positive feedback loop in the rodent liver circadian clock. Mol Endocrinol, 20 (8), 1715–1727.

Canto, C.Auwerx, J., 2009. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr Opin Lipidol, 20 (2), 98–105.

Chepesiuk, R., 2009. Missing the dark: health effects of light pollution. Environ Health Perspect, 117 (1), A20–27.

Chiba, Y., Cutkomp, L. K.Halberg, F., 1973. Circaseptan (7-day) oviposition rhythm and growth of Spring Tail, Folsomia Candida (Collembola: Isotomidae). Journal of interdisciplinary cycle research, 4, 8.

Curtis, A. M., Seo, S. B., Westgate, E. J., Rudic, R. D., Smyth, E. M., Chakravarti, D., FitzGerald, G. A.McNamara, P., 2004. Histone acetyltransferase-dependent chromatin remodeling and the vascular clock. J Biol Chem, 279 (8), 7091–7097.

Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F.Schibler, U., 2000. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. Genes Dev, 14 (23), 2950–2961.

Decoursey, P. J., 1960. Phase control of activity in a rodent. Cold Spring Harb Symp Quant Biol, 25, 49–55.

DeVecchi, A., Halberg, F., Sothern, R. B., Cantaluppi, A.Ponticelli, C., 1981. Circaseptan rhythmic aspects of rejection in treated patients with kidney transplant. Tallahassee, Florida A&M University Foundation.

Devlin, P. F., 2002. Signs of the time: environmental input to the circadian clock. J Exp Bot, 53 (374), 1535–1550.

Dickmeis, T., 2009. Glucocorticoids and the circadian clock. J Endocrinol, 200 (1), 3–22.

Doi, M., Hirayama, J.Sassone-Corsi, P., 2006. Circadian Regulator CLOCK Is a Histone Acetyltransferase. Cell, 125, 497.

Drake, D. J.Evans, J. W., 1978. Cortisol secretion pattern during prolonged ACTH infusion in dexamethasone treated mares. Journal of interdisciplinary cycle research, 9, 8.

Dunlap, J. C., Loros, J. J.DeCoursey, P. J., 2004. The behavioral ecology and evolution of biological timing systems. Sunderland, Massachusetts, USA, Sinauer Associates.

Durgan, D. J., Trexler, N. A., Egbejimi, O., McElfresh, T. A., Suk, H. Y., Petterson, L. E., Shaw, C. A., Hardin, P. E., Bray, M. S., Chandler, M. P., Chow, C. W.Young, M. E., 2006. The circadian clock within the cardiomyocyte is essential for responsiveness of the heart to fatty acids. J Biol Chem, 281 (34), 24254–24269.

Froy, O., 2011. The circadian clock and metabolism. Clin Sci (Lond), 120 (2), 65–72.

Froy, O., Gotter, A. L., Casselman, A. L.Reppert, S. M., 2003. Illuminating the circadian clock in monarch butterfly migration. Science, 300 (5623), 1303–1305.

Golombek, D. A.Rosenstein, R. E., 2010. Physiology of circadian entrainment. Physiol Rev, 90 (3), 1063–1102.

Green, C. B., Takahashi, J. S.Bass, J., 2008. The meter of metabolism. Cell, 134 (5), 728–742.

Gullion, G. W., 1982. Forest wildlife interactions. New York, Wiley.

Gwinner, E., 1977. Circannual rhythms in bird migration. Annual Review of Ecology, Evolution, and Systematics, 8, 25.

Halberg, F., Halberg, F., Sothern, R. B., Pearse, J. S., Pearse, V. B., Shankaraiah, K.Giese, A. C., 1987. Consistent synchronization and circaseptennian (about 7-yearly) modulation of circannual gonadal index rhythm of two marine invertebrates. New York, Alan R. Liss.

Henson, C. A., Duke, S. H.Koukkari, W. L., 1986. Rhythmic oscillations in starch concentration and activities of amylolytic enzymes and invertase in Medicago sativa nodules. Plant and Cell Physiology, 27, 10.

Herzog, E. D.Huckfeldt, R. M., 2003. Circadian entrainment to temperature, but not light, in the isolated suprachiasmatic nucleus. J Neurophysiol, 90 (2), 763–770.

Ho, K. Y., Veldhuis, J. D., Johnson, M. L., Furlanetto, R., Evans, W. S., Alberti, K. G.Thorner, M. O., 1988. Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. J Clin Invest, 81 (4), 968–975.

Janzen, D. H., 1976. Why bamboos wait so long to flower. Annual Review of Ecology, Evolution, and Systematics, 7, 45.

Johnson, C. H., Golden, S. S.Kondo, T., 1998. Adaptive significance of circadian programs in cyanobacteria. Trends Microbiol, 6 (10), 407–410.

Johnsson, A., 1973. Oscillatory transpiration and water uptake of Avena plants: I. Preliminary observations. Physiologia Plantarum, 28, 11.

