Published in final edited form as:

Adv Drug Deliv Rev. 2010 July 31; 62(9-10): 904–917. doi:10.1016/j.addr.2010.05.009.

Circadian Rhythms in Gene Expression: Relationship to Physiology, Disease, Drug Disposition and Drug Action

Siddharth Sukumaran¹, Richard R. Almon^{1,2}, Debra C. DuBois^{1,2}, and William J. Jusko²

¹Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260

²Department of Pharmaceutical Sciences, State University of New York at Buffalo, Buffalo, NY 14260

Abstract

Circadian rhythms (24 h cycles) are observed in virtually all aspects of mammalian function from expression of genes to complex physiological processes. The master clock is present in the suprachiasmatic nucleus (SCN) in the anterior part of the hypothalamus and controls peripheral clocks present in other parts of the body. Components of this core clock mechanism regulate the circadian rhythms in genome-wide mRNA expression, which in turn regulate various biological processes. Disruption of circadian rhythms can be either the cause or the effect of various disorders including metabolic syndrome, inflammatory diseases and cancer. Furthermore, circadian rhythms in gene expression regulate both the action and disposition of various drugs and affect therapeutic efficacy and toxicity based on dosing time. Understanding the regulation of circadian rhythms in gene expression plays an important role in both optimizing the dosing time for existing drugs and in development of new therapeutics targeting the molecular clock.

Keywords

molecular clocks; metabolic disease; inflammation; cancer; drug targets; pharmacokinetics

1. Introduction

Biological rhythms are ubiquitous phenomena present in almost all living organisms. Rhythms can be classified into circadian, ultradian, or infradian based on the period length of the rhythm. Rhythms having 24 h periods are classified as circadian, while ones having more than a day or less than a day are referred to as infradian and ultradian. These biological rhythms synchronize various behavioral, biochemical and physiological process with changes in environmental factors thus allowing the organism to adapt, anticipate, and respond to changes effectively. It is well established that many physiological processes and parameters such as body temperature, sleep-wake cycles, feeding behaviors, endocrine functions, hepatic metabolism, renal functions, and many others show rhythmicity [1]. Furthermore, rhythmicity is observed in the transcriptional expression of a wide range of genes which control the rhythmicity of these above mentioned processes. For example, Fig.

© 2010 Elsevier B.V. All rights reserved.

Corresponding Author: Dr. William Jusko, Department of Pharmaceutical Sciences, 565 Hochstetter Hall, State University of New York at Buffalo, Buffalo, NY 14260, wjjusko@buffalo.edu, Phone: 716-645-2855, FAX: 716-645-3693.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 illustrates that in rat skeletal muscle, 109 genes were identified as having a robust circadian rhythm in the magnitude of their expression [2]. However, as also illustrated in that figure, temporal clustering demonstrates that the time course of expression around the 24 h cycle varies among different genes.

1.1. The master clock in the suprachiasmatic nucleus

In mammals, the brain plays an important role in controlling and coordinating circadian rhythms. The central core of the circadian clock, also called the “master clock”, is located in the suprachiasmatic nucleus (SCN) in the anterior part of the hypothalamus [3]. This clock oscillates with an approximately 24 h period even in the absence of external factors. Other circadian oscillators that are present in other parts of the brain and in other organs are referred to as “peripheral clocks” and are controlled by the central master clock. Evidence demonstrating that the master clock is present in the SCN comes from studies where surgical removal resulted in arrhythmicity in various physiological, behavioral, and biochemical process while transplantation of this region back from another donor animal resulted in restoration of the rhythmicity in these processes [4,5]. In addition, isolated SCN regions in cultures maintain rhythmicity in physiological and molecular processes [6].

The SCN receives direct input from the melanopsin ganglion through the retinohypothalamic tract, which entrains and synchronizes the central clock to the external light-dark cycle [7,8]. Outputs from the SCN are directed to other parts of the hypothalamus that control both anterior and posterior pituitary hormones [9], as well as to the area of the hypothalamus and medulla that control the autonomic nervous system [6]. It is these hormonal and autonomic outputs that to a large extent convey the rhythmicity in light-dark cycles to the rest of the body. Besides receiving inputs from the retina, the SCN also receives inputs from forebrain areas and the locus coeruleus that modulate the influence of the SCN [10,11]. The non-retinal inputs to the SCN provide the flexibility for the existence of diurnal and nocturnal animals as well as for animals being able, when necessary, to shift sleep-wake periods from night to day and vice versa [12]. An important consideration that should be kept in mind when evaluating circadian literature is the fact that rats and mice are nocturnal animals (i.e., they are active during the dark period and mainly feed during this period). On the other hand, humans are diurnal animals, in which case the light period represents the main period of activity.

