Chapter 11
Adenosine and Other Purinergic Products in Circadian Timing

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Abstract The circadian oscillator plays an important role in behavior and metabolic physiology. In turn, adenosine occupies a unique position as both a fundamental neuromodulator and a basic building block of cellular metabolism. Multiple connections exist between the two, both through the direct actions of adenosine and through the cellular signaling cascades regulating and regulated by its availability. Specifically, we show that the circadian clock is connected to adenosine and other purinergic products on three levels. At the level of circadian signaling, the adenosine-derived signaling molecule cAMP is itself a circadian clock component that indirectly induces transcription of many circadian genes, as well as influencing cell cycle timing. At the level of metabolism, AMP kinase, a cellular energy sensor dependent upon AMP, can phosphorylate multiple clock proteins. It phosphorylates cryptochromes and thereby enhances the activity of the inhibitory clock protein complex that contains them. The histone and clock protein deacetylase SIRT1 is also phosphorylated and upregulated by AMPK, leading to increased clock protein degradation and chromatin repression. SIRT1 activity is also regulated by NAD^+ cofactors, whose levels are themselves under both circadian and metabolic control. Finally, multiple adenosine receptor subtypes can control clock function. A3 receptors influence mammalian temperature control and therefore possibly the circadian oscillator. A1 receptor transcription can be induced indirectly via glucocorticoids which are under circadian control. In addition, A1 receptors modulate light responsiveness of the circadian clock. Taken together, this intricate regulatory web likely permits a complex dialogue between metabolism and diurnal behavior and physiology that allows organisms to exploit their circadian geophysical environment optimally.

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In this chapter, we not only outline the existing connections between adenosine and the circadian oscillator defined by research of numerous different laboratories, but also show the vast potential for therapeutically relevant connections whose mechanisms remain to be elucidated. These connections are bidirectional: on the one hand, the circadian clock controls adenosine and its downstream products, which are also important signaling molecules; on the other, adenosine and related molecules directly regulate the circadian oscillator. The drama of this interrelationship plays at the level of both the single cell and the whole organism, in both brain and peripheral tissues. Below, after a general introduction to the circadian oscillator in which we briefly mention these connections, we discuss each of these links in detail, looking first at adenosine itself, and then at several of its products, with a focus on the phosphorylated nucleotides ATP and AMP.

Of course, no discussion of this topic would be complete without a consideration of the important role that adenosine also plays in sleep–wake behavior, the major behavioral output of the circadian clock. This complex and fascinating topic is the subject of a separate chapter.

11.1 Introduction to the Circadian Clock

Most life on earth is governed by 24 h light–dark changes. An internal clock has evolved in both unicellular and multicellular organisms in order to perceive diurnal environmental cues of light, temperature, and nutrients and to control behavioral and physiological output in order to anticipate these environmental changes. This "circadian" clock (from the Latin *circa diem*, "approximately a day"), is found in nearly all cells of multicellular organisms.

In complex organisms, the multitude of circadian oscillators in peripheral cells is controlled by a superior clock in the brain. Transplantation experiments in the late 1980s by Lehman et al. using Syrian hamsters revealed the suprachiasmatic nucleus (SCN) of the hypothalamus as the master circadian clock: animals with lesions in the SCN completely lost rhythmic daily behavior, and implantation of fetal SCN tissue was able to restore this circadian locomotor activity entirely (Lehman et al. 1987).

In order to be functionally useful, the circadian clock must be able to do more than just oscillate with a period of approximately 24 h. It should also be able to sense environmental cues and changes, and further to transmit this information on a behavioral and physiological level. Conceptually, the molecular circuitry responsible for each of these processes is separable. Thus, the circadian clock mechanism in general can be divided in three parts: an input part, where incoming stimuli are processed and passed on, a core part with a ticking molecular clock, and an output part, which transmits information to all target pathways of the circadian oscillator

throughout the body. As we shall see, adenosine and its metabolites can influence all three of these parts independently. In turn, the levels of these compounds are influenced both directly and indirectly by the circadian oscillator.

11.2 How the Rising Sun Stimulates Signal Transmission in the Brain: The Morphology of Clock Input

In mammals, the most important and potent "zeitgeber" or timing cue from the environment, is light. After light is perceived in the retina by both conventional rods and cones and specialized retinal ganglion cells containing the alternative photopigment melanopsin (Berson et al. 2002; Drouyer et al. 2007), it is signaled glutamatergically directly via the retinohypothalamic tract (RHT) to the SCN (Castel et al. 1993). The SCN itself is a heterologous tissue consisting of at least two regions: a core and a shell. While the latter is thought to be primarily an oscillator, the former shows weak intrinsic oscillation but significant induction by RHT stimulation, and is believed to be crucial to clock input (Foley et al. 2011; Moore et al. 2002)

Brain region-specific lesion experiments in rats could show that in addition to the SCN at least two other nuclei in the brain are important for input processing: the intergeniculate leaflets (IGL) and the dorsale raphe nuclei (DRN). Although light signals are transmitted directly to the SCN by the RHT, all other nonlight signals are thought to proceed indirectly by first reaching the DRN (Moga and Moore 1997). The IGL seems to have a more general control function: light signals from the RHT and nonphotic input from the DRN are projected to the IGL before the information is passed on again to SCN neurons, giving rising evidence for an integrative function of the IGL for all environmental cue signaling. Nevertheless, its detailed function remains to be elucidated.

As discussed more completely below, one obvious way in which adenosine affects the circadian oscillator is via its actions as an inhibitory neurotransmitter that modulates the activities of these brain nuclei.

11.3 The Ticking Molecular Core Clock in the SCN

In mammals, the circadian clock mechanism itself depends upon a set of regulatory feedback loops containing both transcriptional and posttranslational components. The CLOCK/BMAL DNA binding complex enhances initially the transcription of Period genes (*Per1*, *Per2*, and *Per3*) and Cryptochromes (*Cry1* and *Cry2*). The PER and CRY proteins are translocated back to the nucleus and inhibit their own transcription by binding to the nuclear CLOCK/BMAL complex. Since both PER and CRY protein and RNA are unstable, the nuclear mRNA and protein level of both proteins drop as soon as this inhibition occurs. When their levels drop low enough, inhibition ceases and transcription of *PER* and *CRY* starts again (for review see Reppert and Weaver 2001).

