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Barbora Černá

Vzájemný vztah mezi cirkadiánními hodinami, příjmem potravy a obezitou

Mutual relationship between circadian clock, feeding and obesity

Bakalářská práce

Školitel: PharmDr. Alena Sumová, DSc.

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Poděkování

Chci poděkovat všem, kteří mě při psaní této práce podporovali, zejména své školitelce PharmDr. Aleně Sumové, DSc. za trpělivost a ochotu.

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Podpis

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Abbreviations

AMPK	AMP activated protein kinase
ANS	Autonomic nervous system
BMAL1	Brain and muscle Arnt-like protein
CK1δ/ε	Casein kinase1 delta/epsilon
CLOCK	Circadian locomotor output cycles kaput
CRY	Cryptochrome
DMV	Dorsal motor nucleus of the vagus
GABA	Gamma-aminobutyric acid
GK	Glucokinase
GLUT2	Glucose transporter 2
GSK-3β	Glycogen synthase kinase-3β
GYS2	Glycogen synthase 2
IML	Intermediolateral column of the spinal chord
NAM	Nicotinamide
NAMPT	Nicotinamide phosphorybosyl transferase
PEPCK	Phosphoenolpyruvate kinase
PER	Period
PF	Perifornical area
PGC-1α	Peroxisome proliferator-activated receptor γcoactivator1-α
PPAR	Peroxisome proliferator-activated receptor
PVN	Paraventricular nucleus
ROR	Retinoid related orphan receptor
SCN	Suprachiasmatic nucleus
SIRT1	Sirtuin

Abstract

Circadian clocks synchronize behavioral and biochemical rhythms with environmental factors, such as day/night cycle and changing seasons. The circadian clock mechanism is provided at the molecular level by rhythmic clock genes expression and regulated clock protein modification and degradation. Rhythmicity in our body relates closely to feeding behavior, nutrient utilization and energy storage which comprises carbohydrate and lipid metabolism. The cell has at its disposal specific set of genes, which connect circadian gene machinery with the actual energy state

Disruption in feeding rhythm may lead to development of various metabolic diseases. Modern lifestyle, which allows disturbances of the circadian system by irregular daily routine, e.g., due to shiftwork or traveling across the time zones, has been considered as risk factor for appearance of obesity and metabolic syndrome. The aim of this thesis is to summarize the current knowledge on the mutual interaction between the circadian mechanism and nutrition and on consequences of its disruption with emphasis to obesity, which tends to be a global problem.

Keywords: circadian rhythm, nutrition, metabolism, obesity, AMPK, SIRT1

Abstrakt

Cirkadiánní hodiny synchronizují behaviorální a biochemické rytmy s faktory vnějšího prostředí, jako je střídání dne a noci a změna ročních období. Mechanismus cirkadiánních hodin je na molekulární úrovni řízen rytmickou expresí hodinových genů, regulovanou modifikací a degradací hodinových proteinů. Rytmicita v těle je úzce spojena s příjmem potravy a jejím využitím, které zahrnuje jak metabolismus karbohydrátů, tak i lipidů. Buňka disponuje specifickou sérií genů, které propojují cirkadiánní hodiny s jejím aktuálním energetickým stavem.

Narušení rytmů v příjmu potravy může vést k vývoji různých metabolických chorob. Moderní životní styl, který zvyšuje pravděpodobnost výskytu poruch cirkadiánního systému nepravidelnou denní rutinou, například prací na směny nebo cestováním přes časová pásma, je považován za rizikový faktor pro vznik obezity a metabolického syndromu. Cíle této práce jsou shrnout aktuální poznatky o vzájemných interakcích v oblasti mechanismu cirkadiánních hodin a výživy a o důsledcích jejich narušení s důrazem na obezitu, která má tendenci se stát globálním problémem.

Klíčová slova: cirkadiánní rytmy, výživa, metabolismus, obezita, AMPK, SIRT1

Introduction

There are many cyclical changes in the environment, which affect our bodies and behavior, such as the alternation of day and night and seasons. It is important for the organism to equalize with these changes as effectively as possible and predict them. The most significant changes are alternation of day and night with 24 h period of a solar day.

Nearly every living organism is equipped with an internal timekeeping system which rhythmically drives processes in the body so that they exhibit about 24 h (circadian) rhythms. This system works at the molecular level and is composed of cellular clocks connected by many neural and hormonal pathways in our body.

The circadian clocks are able to generate rhythmic signals which control various physiological processes, including behavior. The organism thus exhibits two separate phase: the activity and the rest. The distribution of these phases does not only relate to activity and sleep, but also to timing of feeding.

In general, there are two different metabolic processes in out body: catabolic and anabolic reactions. The catabolic reactions are represented by degradation and heat generation. They proceed throughout the day, but prevail mostly during the rest phase, when active energy intake from food is limited and homeostasis must be maintained from energy supply. Anabolic processes predominately occur during the active period. In this part of the day, the organism gains energy actively from food and the excess of it is stored for later utilization in the form of glycogen or fat stores.