1.2. Genetic components of the molecular clock

In the past two decades significant progress has been made in understanding the molecular process that are responsible for the generation of rhythmicity in the SCN. It is now well accepted that the central clock mechanism involves a transcriptional, post-transcriptional, and translational auto-regulatory feedback loop with a periodicity of approximately 24 h (Fig.2) [7,13,14]. The elements of this feedback loops consist of several transcription factors including CLOCK (Circadian Locomotor Output Cycles Kaput) and BMAL1 (Brain-muscle Arnt like 1) which form the positive arm and PERIOD (PER 1,2,3) and CRYPTOCHROME (CRY 1,2) which form the negative arm of the feedback loop. CLOCK, which is a PAS-basic helix-loop-helix transcription factor (PAS-bHLH), heterodimerizes with BMAL1 (another PAS-bHLH transcription factor) to transactivate the expression of *Per* and *Cry* genes by binding to the E-box element present in the promoter of these genes. In turn PER and CRY proteins, after reaching a critical concentration, form heterotypic complexes and repress the transcriptional activity of the CLOCK:BMAL complexes. This core system is entrained to the light-dark cycle with CLOCK:BMAL being high during the light period and PER:CRY being high during the dark period regardless of whether the animal is diurnal or nocturnal. Other transcription factors that are associated with this process include the orphan receptor REV-ERB alpha (NR1D1), its paralog REV-ERB beta (NR1D2) and retinoic acid

related-orphan receptors RORs [15–17]. *Rev-Erbs* and *RORs* are transcriptionally controlled by the same mechanism as that of *Per* and *Cry* genes. While REV-ERBs negatively regulate the gene expression of BMAL, RORs positively regulate the expression, both through the presence of ROR binding elements (RORE). Although these transcription factors are not involved in rhythm generation, they are very important in controlling the phase and the amplitude of gene expression [14]. In addition to these controls, Casein kinase 1 epsilon (CK1 ξ) phosphorylates PER proteins and tags them for degradation, thereby reducing their stability [18]. It is also known that CK1 ξ can positively regulate BMAL activity by phosphorylation. Furthermore, PAR bZIP transcription factor family proteins which includes hepatic leukemia factor (HLF), thyrotroph embryonic factor (TEF), and D-site binding protein (DBP) are under the direct control of the core circulatory loop and form the clock-controlled output genes [19]. These clock-controlled output transcription factors along with the transcription factors involved in the core circulatory loop control the circadian expression of the transcriptome.

1.3. The circadian clock in peripheral tissues

Studies conducted in cell culture demonstrate that rhythmicity in both gene expression and functions exist in cells of peripheral tissues. For example, serum shock synchronizes circadian gene expression in immortalized fibroblast cell cultures, with synchronized rhythmicity lasting for several days [20]. Data from individual cultured fibroblasts show that the cells have autonomous and persistent oscillators similar to those in the SCN [21]. However, after a few cell divisions they lose synchronization in rhythmic expression. In this study, the rhythmic expression of the population of cells was dampened and eventually lost, even though the rhythmicity of individual cells was maintained. Although much is known about the central control of circadian rhythms by the SCN, it is still not entirely understood how central control is transferred to the periphery. In essence, although peripheral clocks can generate circadian oscillations in gene expression on their own, the SCN plays an important role in coordinating and synchronizing rhythmic behavior throughout the body. Studies performed using microarrays or other gene expression techniques demonstrate that the molecular components of the clocks in peripheral tissues are quite similar to that of the central clock present in the SCN [2,22,23]. For example, Fig. 3 illustrates the pattern (time and amplitude) of expression of six clock genes in rat skeletal muscle. Apart from the central clock and the light/dark cycle, other factors such as food, stress, immune challenges, and certain hormones can also potentially regulate and entrain oscillations in the clocks in the periphery [24–27].