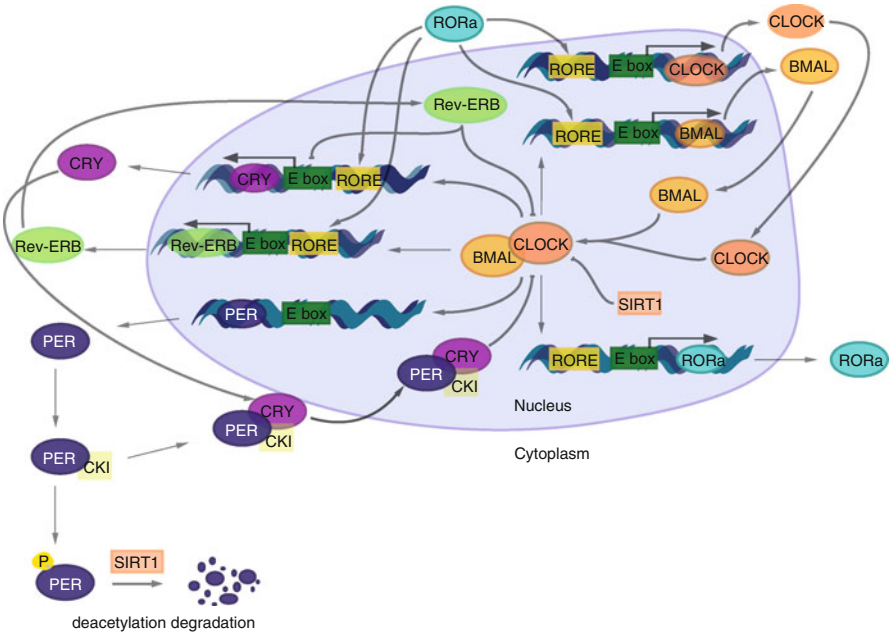

Fig. 11.1 Mechanistic overview of the circadian clock. The mammalian molecular clock responsible for diurnal behavior consists of several interlocked feedback loops of transcription and translation. A cycle begins with the enhanced transcription of Period (*Per1*, *Per2*, and *Per3*) and Cryptochrome (*Cry1* and *Cry2*) by binding of the CLOCK/BMAL heterodimer to DNA. The PER and CRY proteins are translated and then translocated back to the nucleus to inhibit their own transcription. Meanwhile, posttranslational modifications by casein kinase 1ε (CK1ε) and other kinases modify these proteins and target them for ubiquitination and proteosome-mediated degradation. As a result, the nuclear mRNA and protein level of both proteins drop to a level at which inhibition is reversed and transcription of PER and CRY starts again. A second interlocked feedback loop is formed by the action of REV-ERBα, the orphan nuclear receptor and repressor. Its transcription is enhanced by CLOCK/BMAL, but its protein product inhibits *Bmal1* transcription. Opposing it is RORα, whose transcription is also enhanced by CLOCK/BMAL, but whose activity activates *Bmal1* transcription. Metabolic feedback possibly occurs at the level of SIRT1, whose NAD^+-dependent histone deacetylation activity can associate with CLOCK proteins to deacetylate chromatin, BMAL, or PER. Adapted from Fu and Lee (2003)

Several mechanisms stabilize the oscillation of this core loop. For example, a second interlocked feedback loop is formed by the action of REV-ERBα, the orphan nuclear receptor and repressor, via inhibition of *Bmal1* transcription. Posttranslational modification of PER proteins, first to stabilize and then to destabilize them, likely also plays a key role via a number of kinases such as casein kinase 1ε (CK1ε) (Ripperger and Merrow 2011). A host of accessory factors involved in chromatin modification (WDR5, NONO, SFPQ, MLL) also participate (Brown et al. 2005; Duong et al. 2011; Katada and Sassone-Corsi 2010). Among these are the sirtuins, which are sensitive to cellular redox potential via their use of NAD^+ cofactors (Imai et al. 2000) (Fig. 11.1).

Recently, a separate independent circadian mechanism based upon redox modification of proteins has also been discovered. Its mechanism is as yet unknown. Because it is completely independent of either transcription or of known clock proteins, and in addition induces cyclic oscillations of a number of proteins important in buffering cellular redox capacity, it could prove important for many aspects of circadian metabolism (O'Neill et al. 2011). Hence, a second general way in which adenosine affects the circadian oscillator is via its effects upon cellular redox mechanisms. Since these mechanisms also affect adenosine levels, this pathway is bidirectional.

Although the SCN is the seat of the master circadian clock in the brain, the molecular clock mechanisms described above are in fact present in all of the cells of the body. First evidence for extra-SCN clocks came from experiments with retinal clocks identified in *X. laevis* (Besharse and Iuvone 1983) and in rabbits (Brandenburg et al. 1983). Light-induced entrainment of these oscillators could also be demonstrated (Tosini and Menaker 1996). These retinal clocks are independent of SCN rhythms and are important for proper photoreceptor metabolism (Besharse and Dunis 1983) and rod and cone shedding (Green and Besharse 2004). Adenosine plays a key role in the regulation of this process (Ribelayga and Mangel 2005).

11.4 Clock Output, from SCN to Behavior and Physiology

Subsequently, clocks were discovered in nearly all cells of the body, and therefore peripheral tissue like fibroblasts are often used as cellular models to monitor oscillator function (Cuninkova and Brown 2008). More directly, however, one way that the SCN controls circadian physiology is through the synchronization of these peripheral oscillators. Interestingly, the signals by which it does this are a mixture of direct and indirect ones: neural control of circadian behavior affects food intake and body temperature, both of which synchronize circadian clocks; SCN control of endocrine function provides hormonal synchronizing stimuli; and the sympathetic nervous system can also provide synchronizing cues (reviewed in Dibner et al. 2010; Hastings et al. 2007; Schibler et al. 2003).

In order to control behavior like rest–wake activity (Moore 2007) or memory consolidation (Eckel-Mahan and Storm 2009; Ruby et al. 2008) the SCN was shown to have several efferents to the diencephalon, into distinct nuclei in the hypothalamus like the supraventricular zone or the preoptic zone, as well as thalamic regions and the pineal gland (for review see Card 2000). Second, the SCN innervates several organs through the autonomic nervous system (reviewed in Bartness et al. 2001) by which it controls physiological aspects such as heart rate or liver activity (for review see Rüger and Scheer 2009; Shibata 2004). The adrenal cortex (for review see Dickmeis 2009) and glucocorticoid secretion (reviewed in Kalsbeek et al. 2012) is under direct SCN control via the hypothalamic pituitary adrenal gland axis and can furthermore be directly influenced by light (Ishida et al. 2005). Several other hormones like melatonin or luteinizing hormone are also under circadian control

(Lucas et al. 1999). Since adenosinergic modulation of neurotransmission occurs at most brain loci, it is plausible that adenosine might influence a number of these regulatory points.

The neural and hormonal pathways discussed above are directly responsible for a portion of circadian physiology. Another portion, however, is indirectly regulated by food intake. Even though it is tightly controlled, the circadian clock is still a flexible system. Light is known to be the strongest synchronizer, which means concretely that the clock can adjust to changes in seasonal light availability, but food also has a quite strong impact on circadian timing, especially upon peripheral oscillators. Normally, feeding time is controlled by behavior, which is SCN-governed in keeping with light. Thus, it is a potent timing cue from SCN to peripheral clocks. If feeding time is constrained, however, peripheral circadian physiology will adjust to match (Damiola et al. 2000; Stokkan et al. 2001) and in the absence of dominant light cues, behavior will follow. Feeding experiments with different diet-forms, high caloric, hypocaloric, or normocaloric, showed that the locomotor behavior adapted to the new Zeitgeber time (Challet 2010). It remains unclear what exactly in food in general has this strong shifting ability, and how this feedback reaches the clock. On the one hand, hormonal or neural signals in response to food probably play an essential role (Mistlberger 2011). On a cellular level, there is also rising evidence that factors from the sirtuin family link metabolism and therefore probably feeding to the circadian clock (for review see Asher and Schibler 2011; Bellet and Sassone-Corsi 2010), mainly by acting posttranslationally upon histones or clock proteins to modify transcription. All sirtuin families require nicotinamide adenine dinucleotide (NAD^+) as cofactor for their enzymatic activities of ADP-ribosylation and deacetylation (Imai et al. 2000). As mentioned earlier in the context of the core oscillator, the sirtuin SIRT1 interacts directly with clock proteins (Asher et al. 2008). The fact that SIRT1 is dependent on NAD^+ makes it a sensor of the metabolic state of the cell similar to ATP.