Circadian clocks at the molecular level separate anabolic and catabolic reactions according to the environment via affecting expression of various genes, which participate in energy storage and utilization. Concurrently, the current energy state of the cell affects circadian clocks. Obesity and unbalanced glucose plasma level are associated with disruptions at the molecular and behavioral levels. Irregular daily rhythm in feeding can affect clock genes machinery and lead to metabolic diseases. Defects at the molecular level disrupt rhythmic switching of genes, which participate in maintaining an internal clock, and disconnect circadian clocks and metabolism. The result of this disconnection is unbalanced energy storage and obesity.

1 Mechanism of circadian clocks at the molecular level

At the molecular level, the circadian clocks operate via the system of feedback loops regulating expression of clock genes and controlling degradation of their protein products (Fig.1).

Among the clock components, there are two important transcription factors, called BMAL1 (brain and muscle Arnt-like protein) and CLOCK (circadian locomotor output cycles kaput). These two proteins dimerize and in the nucleus, they bind to the E-box promoter sequence on promoters of other clock genes. After the binding the transcription of clock genes *Period* (*Per1/2*) and *Cryptochrome* (*Cry1/2*) genes is initiated. In the cytoplasm, PER1/2 and CRY1/2 proteins are translated and form PER:CRY complexes which translocate to the nucleus and inhibit the BMAL:CLOCK activity leading to inhibition of *Per* and *Cry* transcription (Gekakis et al., 1998; Kume et al., 1999).

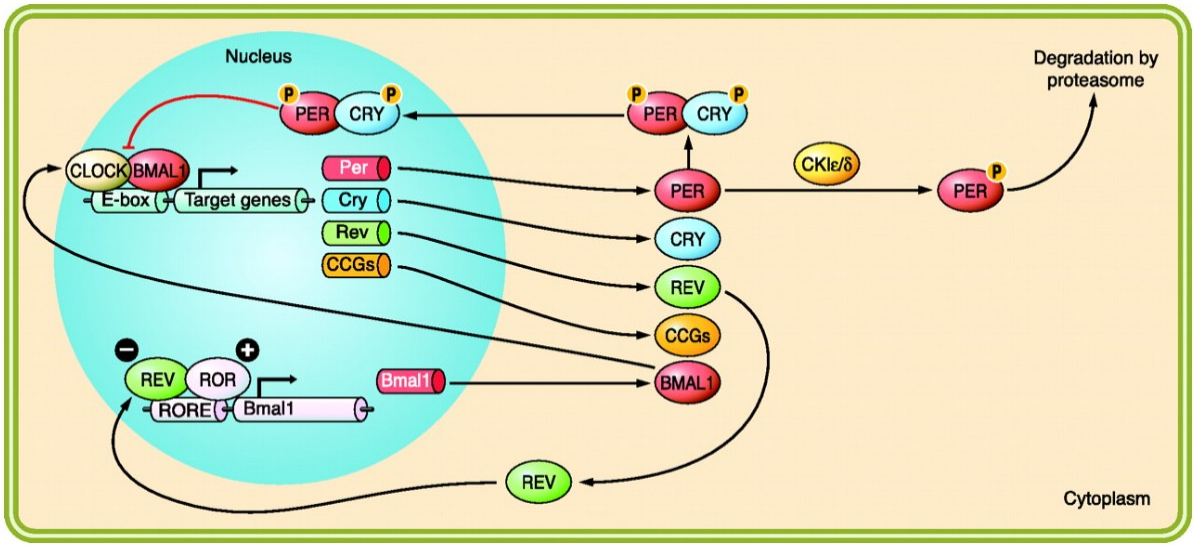

Figure 1: General representation of two basic feedback loops in the circadian gene machinery *(Golombek and Rosenstein, 2010)*

The next feedback loop consists of *Bmal1* expression activator and repressor. Except the *Per* and *Cry*, the BMAL:CLOCK complex initiate retinoid related orphan receptor (ROR) and *Rev-erbα* transcription. ROR and REV-ERBα products compete in binding to orphan receptor response elements, which take place in *Bmal1* promoter. Whereas REV-ERBα

participates in decrease of *Bmal1* transcription, ROR supports it (Guillaumond et al., 2005; Ueda et al., 2002).

These positive and negative feedback loops are crucial within the circadian clock mechanism.

The protein modifications have significant roles in clock protein cellular localization and their degradation. Without clock protein modifications, especially phosphorylation, the circadian clock would provide oscillations with period much shorter than 24 hours (Shanware et al., 2011).

Casein kinase1 exists in two forms: delta (CK1δ) and epsilon (CK1ε). CK1ε is the one of the clock protein kinases, which plays a role in timing of the negative regulation of *Clock* and *Bmal1* gene transcription via regulation of PER nuclear entry and degradation (Camacho et al., 2001). Apart from PER proteins, CK1ε also interacts with BMAL1 and CRY (Eide et al., 2002).

The effect of CK1ε is related with *tau* mutation first discovered in golden hamsters. These animals show a significantly shorter period of free-running locomotor activity, which is only 20 h when they carry the tau mutation homozygously (Ralph and Menaker, 1988). Except rodents, CK1δ mutation was identified in humans who suffered from advanced sleep-wake cycles. This abnormality is called familial advanced sleep phase syndrome (FASPS). In FASPS, stability of PER2 protein is changed due to CK1 delta mutation (Shanware et al., 2011; Xu et al., 2005) or mutation of PER2 phosphorylation site (Vanselow et al., 2006).