Kishi, A., Yasuda, H., Matsumoto, T., Inami, Y., Horiguchi, J., Tamaki, M., Struzik, Z. R.Yamamoto, Y., 2011. NREM sleep stage transitions control ultradian REM sleep rhythm. Sleep, 34 (10), 1423–1432.

Klarsfeld, A.Rouyer, F., 1998. Effects of circadian mutations and LD periodicity on the life span of Drosophila melanogaster. J Biol Rhythms, 13 (6), 471–478.

Kleitman, N., 1963. Sleep and Wakefulness,. Chicago, University of Chicago Press.

Kloog, I., Portnov, B. A., Rennert, H. S.Haim, A., 2011. Does the modern urbanized sleeping habitat pose a breast cancer risk? Chronobiol Int, 28 (1), 76–80.

Ko, C. H.Takahashi, J. S., 2006. Molecular components of the mammalian circadian clock. Hum Mol Genet, 15 Spec No 2, R271–277.

Konopka, R. J.Benzer, S., 1971. Clock mutants of Drosophila melanogaster. Proc Natl Acad Sci U S A, 68 (9), 2112–2116.

Kosir, R., Spaninger, K.Rozman, D., 2013. Circadian events in human diseases and in cytochrome P450-related drug metabolism and therapy. IUBMB Life.

Kosir, R., Zmrzljak, U. P., Bele, T., Acimovic, J., Perse, M., Majdic, G., Prehn, C., Adamski, J.Rozman, D., 2012. Circadian expression of steroidogenic cytochromes P450 in the mouse adrenal gland--involvement of cAMP-responsive element modulator in epigenetic regulation of Cyp17a1. FEBS J, 279 (9), 1584–1593.

Koukkari, W. L., Duke, S. H.Bonzon, M. V., 1985. Circadian rhythms and their relationships to ultradian and high frequency oscillations. Genève, Université de Genève.

Koukkari, W. L., Duke, S. H., Halberg, F.Lee, J. K., 1974. Circadian rhythmic leaflet movements: student exercise in chronobiology. Chronobiologia, 1 (3), 281–302.

Koukkari, W. L.Sothern, R. B., 2006. Introducing Biological Rhythms, Springer Netherlands.

Kripke, D. F., 1972. An ultradian biological rhythm associated with perceptual deprivation and REM sleep. Psychosomatic Medicine, 34 (3), 14.

Lindsley, G., Dowse, H. B., Burgoon, P. W., Kolka, M. A.Stephenson, L. A., 1999. A persistent circhoral ultradian rhythm is identified in human core temperature. Chronobiol Int, 16 (1), 69–78.

McClung, C. R., 2011. The genetics of plant clocks. Adv Genet, 74, 105–139.
McClung, C. R., 2013. Beyond Arabidopsis: The circadian clock in non-model plant species. Semin Cell Dev Biol.
Mittag, M., Kiaulehn, S.Johnson, C. H., 2005. The circadian clock in Chlamydomonas reinhardtii. What is it for? What is it similar to? Plant Physiol, 137 (2), 399–409.
Moore, D., Angel, J. E., Cheeseman, I. M., Fahrbach, S. E.Robinson, G. E., 1998. Timekeeping in the honey bee colony: integration of circadian rhythms and division of labor. Behavioral Ecology and Sociobiology, 43 (3), 147–160.
Moore, J. G., 1992. Biologic Rhythms in Clinical and Laboratory Medicine. Berlin Heidelberg, Springer-Verlag.
Naik, A., Kosir, R.Rozman, D., 2013. Genomic aspects of NAFLD pathogenesis. Genomics.
Oishi, T., Nagai, K., Harada, Y., Naruse, M., Ohtani, M., Kawano, E.Tamotsu, S., 2010. Circadian Rhythms in Amphibians and Reptiles: Ecological Implications. Biological Rhythm Research, 35 (1–2), 105–120.
Onai, K., Morishita, M., Itoh, S., Okamoto, K.Ishiura, M., 2004. Circadian rhythms in the thermophilic cyanobacterium Thermosynechococcus elongatus: compensation of period length over a wide temperature range. J Bacteriol., 186 (15), 4972–4977.
Panda, S., Hogenesch, J. B.Kay, S. A., 2002. Circadian rhythms from flies to human. Nature, 417, 329–335.
Pengelley, E. T.Fisher, K. C., 1963. The effect of temperature and photoperiod on the yearly hibernating behavior of captive golden-mantled ground squirrels (Citellus lateralis testcorum). Canadian Journal of Zoology, 41, 18.
Pittendrigh, C. S., 1960. Circadian rhythms and the circadian organization of living systems. Cold Spring Harb Symp Quant Biol, 25, 159–184.
Pittendrigh, C. S., Bruce, V. G., Rosensweig, N. S.Rubin, M. L., 1959. Growth patterns in Neurospora: A biological clock in Neurospora. Nature, 184, 169–170.
Preitner, N., Damiola, F., Lopez-Molina, L., Zakany, J., Duboule, D., Albrecht, U.Schibler, U., 2002. The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell, 110 (2), 251–260.
Presser, H. B., 1974. Temporal data relating to the human menstrual cycle. New York, Wiley.
Ramsey, K. M., Marcheva, B., Kohsaka, A.Bass, J., 2007. The clockwork of metabolism. Annu Rev Nutr, 27, 219–240.
Reppert, S. M.Weaver, D. R., 2002. Coordination of circadian timing in mammals. Nature, 418 (6901), 935–941.
Rodriguez-Illamola, A., Lopez Patino, M. A., Soengas, J. L., Ceinos, R. M.Miguez, J. M., 2011. Diurnal rhythms in hypothalamic/pituitary AVT synthesis and secretion in rainbow trout: evidence for a circadian regulation. Gen Comp Endocrinol, 170 (3), 541–549.
Roenneberg, T.Merrow, M., 2002. Life before the clock: modeling circadian evolution. J Biol Rhythms, 17 (6), 495–505.
Ruby, N. F., Burns, D. E.Heller, H. C., 1999. Circadian rhythms in the suprachiasmatic nucleus are temperature-compensated and phase-shifted by heat pulses in vitro. J Neurosci, 19 (19), 8630–8636.
Saad, M. F., Riad-Gabriel, M. G., Khan, A., Sharma, A., Michael, R., Jinagouda, S. D., Boyadjian, R.Steil, G. M., 1998. Diurnal and ultradian rhythmicity of plasma leptin: effects of gender and adiposity. J Clin Endocrinol Metab, 83 (2), 453–459.
Sahar, S.Sassone-Corsi, P., 2012. Regulation of metabolism: the circadian clock dictates the time. Trends Endocrinol Metab, 23 (1), 1–8.
Sharma, V. K., Lone, S. R., Goel, A.Chandrashekaran, M. K., 2004. Circadian consequences of social organization in the ant species Camponotus compressus. Naturwissenschaften, 91 (8), 386–390.