11.5 The Many Faces of Adenosine

Adenosine is well established as one of the principal inhibitory neuromodulators of the central nervous system. Numerous studies establish that it is critical in maintaining the delicate neurochemical balance that permits conscious thought, rest, and the maintenance of physiological equilibrium. Equally importantly, it is a precursor of intracellular purinergic signaling pathways and a basic building block of ATP, the energy currency of the cell (reviewed in Dunwiddie and Masino 2001). The functions of adenosine are therefore ubiquitous on both cellular and systemic levels. Interestingly, all of the aforementioned processes—energy regulation and metabolism, physiology, rest and activity, and memory consolidation—are regulated in mammals by the circadian oscillator via a complex bidirectional web of both intra- and inter-cellular signaling pathways. Not surprisingly, as we discuss below, adenosine figures prominently within these pathways.

Both the functions of adenosine and its sources in the CNS vary widely. Research in the past 30 years revealed many different functions and aspects of adenosine activity in the cell as well as in the extracellular matrix, be it in the form of a direct neuromodulator or as modifier of the activity of other receptors like D1 or NMDA (Manzoni et al. 1994; Popoli et al. 1996), to mention two examples. Due to its wide functionality, the metabolism of adenosine is a well-studied topic, and all precursors and breakdown products are known. Extracellular adenosine is dependent on two main processes, first on the degradation of extracellular AMP to adenosine by ecto-5′nucleotidase and second on the secretion of intracellular adenosine, which itself has two different origins. The intracellular adenosine level depends on the degradation of ATP to AMP and finally to adenosine via the cytoplasmic 5′nucleotidase as well as on the hydrolysis of S-adenosylhomocysteine (SAH). Interestingly the ATP-AMP dephosphorylation process is highly concentration-dependent and therefore changes in intracellular adenosine levels can reflect changes in metabolic rate. More specifically, the formation of adenosine from AMP occurs as the concentration of ATP changes in a physiological range, most likely due to the activity of a specific subtype of cytoplasmic 5′nucleotidase (for review see Latini and Pedata 2001) (Fig. 11.2). However, as ATP is not the only source of adenosine, the relationship between adenosine and ATP concentration is most probably not a linear one.

On the contrary, to reduce levels of adenosine two major reactions can occur, either via phosphorylation of adenosine by adenosine kinase (ADK) resulting in raising concentrations of 5′AMP, or via the generation of inosine and later on inosine monophosphate (IMP) by an irreversible reaction of adenosine with adenosine deaminase (ADA). Both reactions are necessary tools to stabilize intra-and extracellular pools of adenosine (for review see Dunwiddie and Masino 2001).

11.6 From Bottom to Top: Adenosine as Regulator of the Circadian Clock

11.6.1 Adenosine Receptors

The first adenosine agonist experiments more than 15 years ago were carried out with hamsters, where application of the selective adenosine agonist N6-cyclohexyladenosine (CHA) revealed a regulative aspect of adenosine for the clock (Watanabe et al. 1996). In particular, phase advances induced by changed light settings were diminished after CHA application to the SCN (Elliott et al. 2001). Administration of the adenosine antagonist caffeine did not affect the circadian clock, though it did diminish sleep and stimulate arousal (Antle et al. 2001).

Surprisingly, subsequent experiments in mice showed that the observed changes in phase-shifting were regulated by A1 adenosine receptors (Sigworth and Rea 2003). It was already known that relatively few adenosine A1 receptor mRNAs are present in the hypothalamus or SCN, so it remained unclear how A1 receptor

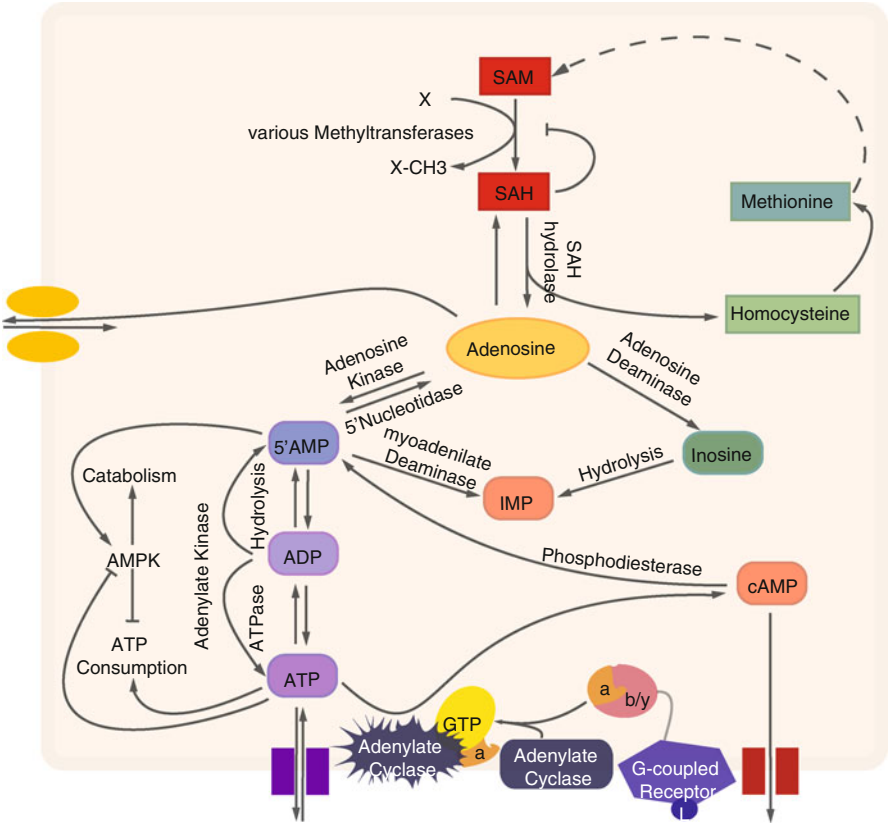

Fig. 11.2 Adenosine metabolism. Adenosine is formed by the sequential breakdown of ATP into first ADP and then 5′AMP via the enzymatic activity of a cytosolic 5′nucleotidase. To a lesser extent, S-adenosylhomocystein (SAH) derived from S-adenosylmethionine (SAM) also serves as a source of cellular adenosine. Adenosine kinase (ADK) and adenosine deaminase (ADA) instead promote the breakdown reaction of adenosine. ADK activity leads to the production of 5′AMP by adenosine phosphorylation. ADA converts adenosine to inosine in an irreversible reaction. Subsequent hydrolyis of inosine results in inosine monophosphate (IMP), an intermediate of purine metabolism. In addition to sequential breakdown to form adenosine, ATP is also a substrate for adenylate cyclase, whose activation by G-coupled receptors promotes the formation of the signaling molecule cAMP, which can itself be transformed into 5′AMP. The "energy metabolites" of the cell (ATP, ADP, and AMP) can bind to AMP kinase (AMPK) to regulate its activity. AMPK is itself a sensor of the cellular energy status and can promote ATP formation

signaling acts on SCN clock activity. Work by Hallworth and coworkers was able to show that A1 receptors located in the retinohypothalamic tract play an important role in attenuation of the release of glutamate and therefore regulate the transmission of light information to the SCN (Hallworth et al. 2002).

The A2a and A2b receptors are both expressed in the brain—A2a rather ubiquitously, and A2b in specific regions—and both are also expressed in somatic tissue

(for details see review Dunwiddie and Masino 2001). For both of them, agonists show no effects upon the circadian clock and there is no further indication that they might play a role in controlling the transmission of input cues to or within the SCN (Fredholm et al. 2000; Hallworth et al. 2002; Latini and Pedata 2001). The A3 receptor was also tested with several different agonists but light-induced phase advances were not observed (Elliott et al. 2001).