Except CK1, also casein kinase2 (CK2) is important within the circadian clock mechanism. This kinase affects feedback loops by BMAL1 phosphorylation, which disrupts BMAL1 nuclear accumulation (Tamaru et al., 2009).

Cry1, *Cry2* and *Per2* levels are regulated by activated AMP protein kinase (AMPK). AMPK induces phosphorylation of these clock genes and leads them to degradation (Camacho et al., 2001; Lamia et al., 2009; Um et al., 2007).

2 The SCN

In mammals, the circadian system is hierarchically organized with the central pacemaker, situated in the suprachiasmatic nucleus (SCN) of anterior hypothalamus and subordinated clocks in other brain areas and periphery (Balsalobre et al., 1998; Yamazaki et al., 2000). The SCN consists of paired structures, nuclei, and works as the principal synchronizer of the rhythmic behavioral and physiological processes with the environment (Card and Moore, 1984). The temporal regulation of these processes is dependent on correct setting of the SCN phase relative to daytime. In case of the SCN damage, these rhythms are lost (Edgar et al., 1993; Stephan and Zucker, 1972).

The synchronization with the external environment is mainly achieved by light information provided through the retina. The retina is interconnected with the SCN by the monosynaptic pathway, the retinohypothalamic tract, which innervates subpopulation of the SCN cells called the core. The other pathway is indirect and involves intergeniculate leaflet, which provides secondary visual informantion to the SCN through geniculohypothalamic tract (Abe and Rusak, 1992; Edelstein and Amir, 1999). The reception of photic input from the retina distinguishes the central clock from the other clocks in the body (Abrahamson and Moore, 2001).

3 Peripheral clock

Clocks in the peripheral tissues are also able to generate endogenously the rhythmic signal and drive the tissue specific processes. For example circadian gene expression is shown in the liver (Tahara et al., 2012), pancreatic tissue (Muhlbauer et al., 2004) or adipose tissue (Ando et al., 2005).

The peripheral clocks need to be synchronized by the central clock in SCN, which provides them the information about daytime. In case of SCN lesions, the peripheral clocks persist in generation of their rhythms. The rhythm was recorded for about 20 days at the organ level, however, in absence of the entraining signal from the central clock, its amplitude gradually decreased due to desynchrony among the cellular clocks (Yoo et al., 2004).

3.1 Communication of the SCN with peripheral clocks

To maintain all the body synchronized with the light/dark cycle, the SCN must communicate with the clocks in the peripheral organs. This connection involves neuro-endocrine and autonomic pathways, which are controlled by the SCN activity. The SCN projects its output via neurotransmitters, namely gamma-aminobutyric acid (GABA), vasopressin, vasoactive intestinal polypeptide (Abrahamson and Moore, 2001; TeclemariamMesbah et al., 1997), prokineticin 2 (Prosser et al., 2007) and glutamate (Kalsbeek et al., 2008).

The autonomic nervous system is controlled by the SCN via its projections to the paraventricular nucleus of the hypothalamus (PVN) (Fig.2) (Wang et al., 2003). From the PVN, the neurons project to the intermedio-lateral column of the spinal chord, as the pre-ganglionic sympathetic neurons, or to the dorsal motor nucleus of the nervus vagus.

The neuro-endocrine output of the SCN consists of regulation of hormonal levels, namely the melatonin and corticosterone. Production of melatonin is controlled by the SCN via projections to the PVN and then by a multisynaptic pathway to the pineal gland (Perreau-Lenz et al., 2005). Corticosterone level shows circadian rhythm with the peak at the beginning of the night and its production is regulated by the hypothalamic-pituitary-adrenal axis (Buijs et al., 1999; Kalsbeek et al., 1996) and by sympathetic pathways (Oster et al., 2006). These hormones participate as a signal transformers for all the body.