Simon, C.Brandenberger, G., 2002. Ultradian oscillations of insulin secretion in humans. Diabetes, 51 Suppl 1, S258–261.

Soriano, M. I., Roibas, B., Garcia, A. B.Espinosa-Urgel, M., 2010. Evidence of circadian rhythms in non-photosynthetic bacteria? J Circadian Rhythms, 8, 8.

Spiegel, K., Leproult, R., L'Hermite-Baleriaux, M., Copinschi, G., Penev, P. D.Van Cauter, E., 2004. Leptin levels are dependent on sleep duration: relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. J Clin Endocrinol Metab, 89 (11), 5762–5771.

Spruyt, E., Verbelen, J. P.De Greef, J. A., 1987. Expression of circaseptan and circannual rhythmicity in the imbibition of dry stored bean seeds. Plant Physiol, 84 (3), 707–710.

Spruyt, E., Verbelen, J. P.De Greef, J. A., 1988. Ultradian and circannual rhythmicity in germination of Phaseolus seeds. Journal of Plant Physiology, 132, 5.

Svanes, C., Sothern, R. B.Sorbye, H., 1998. Rhythmic patterns in incidence of peptic ulcer perforation over 5.5 decades in Norway. Chronobiol Int, 15 (3), 241–264.

Takahashi, J. S., 2004. Finding new clock components: past and future. J Biol Rhythms, 19 (5), 339–347.

Takahashi, J. S., Hong, H. K., Ko, C. H.McDearmon, E. L., 2008. The genetics of mammalian circadian order and disorder: implications for physiology and disease. Nat Rev Genet, 9 (10), 764–775.

Tzameli, I., 2012. Special focus: Mammalian circadian rhythms and metabolism. Trends Endocrinol Metab, 23 (7), 311.

Vitaterna, M. H., Takahashi, J. S.Turek, F. W., 2001. Overview of circadian rhythms. Alcohol Res Health, 25 (2), 85–93.

Whitehead, K., Pan, M., Masumura, K., Bonneau, R.Baliga, N. S., 2009. Diurnally entrained anticipatory behavior in archaea. PLoS One, 4 (5), e5485.

Zhang, E. E.Kay, S. A., 2010. Clocks not winding down: unravelling circadian networks. Nat Rev Mol Cell Biol, 11 (11), 764–776.

Zimmerman, W. F., Pittendrigh, C. S.Pavlidis, T., 1968. Temperature compensation of the circadian oscillation in drosophila pseudoobscura and its entrainment by temperature cycles. J Insect Physiol, 14 (5), 669–684.

Zmrzljak, U. P.Rozman, D., 2012. Circadian regulation of the hepatic endobiotic and xenobitoic detoxification pathways: the time matters. Chem Res Toxicol, 25 (4), 811–824.

Zvonic, S., Ptitsyn, A. A., Conrad, S. A., Scott, L. K., Floyd, Z. E., Kilroy, G., Wu, X., Goh, B. C., Mynatt, R. L.Gimble, J. M., 2006. Characterization of peripheral circadian clocks in adipose tissues. Diabetes, 55 (4), 962–970.