11.6.2 Adenosine Monophosphate

The first metabolic product of adenosine by adenosine kinase (ADK) is AMP, and in subsequent enzymatic steps ADP and ATP are produced. Together with adenosine deaminase, ADK is the principal regulator of intracellular adenosine availability. AMP levels themselves, or more specifically the cellular ratio of AMP–ATP, is highly controlled. AMP protein kinase (AMPK) is a direct sensor for changed AMP–ATP ratios, or better, energy levels in the cell in general (reviewed in Hardie 2007). Due to the fact that the adenylate kinase enzyme keeps the catalysis of $2\text{ADP} \rightarrow 1\text{ATP} + 1\text{AMP}$ near to equilibrium, the ATP–AMP ratio varies approximately as the square of the ATP–ADP ratios, which makes the AMP–ATP ratio a reliable index for metabolic processes in the cell (Kahn et al. 2005). Moreover, AMP has the ability to activate AMPK through three different mechanisms—allosteric activation, phosphorylation, and inhibition of dephosphorylation—AMPK is therefore a very sensitive sensor for changes in cellular energy status (reviewed in Hardie 2007). In its turn, through phosphorylation of a diversity of targets, AMPK can organize metabolic pathways depending upon hormonal inputs like leptin or adiponectin, as well as coordinate the response to stress-induced depletion of ATP (Kemp et al. 1999).

What does this have to do with the regulation of circadian clocks? In fact, one of the targets of AMPK is the circadian clock! In 2009 Lamia and coauthors demonstrated nicely by using the agonist aminoimidazole carboxamide ribonucleotide (AICA), that AMPK phosphorylates the circadian repressor protein CRY1, thereby stabilizing its binding to PER2 while decreasing its degradation. As a result, the transcription normally induced by the CLOCK/BMAL complex is suppressed, and the "trigger" for a new clock cycle is inactivated (Lamia et al. 2009). In a related story 2 years before, another group showed that AMPK phosphorylates CK1ε, which itself controls the degradation of PER2 (Um et al. 2007). The same group published data this year about tissue- and isoform-specific activity of AMPK and concluded that AMPK controls expression of peripheral clock genes (Um et al. 2011). Beside this direct interaction of AMPK on the circadian clock, it has been observed in muscle tissue that AMPK also triggers the activation of SIRT1 (Cantó et al. 2009, 2010). SIRT1, as discussed previously, deacetylates PER2 in order to degrade PER2 protein and create the environment for a new cycle (Asher et al. 2008).

11.6.3 Adenosine Triphosphate

Recent research shows that adenosine in the form of ATP also seems to be an important factor to link metabolism to clock function. In the simplest existing circadian oscillator, found in the cyanobacterium *Synechococcus elongates*, the protein products of the *KaiABC* locus, together with ATP, are themselves sufficient to generate a 24 h rhythm, even in vitro. One of those proteins, KaiC, has two ATP binding sites (Ishiura et al. 1998) and was later on found to display ATPase activity under the control of KaiA (Pattanayek et al. 2009). In fact, the rate of ATP hydrolysis is crucial to set clock speed (Rust et al. 2011).

Even if the KaiABC clock is not conserved directly in mammalian cells, ATP hydrolysis equally provides timing and directionality to circadian processes in mammals. For example, diurnal modulation of a Na^+/K^+-ATPase in rat neurons directly influences firing rate (Wang and Huang 2004). Highest ATPase enzyme activity was found during the subjective day, when calcium concentrations were also increased. In fact, the membrane potential and firing of rat SCN neurons is highly dependent on the actual activity status of the Na^+/K^+-ATPase (Wang and Huang 2006). Similar regulation of firing could explain the spontaneous circadian electrical activity of SCN neurons.

Although the ATPase of the previous paragraph was governed by circadian mechanisms, ATP itself is mostly regulated by metabolic function and energy consumption. Briefly, ATP is generated or (more accurately) recycled in several catabolic subprocesses during glycolysis or in the respiration chain reaction in mitochondria. In the same process in which ATP is synthesized, NAD^+ is oxygenated and vice versa. In the context of circadian clocks, it has been suggested that food/energy uptake modulates ATP and NAD^+ levels (Ishikawa and Shimanzu 1976; Mistlberger 2011) thereby regulating clock function. The effects of this energy input on clock output are discussed in the next paragraph. Apart from these metabolic considerations, however, ATP may directly affect clock function via P2X7 purinergic receptors, a class of ligand-gated ion channel whose ATP-dependent activation results in subsequent upregulation of *Per1* clock gene expression in some tissues (Nakazato et al. 2011).

11.7 Metabolism and Clock Output: NAD^+ as a Linker

The ability of food to entrain the circadian clock has itself hinted repeatedly at a long-suspected connection between metabolism and circadian function (reviewed in Asher and Schibler 2011). Nevertheless, the molecular currency of this link is not clear. Beside ATP, nicotinamide adenine dinucleotide (NAD^+) is a second potent linker between energy metabolism and the circadian clock. As stated above, recent data has shown that the histone acetylase SIRT1 is able to counterbalance the activity

of CLOCK on a chromatin level (Nakahata et al. 2009), and also to modulate PER protein function (Asher et al. 2008). In turn, SIRT1 activity is directly modulated by intracellular levels of NAD^+. (As explained below, because NAD^+ salvage is itself under circadian control via the CLOCK/BMAL-controlled transcription of nicotinamide phosphoribosyltransferase (NAMPT) (Nakahata et al. 2009; Revollo et al. 2004), this pathway is bidirectional.)

11.8 From Top to Bottom: Diurnal Regulation of Adenosine and Adenosinergic Factors

As discussed above, adenosine levels in the retinohypothalamic tract are critical to regulating light-induced phase-shifting in the SCN. In studying these adenosine-dependent currents, however, Ribelayga found in a rabbit retinal model system additionally a strong direct dependency upon light and darkness (Ribelayga and Mangel 2005). Thus, adenosine levels in the extracellular matrix of the rabbit retina are highly sensitive to light, and this sensitivity is more pronounced during the subjective night than day (Brandenburg et al. 1983). The authors therefore suggested a threshold light intensity for the modulation of adenosine as a function of the circadian clock. Concretely, this double regulation means that extracellular baseline levels of adenosine are circadian, with high levels at the subjective night and lower levels during the day. The underlying mechanism is still not fully understood, but it appears that not only less adenosine is transported to the intracellular compartment but also the intracellular purinergic fraction is increased. Both factors lead to an extracellular accumulation of adenosine in the observed circadian pattern (Ribelayga and Mangel 2005). The physiological effects of this regulation upon neural activity remain unclear. Of course, adenosine is also an important regulator of sleep–wake behavior—the most important behavioral output of the circadian system—but this topic is treated in a separate chapter.

Metabolic products of adenosine are also regulated in circadian fashion, most notably ATP, in many examined tissues. For example, Womac et al. reported circadian ATP fluctuations in brain, with highest levels at night. The authors suggest that one consequence of this oscillation would be a circadian modulation of intracellular signaling between astrocytes and neurons in the brain (Womac et al. 2009). In this context, ATP serves as an auto- as well as a paracrine factor, which is thought to regulate intracellular calcium waves, the interconnection between astrocytes and neurons, and even brain metabolism in general (Bernardinelli et al. 2004; Haydon 2001; Womac et al. 2009).

More generally still, metabolomic analysis of cultured human or mouse cells (R. Dallmann, personal communication) shows circadian levels of ATP as well as most redox molecules. This suggests a global circadian regulation of mitochondrial function, which in turn affects ATP and adenosine levels.