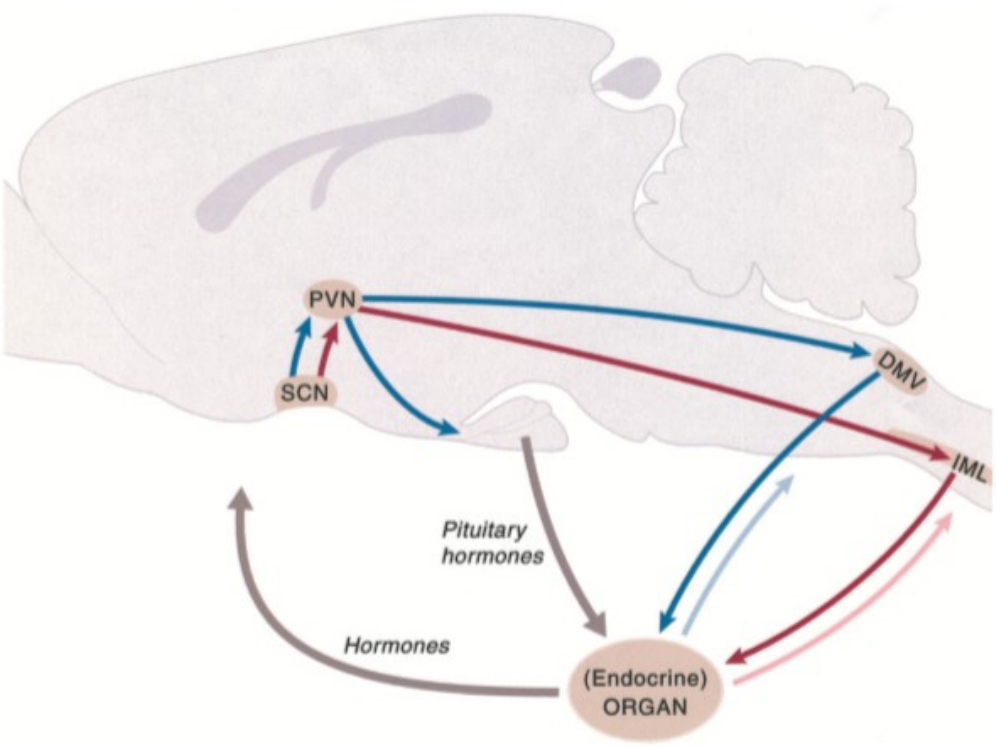

Figure 2: Connection of the SCN with the body via autonomic nervous system and pituitary gland

The SCN projects to the PVN, where three different pathway are separated: (1) the endocrine pathway, associated with melatonin secretion, (2) parasympathetic pathway, which affects the body via dorsal motor nucleus of the vagus (DMV) and (3) sympathetic pathway, which leads from the PVN to preganglionic neurons in the intermediolateral column of the spinal cord (IML) (Buijs et al., 2003).

3.2 The liver

As mentioned before, hepatocytes show circadian clock gene rhythms in vitro and in vivo (Balsalobre et al., 1998; Tahara et al., 2012). Due to synchronization with the activity and rest phases, the liver is connected with the SCN through neural (Kalsbeek et al., 2004) and humoral pathway (Gomez et al., 1997).

The neural connection of the liver with the SCN can be demonstrated on maintaining of the diurnal glucose rhythm, peaking at the end of the active period (Fig.3). The glucose rhythm sustains under food deprivation (Kalsbeek et al., 2008).

The SCN sends an inhibitory GABAergic and stimulatory glutamate input to orexin-containing neurons in hypothalamus and regulates orexin releasing. The GABAergic signalization is associated with its rhythmic withdrawal at the end of the active period,

whereas glutamate signal shows constant stimulation (Kalsbeek et al., 2008). Due to the rhythmic inhibitory GABAergic effect, orexin release from the hypothalamus shows a circadian rhythm with its peak at the end of the activity period (Zhang et al., 2004). Release of orexin stimulates the sympathetic branch of the autonomic nervous system (ANS) and induces hepatic glucose output (Yi et al., 2009).