11.9 Outlook: The Extending Front of Circadian Adenosine Regulation

11.9.1 Adenosine and cAMP Signaling Pathways in the Central Nervous System

So far, we have considered the circadian effects of adenosine and the metabolic products whose production is directly dependent upon it—AMP and ATP. However, these products are themselves the source of signaling molecules crucial to circadian function. ATP is not only a source of adenosine, but also cAMP, which is a cellular signaling molecule for both the circadian oscillator and other processes. For example, a study focusing on melatonin synthesis in the pineal gland revealed a special role for cAMP in controlling the circadian expression of genes (Kim et al. 2005). Melatonin synthesis requires (S)-Adenosylmethionine (SAM) activity, which in turn is dependent on methionine adenosyltransferase II (METII), an enzyme with increased activity during the night. It is clear that the circadian expression of METII is directly under the control of cAMP signaling pathways, though various mechanisms have been proposed (Chik et al. 2007; Ho et al. 2007; Kim et al. 2005). A study some years later was even able to show that the adrenergic/cAMP signaling pathway controls more than 600 genes in the pineal gland and drives their nocturnal expression (Bailey et al. 2009). Another study published comparable results, showing that a homeobox gene with strong circadian expression in the pineal gland itself serves as a transcription factor in the adult pineal gland. Interestingly, the authors as well postulated adrenergic/cAMP signaling mechanisms as mediators of the circadian oscillator (Rath et al. 2009).

A completely different aspect of clock-controlled cAMP is its direct interference in glucogenesis. The impact of the circadian pacemaker on liver glucogenesis is generally accepted, but Zhang et al. recently showed that CRY1, a core clock protein, is able to block cAMP increase and as a consequence pCREB-induced gene expression. CRY1 inhibition interferes upstream of cAMP synthesis by repression of the Gs alpha subunit from activated G protein-coupled receptors and therefore inhibits the activity of the adenylate cyclase (Zhang et al. 2010).

At the same time, cAMP is an important regulator of circadian function. Indeed, inhibitors of cAMP signaling disrupt circadian oscillations at a cellular level and cAMP levels themselves oscillate in circadian fashion. Thus, purinergic signaling in the form of cAMP forms an auxiliary feedback loop within the circadian clock itself (O'Neill et al. 2008). Similarly, Amelio et al. discovered in 2007 a NONO-TORC2 complex which activates CREB targets dependent on cAMP levels (Amelio et al. 2007). NONO itself plays a crucial part of the internal circadian clock (Brown et al. 2005) and might therefore be a clock output transmitter to transcription via cAMP.

11.10 Feedback Loops from Adenosine to the Clock and Back

In the case just described, cAMP is both an effector of circadian output, and itself a regulator of clock function. Such a feedback loop forms a mechanism by which pathways controlled by the clock can themselves influence clock timing, and has become a common theme in the circadian regulation of physiology. It is possible that two evolutionary benefits are obtained by this strategy. First, by coupling multiple feedback loops of cellular signaling, additional resilience is achieved. Very simply, two interlocked feedback loops are more robust to perturbation than just one. In this way, at a cellular level the timing of the circadian oscillator even withstands cell division (Nagoshi et al. 2004). Second, through clock control of a given pathway, an organism can anticipate an environmental change and induce signaling cascades to respond appropriately. By linking some output of this change back to clock timing, repeatedly mistimed inductions can change the phase of the clock to better anticipate these changes. In this way, for example, the circadian clock regulates feeding behavior, but repeatedly mistimed feeding resets the phase of the clock (Damiola et al. 2000; Stokkan et al. 2001).

For all of the cases above, it is likely that adenosine and the purinergic signaling that it governs form parts of such clock-controlled feedback loops. In the case of cAMP, which is one of the most important signaling metabolites in the CNS and peripheral tissues, the case is clear. On the one hand, it shows circadian expression, with highest concentrations in the subjective night. The effects of this are wide-ranging, as we discuss above. Not only is it responsible for different types of indirect circadian signaling (An et al. 2011; Atkinson et al. 2011), but it also activates transcription at *Per* clock gene promoters themselves and is itself therefore necessary for robust clock oscillations (Hastings et al. 2007).

Adenosine itself occupies a similar central position. For example, in retinal cells, its levels vary directly with light (Ribelayga and Mangel 2005). At the same time, however, its abundance affects the ability of the circadian oscillator to be phase-shifted by light (Sigworth and Rea 2003). The elegant result is that the more light there is in the environment, the less the clock is systematically affected by it! Adding additional complexity, an alternative promoter of the A1 receptor itself is induced by glucocorticoids themselves (Ren and Stiles 1999). The physiological implications of this further feedback are as yet unclear.

A final and more complex example is furnished by AMP and ATP in metabolism. Both extracellular and intracellular ATP levels vary in circadian fashion, both in brain regions including the SCN and in retina (Burkeen et al. 2011; Womac et al. 2009), and in cultured astrocytes (Marpegan et al. 2011). Similarly, there exists a global circadian regulation of redox molecules like NAD^+ (Nakahata et al. 2009). Extracellular ATP acts as a signaling molecule for glia, and is thought to play a role in the regulation of brain metabolism (Womac et al. 2009). Meanwhile, as discussed earlier, the AMP–ATP ratio is a cellular "thermometer" for energy levels and regulates mitochondrial function. Lastly, AMP (via AMPK) and NAD^+ (via SIRT1)

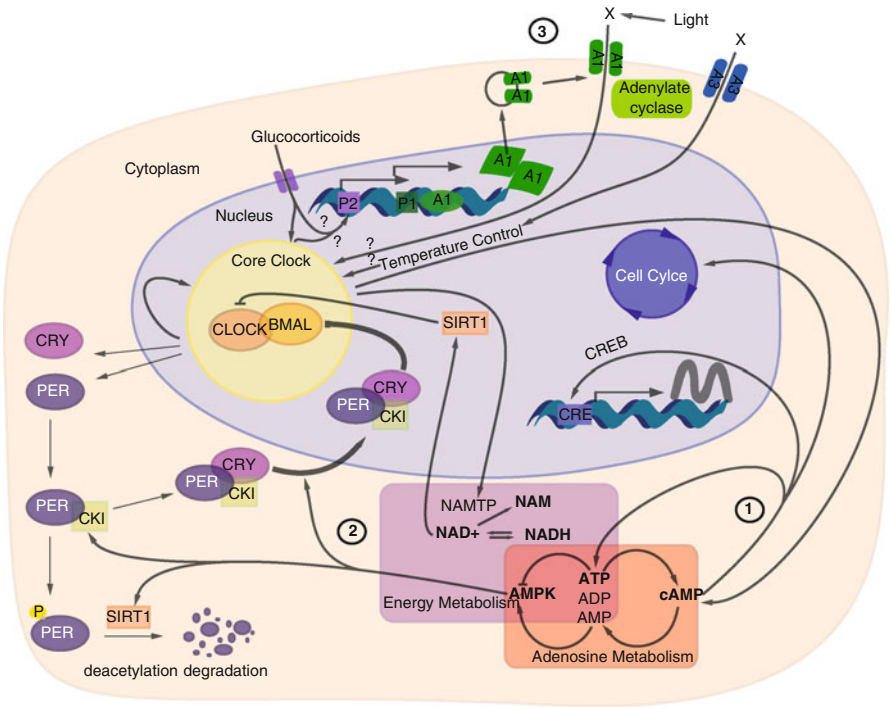

Fig. 11.3 Adenosine and the circadian clock: a model. In this review, we propose that the circadian clock and adenosine are connected on three levels. (1) Circadian signaling pathways. cAMP is itself a circadian signaling molecule and induces transcription of several circadian genes, as well as influencing cell cycle timing. (2) Metabolic clock feedback. AMP kinase, a cellular energy sensor, phosphorylates CRY and thereby enhances the activity of the PER/CRY inhibitory clock protein complex. Moreover, SIRT1 deacetylase activity is upregulated by AMPK, leading to increased PER degradation and chromatin repression. SIRT1 activity is also regulated by NAD+ cofactors, whose levels are themselves under both circadian and metabolic control. (3) Adenosine receptor control of clock function. A3 receptor subtypes influence mammalian temperature control and therefore possibly the circadian oscillator. A1 receptor subtype transcription can be induced indirectly via glucocorticoids which are under circadian control. In addition, A1 receptors modulate light responsiveness of the circadian clock

directly regulate circadian function (Asher et al. 2008; Lamia et al. 2009; Nakahata et al. 2009; Um et al. 2011). Circadian entrainment to feeding is additionally regulated via NAD^+ through the activity of poly-ADP ribose polymerase 1 (PARP1) (Asher et al. 2010), and AMPK itself regulates SIRT1 (Cantó et al. 2009, 2010). These interconnections form in effect another feedback loop (Fig. 11.3). Since ATP signaling also regulates intracellular Ca^{2+} waves, this loop might have more than just metabolic implications: it could also be an important factor for synaptic plasticity and long-term potentiation, regulated in parallel by the homeostatic process of sleep itself (Tononi and Cirelli 2006).

11.11 The Tip of the Iceberg

As discussed extensively elsewhere in this book, adenosine receptors exist in many brain tissues that are also enervated by the SCN, including the hypothalamus, thalamus, or the amygdale. Thus, in the brain alone, the potential exists for many more links between the circadian clock and adenosine that await discovery. For example, the adenosine A3 receptor plays an important role in thermoregulation in mammals (Yang et al. 2010). In fact, however, mammalian body temperature varies slightly in circadian fashion, probably via rest–activity regulation, and these fluctuations are sufficient to entrain and phase-shift peripheral circadian oscillators (Brown et al. 2002). Since circadian body temperature is one of the cues by which the SCN entrains peripheral circadian oscillators, adenosine might play an important unexplored role in this process.

Peripheral tissues also express a variety of adenosine receptors, like A2a, A2b or A3. Some tissues, like muscle, are able to link adenosine metabolism to PER2 degradation via SIRT1 activation (Cantó et al. 2009). In cells throughout the body, the molecular basis of circadian clocks is mostly the same. Thus, where it exists, adrenergic input/output could influence many diverse aspects of circadian physiology.

A variety of pathophysiologies have been directly linked to circadian dysfunction, and in parallel to purinergic signaling dysfunction. It is possible that these correlations are marks of the interplay of circadian clocks with adenosine. Circadian disruption from night shiftwork, for example, has been linked to metabolic syndrome (Knutsson et al. 1999; Pietroiusti et al. 2010). Metabolic disorders have also been tied to adenosinergic dysfunction (Figler et al. 2011; Westermeier et al. 2011). Additionally, many psychiatric disorders are linked to a circadian context—for example bipolar disorders (Mansour et al. 2005) or schizophrenia (Lamont et al. 2010; Moons et al. 2011). Similarly, some depressive disorders have been proposed to arise as consequences of manipulations in the adenosine neuromodulatory system (reviewed in Gomes et al. 2011). Of course, experimental evidence is still missing that directly links the circadian clock to purinergic signaling in any major disease, but the plethora of molecular connections between these pathways makes it likely that such connections exist.

To conclude, research in the past 40 years has discovered more and more connections between adenosine, its downstream products, and the circadian oscillator, in the brain as well as in peripheral tissues. The mechanism of this interaction seems to vary from tissue to tissue, and many interesting connections remain to be found.

References

Amelio AL, Miraglia LJ, Conkright JJ, Mercer BA, Batalov S, Cavett V, Orth AP, Busby J, Hogenesch JB, Conkright MD (2007) A coactivator trap identifies NONO (p54nrb) as a component of the cAMP-signaling pathway. Proc Natl Acad Sci USA 104:20314–20319

An S, Irwin RP, Allen CN, Tsai C, Herzog ED (2011) Vasoactive intestinal polypeptide requires parallel changes in adenylate cyclase and phospholipase C to entrain circadian rhythms to a predictable phase. J Neurophysiol 105:2289–2296

Antle MC, Steen NM, Mistlberger RE (2001) Adenosine and caffeine modulate circadian rhythms in the Syrian hamster. Neuroreport 12:2901–2905

Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, Kreppel F, Mostoslavsky R, Alt FW, Schibler U (2008) SIRT1 regulates circadian clock gene expression through PER2 deacetylation. Cell 134:317–328

Asher G, Reinke H, Altmeyer M, Gutierrez-Arcelus M, Hottiger MO, Schibler U (2010) Poly(ADP-ribose) polymerase 1 participates in the phase entrainment of circadian clocks to feeding. Cell 142:943–953

Asher G, Schibler U (2011) Crosstalk between components of circadian and metabolic cycles in mammals. Cell Metab 13:125–137

Atkinson SE, Maywood ES, Chesham JE, Wozny C, Colwell CS, Hastings MH, Williams SR (2011) Cyclic AMP signaling control of action potential firing rate and molecular circadian pacemaking in the suprachiasmatic nucleus. J Biol Rhythms 26:210–220

Bailey MJ, Coon SL, Carter DA, Humphries A, Kim JS, Shi Q, Gaildrat P, Morin F, Ganguly S, Hogenesch JB et al (2009) Night/day changes in pineal expression of >600 genes. J Biol Chem 284:7606–7622

Bartness TJ, Song CK, Demas GE (2001) SCN efferents to peripheral tissues: implications for biological rhythms. J Biol Rhythms 16:196–204

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

Bernardinelli Y, Magistretti PJ, Chatton J-Y (2004) Astrocytes generate Na^+-mediated metabolic waves. Proc Natl Acad Sci USA 101:14937–14942

Berson DM, Dunn FA, Takao M (2002) Phototransduction by retinal ganglion cells that set the circadian clock. Science 295:1070–1073

Besharse J, Dunis D (1983) Methoxyindoles and photoreceptor metabolism: activation of rod shedding. Science 219:1341–1343

Besharse JC, Iuvone PM (1983) Circadian clock in Xenopus eye controlling retinal serotonin N-acetyltransferase. Nature 305:133–135

Brandenburg J, Bobbert AC, Eggelmeyer F (1983) Circadian changes in the response of the rabbit's retina to flashes. Behav Brain Res 7:113–123

Brown SA, Ripperger J, Kadener S, Fleury-Olela F, Vilbois F, Rosbash M, Schibler U (2005) PERIOD1-associated proteins modulate the negative limb of the mammalian circadian oscillator. Science 308:693–696

Brown SA, Zumbrunn G, Fleury-Olela F, Preitner N, Schibler U (2002) Rhythms of mammalian body temperature can sustain peripheral circadian clocks. Curr Biol 12:1574–1583

Burkeen JF, Womac AD, Earnest DJ, Zoran MJ (2011) Mitochondrial calcium signaling mediates rhythmic extracellular ATP accumulation in suprachiasmatic nucleus astrocytes. J Neurosci 31:8432–8440