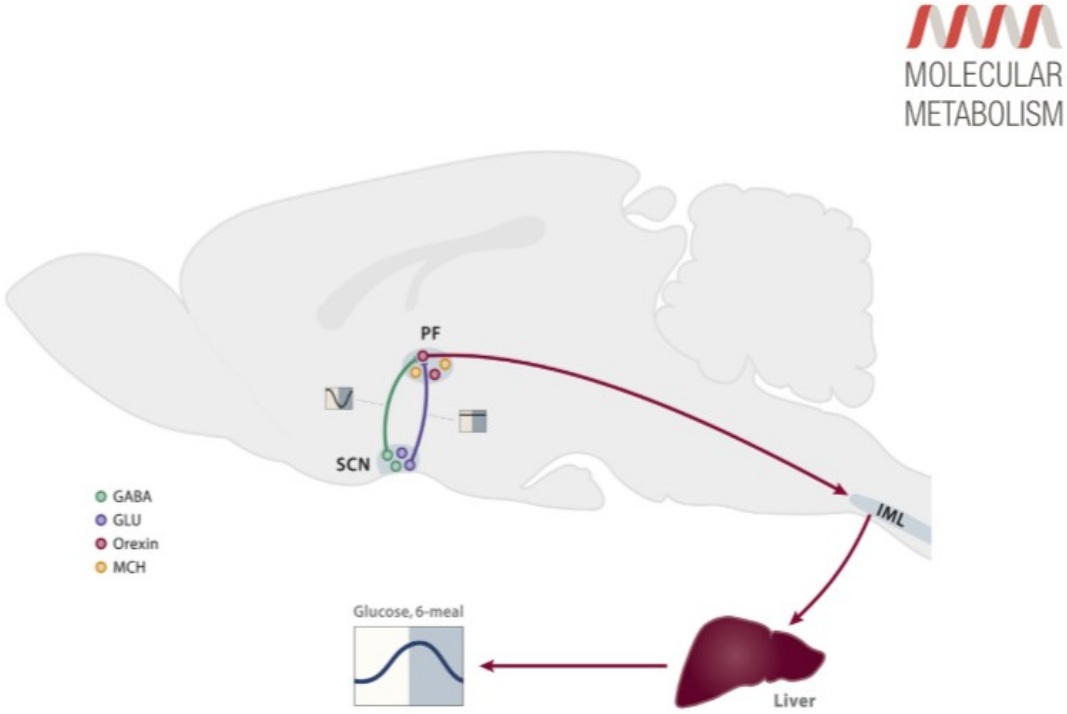

Figure 3: Formation of the diurnal glucose rhythm

The SCN projects by GABAergic (green) and glutamatergic (blue) projections to the orexin containing neurons in the perifornical area (PF). Whereas stimulatory glutamatergic input is constant, the inhibitory GABAergic input to the PF exhibits circadian rhythm. The orexin containing neurons become active and their excitatory effect (red) is conducted to the intermediolateral column of the spinal chord (IML). IML activates sympathetic pathway to the liver and induces hepatic glucose production (Kalsbeek et al., 2010).

Hepatocytes show diurnal rhythm in expression of several hundreds transcripts. Many of them are associated with glucose and cholesterol metabolism and participate in their transport (Panda et al., 2002).

The glycogen level shows diurnal rhythm due to rhythm in glycogen synthase activation (Ishikawa and Shimazu, 1976) and expression (Doi et al., 2010), peaking at the end of the dark period in nocturnal rodents. Glycogen synthase 2 (GYS2) is regulated by circadian

proteins via two E-boxes, which are located in the first intron of the *Gys2* gene. In case of *Clock* gene disruption, *Gys2* expression is damped and rhythm in plasma glucose is unbalanced, due to disturbed rhythm in glycogen production (Doi et al., 2010).

Glycogen metabolism is also affected by AMPK and PPAR, proteins which are associated with signalization of the current energy state of the cell. Role of AMPK and PPAR will be described in chapter 4.

Gluconeogenesis is influenced by circadian clocks via *Cry1* and *Cry2* genes. These genes interact with glucocorticoid receptor in the liver and induce repression of phosphoenolpyruvate kinase (PEPCK). A mouse with *Cry1* and *Cry2* deficiency shows disruptions in glucose homeostasis (Lamia et al., 2011) and the *Bmal1* gene deletion and *Clock* gene mutation lead to inhibition or suppression of gluconeogenesis (Rudic et al., 2004)

Targeted *Bmal1* disruption in the mouse liver affected glucose homeostasis through hepatic glucose export, which was attenuated by deregulation of diurnal expression of glucose transporter 2 (GLUT2) (Lamia et al., 2008)

3.3 Adipose tissue

Adipose tissue shows circadian rhythmicity in clock genes (Zvonic et al., 2006).

Bmal1 gene induces lipid differentiation, stimulates expression of genes, which operate in lipogenesis and suppress expression of *Pepck* gene (Shimba et al., 2005). *Bmal1* affects secretion of adipose hormones and its deletion causes increased adiposity under the normal diet (Kennaway et al., 2013) and obesity. The *Bmal1* gene deletion and *Clock* gene mutation abolish rhythms in triglyceride (Rudic et al., 2004)

Adipogenesis is also regulated by *Per2* gene, interacting with the peroxisome proliferator-activated receptor γ (PPARγ), which is closely associated with fat modulation (He et al., 2003) and represses its expression (PPARγ will be described in detail in chapter 4). *Per2* null mouse gains weight less than wild type and show substantial decrease in plasma levels of triacylglycerol (Grimaldi et al., 2010).

Leptin, which is rhythmically secreted in the adipose tissue (Langendonk et al., 1998) provides information about energy storage of the body to the hypothalamus. In response to leptin concentration, which is higher in obese individual (Langendonk et al., 1998; Yildiz et al., 2004), the hypothalamus reduces appetite. An experiment, with *Bmal1* null mice shows, that *Bmal1* gene is related to leptin oscillations (Kennaway et al., 2013). Adiponectin secretion also shows circadian rhythmicity and its levels show opposite pattern to

leptin secretion (Ando et al., 2005). Adiponectin diurnal cycle is disrupted in obese individuals (Yildiz et al., 2004).

4 Interaction between the circadian clock and the cellular metabolic state

Various proteins, which participate in metabolism, affect the major circadian feedback loop, and form the connection between circadian clocks and energy utilization (Fig.4). This connection is essential for the clock synchronization with metabolism and its disruption results in unbalanced plasma glucose level (Dasgupta et al., 2012), insulin resistance or damaged fat modulation (He et al., 2003).