Cantó C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J (2009) AMPK regulates energy expenditure by modulating NAD^+ metabolism and SIRT1 activity. Nature 458:1056–1060

Cantó C, Jiang LQ, Deshmukh AS, Mataki C, Coste A, Lagouge M, Zierath JR, Auwerx J (2010) Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. Cell Metab 11:213–219

Card JP (2000) Pseudorabies virus and the functional architecture of the circadian timing system. J Biol Rhythms 15:453–461

Castel M, Belenky M, Cohen S, Ottersen OP, Storm-Mathisen J (1993) Glutamate-like immunoreactivity in retinal terminals of the mouse suprachiasmatic nucleus. Eur J Neurosci 5:368–381

Challet E (2010) Interactions between light, mealtime and calorie restriction to control daily timing in mammals. J Comp Physiol B 180:631–644

Chik CL, Arnason TG, Dukewich WG, Price DM, Ranger A, Ho AK (2007) Histone H3 phosphorylation in the rat pineal gland: adrenergic regulation and diurnal variation. Endocrinology 148:1465–1472

Cuninkova L, Brown SA (2008) Peripheral circadian oscillators. Ann N Y Acad Sci 1129:358–370
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 14:2950–2961
Dibner C, Schibler U, Albrecht U (2010) The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu Rev Physiol 72:517–549
Dickmeis T (2009) Glucocorticoids and the circadian clock. J Endocrinol 200:3–22
Drouyer E, Rieux C, Hut RA, Cooper HM (2007) Responses of suprachiasmatic nucleus neurons to light and dark adaptation: relative contributions of melanopsin and rod cone inputs. J Neurosci 27:9623–9631
Dunwiddie TV, Masino SA (2001) The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 24:31–55
Duong HA, Robles MS, Knutti D, Weitz CJ (2011) A molecular mechanism for circadian clock negative feedback. Science 332:1436–1439
Eckel-Mahan KL, Storm DR (2009) Circadian rhythms and memory: not so simple as cogs and gears. EMBO Rep 10:584–591
Elliott KJ, Todd Weber E, Rea MA (2001) Adenosine A1 receptors regulate the response of the hamster circadian clock to light. Eur J Pharmacol 414:45–53
Figler RA, Wang G, Srinivasan S, Jung DY, Zhang Z, Pankow JS, Ravid K, Fredholm B, Hedrick CC, Rich SS et al (2011) Links between insulin resistance, adenosine A2B receptors, and inflammatory markers in mice and humans. Diabetes 60:669–679
Foley NC, Tong TY, Foley D, LeSauter J, Welsh DK, Silver R (2011) Characterization of orderly spatiotemporal patterns of clock gene activation in mammalian suprachiasmatic nucleus. Eur J Neurosci 33:1851–1865
Fredholm BB, Arslan G, Halldner L, Kull B, Schulte G, Wasserman W (2000) Structure and function of adenosine receptors and their genes. Naunyn Schmiedebergs Arch Pharmacol 362:364–374
Fu L, Lee CC (2003) The circadian clock: pacemaker and tumour suppressor. Nat Rev Cancer 3:350–361
Gomes CV, Kaster MP, Tomé AR, Agostinho PM, Cunha RA (2011) Adenosine receptors and brain diseases: neuroprotection and neurodegeneration. Biochim Biophys Acta 1808:1380
Green CB, Besharse JC (2004) Retinal circadian clocks and control of retinal physiology. J Biol Rhythms 19:91–102
Hallworth R, Cato M, Colbert C, Rea MA (2002) Presynaptic adenosine A1 receptors regulate retinohypothalamic neurotransmission in the hamster suprachiasmatic nucleus. J Neurobiol 52:230–240
Hardie DG (2007) AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. Nat Rev Mol Cell Biol 8:774–785
Hastings M, O'Neill JS, Maywood ES (2007) Circadian clocks: regulators of endocrine and metabolic rhythms. J Endocrinol 195:187–198
Haydon PG (2001) Glia: listening and talking to the synapse. Nat Rev Neurosci 2:185–193
Ho AK, Price DM, Dukewich WG, Steinberg N, Arnason TG, Chik CL (2007) Acetylation of histone H3 and adrenergic-regulated gene transcription in rat pinealocytes. Endocrinology 148:4592–4600
Imai SI, Armstrong CM, Kaeberlein M, Guarente L (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403:795–800
Ishida A, Mutoh T, Ueyama T, Bando H, Masubuchi S, Nakahara D, Tsujimoto G, Okamura H (2005) Light activates the adrenal gland: timing of gene expression and glucocorticoid release. Cell Metab 2:297–307
Ishikawa K, Shimanzu T (1976) Daily rhythm of glycogen synthetase and phosphorylase activities in rat liver: influence of food and light. Life Sci 19:1873–1878
Ishiura M, Kutsuna S, Aoki S, Iwasaki H, Andersson CR, Tanabe A, Golden SS, Johnson CH, Kondo T (1998) Expression of a gene cluster kaiABC as a circadian feedback process in cyanobacteria. Science 281:1519–1523

Kahn BB, Alquier T, Carling D, Hardie DG (2005) AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab 1:15–25
Kalsbeek A, van der Spek R, Lei J, Endert E, Buijs RM, Fliers E (2012) Circadian rhythms in the hypothalamo-pituitary-adrenal (HPA) axis. Mol Cell Endocrinol 349(1):20–29
Katada S, Sassone-Corsi P (2010) The histone methyltransferase MLL1 permits the oscillation of circadian gene expression. Nat Struct Mol Biol 17:1414–1421
Kemp BE, Mitchelhill KI, Stapleton D, Michell BJ, Chen Z-P, Witters LA (1999) Dealing with energy demand: the AMP-activated protein kinase. Trends Biochem Sci 24:22–25
Kim J-S, Coon SL, Blackshaw S, Cepko CL, Møller M, Mukda S, Zhao W-Q, Charlton CG, Klein DC (2005) Methionine adenosyltransferase:adrenergic-cAMP mechanism regulates a daily rhythm in pineal expression. J Biol Chem 280:677–684
Knutsson A, Hallquist J, Reuterwall C, Theorell T, Akerstedt T (1999) Shiftwork and myocardial infarction: a case-control study. Occup Environ Med 56:46–50
Lamia KA, Sachdeva UM, DiTacchio L, Williams EC, Alvarez JG, Egan DF, Vasquez DS, Juguilon H, Panda S, Shaw RJ et al (2009) AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science 326:437–440
Lamont E, Coutu D, Cermakian N, Boivin D (2010) Circadian rhythms and clock genes in psychotic disorders. Isr J Psychiatry Relat Sci 47:27–35
Latini S, Pedata F (2001) Adenosine in the central nervous system: release mechanisms and extracellular concentrations. J Neurochem 79:463–484
Lehman MN, Silver R, Gladstone WR, Kahn RM, Gibson M, Bittman EL (1987) Circadian rhythmicity restored by neural transplant. Immunocytochemical characterization of the graft and its integration with the host brain. J Neurosci 7:1626–1638
Lucas RJ, Stirland JA, Darrow JM, Menaker M, Loudon ASI (1999) Free running circadian rhythms of melatonin, luteinizing hormone, and cortisol in Syrian hamsters bearing the circadian tau mutation. Endocrinology 140:758–764
Mansour HA, Monk TH, Nimgaonkar VL (2005) Circadian genes and bipolar disorder. Ann Med 37:196–205
Manzoni OJ, Manabe T, Nicoll RA (1994) Release of adenosine by activation of NMDA receptors in the hippocampus. Science 265:2098–2101
Marpegan L, Swanstrom AE, Chung K, Simon T, Haydon PG, Khan SK, Liu AC, Herzog ED, Beaulé C (2011) Circadian regulation of ATP release in astrocytes. J Neurosci 31:8342–8350
Mistlberger RE (2011) Neurobiology of food anticipatory circadian rhythms. Physiol Behav 104(4):535–545
Moga MM, Moore RY (1997) Organization of neural inputs to the suprachiasmatic nucleus in the rat. J Comp Neurol 389:508–534
Moons T, Claes S, Martens GJM, Peuskens J, Van Loo KMJ, Van Schijndel JE, De Hert M, van Winkel R (2011) Clock genes and body composition in patients with schizophrenia under treatment with antipsychotic drugs. Schizophr Res 125:187–193
Moore R, Speh J, Leak R (2002) Suprachiasmatic nucleus organization. Cell Tissue Res 309:89–98
Moore RY (2007) Suprachiasmatic nucleus in sleep-wake regulation. Sleep Med 8:27–33
Nagoshi E, Saini C, Bauer C, Laroche T, Naef F, Schibler U (2004) Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. Cell 119:693–705
Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P (2009) Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science 324:654–657
Nakazato R, Takarada T, Yamamoto T, Hotta S, Hinoi E, Yoneda Y (2011) Selective upregulation of Per1 mRNA expression by ATP through activation of P2X7 purinergic receptors expressed in microglial cells. J Pharmacol Sciences 116, 350–361
O'Neill JS, Maywood ES, Chesham JE, Takahashi JS, Hastings MH (2008) cAMP-dependent signaling as a core component of the mammalian circadian pacemaker. Science 320:949–953
O'Neill JS, van Ooijen G, Dixon LE, Troein C, Corellou F, Bouget F-Y, Reddy AB, Millar AJ (2011) Circadian rhythms persist without transcription in a eukaryote. Nature 469:554–558