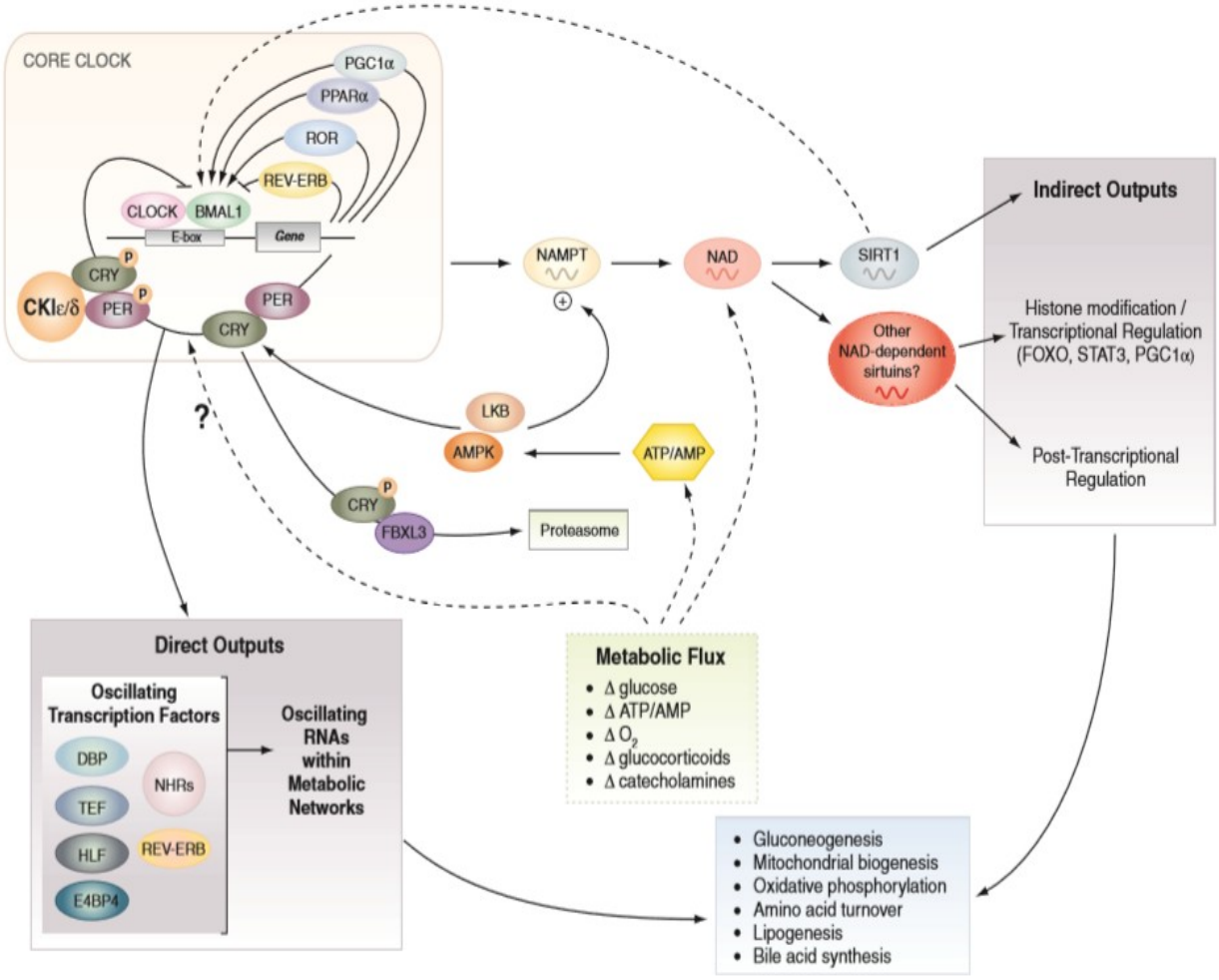

Figure 4: Outputs of the major circadian feedback loop and pathways, which participate in its interaction with and metabolism

The circadian feedback loop synchronize circadian clock with the metabolism by indirect and direct outputs. Concurrently the major clock genes machinery receives signal about current energy state. For more detail, see text. (Bass and Takahashi, 2010)

4.1 AMPK

AMP-activated protein kinase or 5' adenosine monophosphate-activated protein kinase or AMPK is an enzyme, which connects the current energy state and circadian clocks in the cell. AMPK is sensitive to AMP and ADP levels (Carling et al., 1987; Xiao et al., 2011) and therefore can detect fluctuations in the ATP/AMP/ADP ratio. Concurrently, AMPK affects circadian clock feedback loop by tagging *Per2*, *Cry1* and *Cry2* genes for degradation, as mentioned before. AMPK activation is associated with increase in glucose uptake and fatty acid oxidation in skeletal muscle (Merrill et al., 1997).

AMPK participates in food intake regulation through the hypothalamus. Leptin, insulin, high glucose level and refeeding decrease AMPK activity in hypothalamus that results in reducing the appetite. Agouti-related protein (Minokoshi et al., 2004) and ghrelin (Andersson et al., 2004) increase AMPK activity in the hypothalamus, which induces the food intake.

AMPK consists of α, β and γ subunits. The α subunit exhibits catalytic activity and its deletion induces intracellular stress by activating NAD(P)H oxidase (Wang et al., 2010) and insufficient contraction-mediated glucose transport in skeletal muscle (Lefort et al., 2008). The β subunit has a glycogen binding domain (Polekhina et al., 2003). Physical connection between AMPK and glycogen enables the control of the status of cellular energy reserves and regulates AMPK activity, which is reduced after binding to glycogen (McBride et al., 2009).

AMPKβ appears in two isoforms: AMPKβ1 and AMPKβ2, which are located in different parts of the body. Whereas AMPKβ2 is expressed in the skeletal muscle, AMPKβ1 is associated with the liver and adipose tissue (Chen et al., 1999). AMPK β2 deficient mouse is not able to sustain euglycemia during exposure to stress and shows disturbed glycogen metabolism in the skeletal muscle (Dasgupta et al., 2012). AMPK β1 deletion induces reduced gluconeogenesis in the liver and enhanced insulin sensitivity in conjunction with reduced appetite (Dzamko et al., 2010).

The γ subunit is associated with ADP or AMP binding. This connection induces conformational change within the AMPK, which prevents against its inactivation (Adams et al., 2004; Chen et al., 2012; Xiao et al., 2011).

AMPK affects circadian clock by phosphorylation of Ser 389 of CKIε, which results in increasing of CKIε activity (Um et al., 2007). This kinase induces *Per2* degradation through its phosphorylation (Camacho et al., 2001). The circadian period in Rat-1 fibroblasts

is shortened due to exposure of the activator of AMPK metformin (Um et al., 2007). Due to the role of AMPK in metabolism, metformin is used as a drug in the treatment of type 2 diabetes (Consoli et al., 2004).

AMPK may also influence clock genes by activation of SIRT1(Canto et al., 2009), which induces deacetylation and therefore activation of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (Canto et al., 2009), whose function is described in 4.4.

4.2 SIRT1

Sirtuin (SIRT1), a NAD-dependent deacetylase, is a protein whose activity depends on the presence of nicotinamide adenine dinucleotide (NAD+). NAD+ levels show circadian oscillation (Ramsey et al., 2009) and reflect the energy state of the cell via SIR1, connecting the cell metabolism with the major feedback loop of circadian genes via BMAL1 repression and histone deacetylation (Fig. 4) (Nakahata et al., 2008).

As mentioned before, SIRT1 activity is affected by AMPK. AMPK, which is another cell sensor of energy state, induces SIRT1 transition to the active phase by increasing NAD+ level. SIRT1 activity involves downstream SIRT1 targets, for example PGC-1α (Canto et al., 2009). Except AMPK activity, SIRT1 affects its own activation. A product of SIRT1 deacetylation activity, nicotinamide (NAM), is a negative regulator of SIRT1(Bitterman et al., 2002). The nicotinamide phosphoribosyl transferase (NAMPT) is an enzyme, which participates in restoration NAD+ from NAM. The *Nampt* gene is rhythmically expressed due to BMAL1:CLOCK binding to its E-box (Ramsey et al., 2009).

SIRT1 is rhythmically accumulated in the cell, whereas *Sirt1* mRNA values do not show any rhythmic changes. SIRT1 binds to PER2 and CLOCK in a circadian manner (Asher et al., 2008) and cooperates with the acetylase activity of CLOCK (Doi et al., 2006).

Histone acetylase activity of CLOCK exerts a circadian rhythm, which leads to circadian activation of gene expression. SIRT1 and CLOCK exhibit opposing effect on acetylation. Except regulation of histone deacetylation, SIRT1 also deacetylates BMAL1 on Lys 537 and decreases the CRY protein efficiency to silence CLOCK:BMAL1 activation complex. In general, oscillation of NAD+ correlates with active state of SIRT1 and this oscillation is opposite to that of histone and BMAL1 acetylation. SIRT1 also initiate PER2 degradation by its rhythmic deacetylation (Asher et al., 2008).

Sirt1 knock-out mice exert repressed *Bmal*, *Per2* and *Cry1* mRNA levels, whereas *Rev-erbα* mRNA levels are not affected. Levels of nuclear receptor Rorγ are reduced (Asher et al., 2008).

SIRT1 interacts and activates (PGC-1α) by deacetylation (Nemoto et al., 2005).

4.3 PPARs

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptor proteins, which exist in three subtypes: PPARα, PPARβ and PPARγ. PPARα is associated with lipid and carbohydrate metabolism in the liver and induction of fasting response (Aoyama et al., 1998; Leone et al., 1999). PPARγ is expressed in the adipose tissue and the liver (Yang et al., 2006) and involves adipocyte differentiation (Barak et al., 1999) and PPARβ operates with up-regulation of fatty acid synthesis and glycogen production in the liver and muscle (Liu et al., 2011; Yang et al., 2006).

PPARα exhibits diurnal expression with its peak at the beginning of the night (Yang et al., 2006) and interacts with PGC-1α in the liver (Liu et al., 2011), which is described in 4.4.

PPARβ supports fatty acid synthesis and glucose utilization. Overexpression of PPAR induces expression of genes such as glucokinase (GK), glucose transporter 2 (GLUT2) and PGC-1β . Co-activation of PPARβ with PGC-1β induces higher acetyl-CoA carboxylase2 promoter I activity and increase PPARβ-controlled GK promoter activity. *Pparβ* expression is followed by AMPK activation due to PPARβ induced sensitivity to adiponectin and increasing of AMP level (Liu et al., 2011). AMPK may serve as a feedback mechanism to PPARβ effect.