Pattanayek R, Mori T, Xu Y, Pattanayek S, Johnson CH, Egli M (2009) Structures of KaiC circadian clock mutant proteins: a new phosphorylation site at T426 and mechanisms of Kinase, ATPase and phosphatase. PLoS One 4:e7529

Pietroiusti A, Neri A, Somma G, Coppeta L, Iavicoli I, Bergamaschi A, Magrini A (2010) Incidence of metabolic syndrome among night-shift healthcare workers. Occup Environ Med 67:54–57

Popoli P, Ferré S, Pezzola A, Reggio R, Scotti de Carolis A, Fuxe K (1996) Stimulation of adenosine A1 receptors prevents the EEG arousal due to dopamine D1 receptor activation in rabbits. Eur J Pharmacol 305:123–126

Rath MF, Bailey MJ, Kim J-S, Ho AK, Gaildrat P, Coon SL, Møller M, Klein DC (2009) Developmental and diurnal dynamics of Pax4 expression in the mammalian pineal gland: nocturnal down-regulation is mediated by adrenergic-cyclic adenosine 3′,5′-monophosphate signaling. Endocrinology 150:803–811

Ren H, Stiles GL (1999) Dexamethasone stimulates human A1 adenosine receptor (A1AR) gene expression through multiple regulatory sites in promoter B. Mol Pharmacol 55:309–316

Reppert SM, Weaver DR (2001) Molecular analysis of mammalina circadian rhythms. Annu Rev Physiol 63:647–676

Revollo JR, Grimm AA, Imai SI (2004) The NAD biosynthesis pathway mediated by nicotinamide phosphoribosyltransferase regulates Sir2 activity in mammalian cells. J Biol Chem 279:50754–50763

Ribelayga C, Mangel SC (2005) A circadian clock and light/dark adaptation differentially regulate adenosine in the mammalian retina. J Neurosci 25:215–222

Ripperger JA, Merrow M (2011) Perfect timing: epigenetic regulation of the circadian clock. FEBS Lett 585:1406–1411

Ruby NF, Hwang CE, Wessells C, Fernandez F, Zhang P, Sapolsky R, Heller HC (2008) Hippocampal-dependent learning requires a functional circadian system. Proc Natl Acad Sci USA 105:15593–15598

Rüger M, Scheer F (2009) Effects of circadian disruption on the cardiometabolic system. Rev Endocr Metab Disord 10:245–260

Rust MJ, Golden SS, O'Shea EK (2011) Light-driven changes in energy metabolism directly entrain the cyanobacterial circadian oscillator. Science 331:220–223

Schibler U, Ripperger J, Brown SA (2003) Peripheral circadian oscillators in mammals: time and food. J Biol Rhythms 18:250–260

Shibata S (2004) Neural regulation of the hepatic circadian rhythm. Anat Rec A Discov Mol Cell Evol Biol 280A:901–909

Sigworth LA, Rea MA (2003) Adenosine A1 receptors regulate the response of the mouse circadian clock to light. Brain Res 960:246–251

Stokkan K-A, Yamazaki S, Tei H, Sakaki Y, Menaker M (2001) Entrainment of the circadian clock in the liver by feeding. Science 291:490–493

Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. Sleep Med Rev 10:49–62

Tosini G, Menaker M (1996) Circadian rhythms in cultured mammalian retina. Science 272:419–421

Um J-H, Pendergast JS, Springer DA, Foretz M, Viollet B, Brown A, Kim MK, Yamazaki S, Chung JH (2011) AMPK regulates circadian rhythms in a tissue- and isoform-specific manner. PLoS One 6:e18450

Um JH, Yang S, Yamazaki S, Kang H, Viollet B, Foretz M, Chung JH (2007) Activation of 5′-AMP-activated kinase with diabetes drug metformin induces casein kinase Iε (CKIε)-dependent degradation of clock protein mPer2. J Biol Chem 282:20794–20798

Wang H-Y, Huang R-C (2004) Diurnal modulation of the Na+/K+-ATPase and spontaneous firing in the rat retinorecipient clock neurons. J Neurophysiol 92:2295–2301

Wang Y-C, Huang R-C (2006) Effects of sodium pump activity on spontaneous firing in neurons of the rat suprachiasmatic nucleus. J Neurophysiol 96:109–118

Watanabe A, Moriya T, Nisikawa Y, Araki T, Hamada T, Shibata S, Watanabe S (1996) Adenosine A1-receptor agonist attenuates the light-induced phase shifts and fos expression in vivo and optic nerve stimulation-evoked field potentials in the suprachiasmatic nucleus in vitro. Brain Res 740:329–336

Westermeier F, Salomón C, González M, Puebla C, Guzmán-Gutiérrez E, Cifuentes F, Leiva A, Casanello P, Sobrevia L (2011) Insulin restores gestational diabetes mellitus-reduced adenosine transport involving differential expression of insulin receptor isoforms in human umbilical vein endothelium. Diabetes 60:1677–1687

Womac AD, Burkeen JF, Neuendorff N, Earnest DJ, Zoran MJ (2009) Circadian rhythms of extracellular ATP accumulation in suprachiasmatic nucleus cells and cultured astrocytes. Eur J Neurosci 30:869–876

Yang JN, Wang Y, Garcia-Roves PM, Björnholm M, Fredholm BB (2010) Adenosine A3 receptors regulate heart rate, motor activity and body temperature. Acta Physiol 199:221–230

Zhang EE, Liu Y, Dentin R, Pongsawakul PY, Liu AC, Hirota T, Nusinow DA, Sun X, Landais S, Kodama Y et al (2010) Cryptochrome mediates circadian regulation of cAMP signaling and hepatic gluconeogenesis. Nat Med 16:1152–1156