PPARγ is closely connected with adipose tissue. PPARγ is rhythmically expressed in adipocytes and fluctuates in synchrony with leptin and adiponectin secretion (Yang et al., 2006). PPARγ involves fat modulation and its targeted deletion causes adipocyte hypertrophy, elevated levels of triglyceride and plasma free fatty acids in adipose tissue and induces insulin resistance in the liver and adipocytes (He et al., 2003).

4.4 PGC-1α

Transcriptional coactivators, which exhibit the connection between clock gene transcription and nutrition are coactivators of PGC-1 family (Finck and Kelly, 2006). Through coactivation of ROR proteins, the PGC-1α induces expression of *Bmal1* (Fig. 4) and *Rev-erb*

genes (Liu et al., 2007). PGC-1α coactivator is involved in mitochondrial biogenesis in muscle cells (Wu et al., 1999). It is produced in the mice liver during fasting and in mice with insulin deficiency. PGC-1 induction in the liver increases glucose release by involvement of gluconeogenic enzymes (Yoon et al., 2001). Attenuation of PGC-1α expression in mice shows disrupted metabolic cycles due to the clock and metabolic gene abnormalities (Liu et al., 2007).

4.5 Glycogen synthase kinase-3

The glycogen synthase kinase-3β (GSK-3β) is a serin/threonin kinase which participates in glycogen synthesis and is also involved in regulation of circadian rhythms. The GSK3β exhibits a robust circadian rhythm in its phosphorylation at Ser-9; in the phosphorylated state, the enzym is deactivated (Iitaka et al., 2005). It is expressed in the SCN and hepatic cells and the phosporylation peaks at the end of the light period in the liver and at the end of the dark period in the SCN (Iitaka et al., 2005).

The GSK-3 can affect the circadian clock because application of a GSK-3 inhibitor causes subsequent phase-delay in expression *Bmal1* and *Per2* genes (Iitaka et al., 2005).

GSK-3 phosphorylates PER2 protein and affects its nucleus localization because GSK-3 inhibitor, which reduced PER2 phosphorylation to 62,7%, remained its localization predominantly cytoplasmatic (Iitaka et al., 2005). The level of PER2 in the nucleus shows circadian rhythm and the PER2 accumulation in the nucleus peaks at the time, when GSK-3 phosphorylation is minimal (Field et al., 2000).

5 Obesity

Disruptions in food intake, disorganization in feeding rhythm and obesity can affect circadian clock genes expression (Kaneko et al., 2009). On the other hand, disruptions in circadian clocks affects feeding behavior and energy storage through changes in secretion of hormones, which are associated with hunger (Turek et al., 2005). In general, desynchronization of circadian rhythms with food intake may cause disruptions in energy balance and energy storage.

As mentioned before, circadian clock genes are directly or indirectly connected with the current energy state of the cell. In case of clock gene disruptions, the cell lacks correct information about the actual energy state, which may lead to many metabolic syndromes at the level of the organism. The obesity leads to changes in expression of clock genes and PGC-1α (Otway et al., 2011).

Proper function of *Clock* gene is essential for a well-balanced food intake and healthy weight. Homozygous *Clock* mutant mouse exhibits disrupted behavior, which leads to attenuation of diurnal feeding rhythm, and is associated with obesity and metabolic syndrome. This mutant mouse shows disrupted glucose and lipid metabolism, reduced energy expenditure and involves *Per2* gene expression (Turek et al., 2005). Although changes in feeding rhythms lead to obesity, *Clock* gene mutation affects fat absorption via reduction of cholecystokinin-A receptor and lipase gene expression in the pancreas, which leads to attenuation obesity (Oishi et al., 2006).

Bmal1 deficiency does not result in such obesity phenotype as found in *Clock* mutant mouse. *Bmal1* deficient mouse shows similar weight gain under high fat diet conditions as wild type (Hemmeryckx et al., 2011). On the other hand, adipocyte-specific deletion of *Bmal1* caused obesity and a shift in the diurnal rhythm of food intake (Paschos et al., 2012).

Development of obesity is supported by alteration of the time of feeding. Mice consume most of their nutriment during the night. When food presentation takes place during the light phase, mice gained more weight compared to animals, those fed at night (Arble et al., 2009). The same results in present in patients with night eating syndrome. Night eating syndrome is associated with change in natural feeding pattern, where most calories are consumed during the night. Patients suffering from this syndrome tend to higher body mass index and psychological distress (Colles et al., 2007)(Colles et al., 2007).

Desynchronization in the time of feeding and circadian clocks is closely associated with shift work. Shift workers exhibit increased concentrations of plasma triglycerides and higher probability of obesity (Karlsson et al., 2001).

Conclusion

The mammalian cell utilizes various proteins which participate in metabolism and its regulation. Many of these regulatory mechanisms are under circadian control, which restricts their effects to a specific part of the day. This thesis summarizes relationship between the temporal time-keeping mechanism and mechanisms regulating the energetic state at the molecular and organ level. The summarized data show that the current energy state of the cell can affect circadian clocks and vice versa.

In this work, obesity is represented as a result of disruption in circadian clock system at both molecular and behavioral level. Increasing prevalence of obesity in the population due to the modern lifestyle could emphasize this issue and generate considerable interest about this global problem.

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