1.4. Integration of the master clock and the peripheral clocks

The master clock present in the SCN imparts its regulation of the peripheral tissue clocks by at least two possible mechanisms. One mechanism involves the autonomic nervous system which receives input from the hypothalamus. Both sympathetic and parasympathetic neurons innervate almost all peripheral organs and tissues including liver, muscle, kidney, white adipose tissue, heart, pancreas, and ovary [6,28]. Autonomic nerve activity changes with the light/dark cycle, and lesioning the SCN results in the abrogation of this effect. In addition, inputs to the sympathetic branch of the autonomic nervous system are passed on to the superior cervical ganglia, which in turn innervate the pineal gland (a small hormone-producing structure in the epithalamus) [11]. The sympathetic input to the pineal gland imparts the information concerning light/dark cycles necessary for regulating the output of melatonin. Melatonin, secreted from the pineal gland, plays an important role in the regulation of sleep-wake cycles [29,30]. Regardless of whether the animal is diurnal or nocturnal, light suppresses the output of melatonin. Melatonin peaks during the dark period and is present in very low concentrations during the light period. Melatonin receptors are expressed in the cells of the SCN, and melatonin plays a feedback role on the SCN.

Exogenous administration of melatonin can cause phase shifts (either delay or advancement based on the time of administration).

Molecular analysis of the effects of melatonin on the SCN and peripheral tissues has shown that it does not affect the expression of *Per* genes, but rather regulates the phase shift by affecting the expression of *RORs* and *Rev-Erb* genes [31,32]. Melatonin receptors are present on some peripheral tissues as well [29]. Although the effects of melatonin on peripheral tissues are not well characterized, it does appear to modulate circadian rhythmicity. For example, melatonin has a complex influence on insulin secretion from the beta islet cells of the pancreas [33].

Like the pineal gland, the sympathetic nervous system directly innervates the adrenal medulla. Sympathetic innervation via the splanchnic nerve influences the production and release of epinephrine. Besides having wide systemic effects, epinephrine from the adrenal medulla also influences a second major system imparting circadian rhythmicity to peripheral tissues, the hypothalamic/pituitary/adrenal (cortex) axis (HPA) [34]. Hypothalamic neurons that produce corticotrophin-releasing factor (CRF) receive direct innervations from the SCN. The input from the SCN imparts circadian rhythmicity to CRF which in turn imparts rhythmicity to the secretion of adrenocorticotrophic hormone (ACTH). Notwithstanding the previously mentioned modulatory effect of epinephrine from the adrenal medulla, it is ACTH that causes the adrenal cortex to synthesize and release glucocorticoids in a rhythmic manner. However, unlike melatonin (which peaks in the dark period and is low during the light period) the HPA axis is not strictly controlled by light, allowing for both diurnal and nocturnal animals as well as phase shifting. In the case of nocturnal animals (e.g., mice and rats) the level of corticosterone reaches its peak during the transition from the light/inactive period to the dark/active period and in the case of diurnal animals (eg humans) the cortisol peak occurs during the transition from the dark/inactive period to the light/active period [2,12]. In essence, the circadian rhythm in glucocorticoid secretion prepares the animal for the upcoming period of activity and feeding. Glucocorticoids influence gene expression in virtually all tissues and play a major role in imparting coordinated circadian rhythmicity to gene expression throughout the body [35,36]. In addition, there is some evidence suggesting that sex steroids may have a direct modulatory effect on the central clock [9]. Conversely, the central clock influences the output of many hormones [30].

2. Circadian rhythms in gene expression and disease

It is well established that circadian rhythms play an important role in maintaining homeostasis and normal body function. Therefore it follows that disruption of circadian regulation can affect normal physiological and biochemical function and can lead to diseases. The effects of disruption in circadian rhythms can range from minor and easily reversible conditions to severe damage to the system. Jet lag is a very simple and common case where a disruption of circadian rhythms is observed [37]. Changes in time zones often cause sleep disorders (night-time insomnia or abnormal day-time sleeping) and temporary cognitive defects. Similar observations were made when rats were subjected to an abrupt shift in the light/dark cycle, in which case the animals displayed an increase in night time (active period) rest [38]. A series of studies performed by Cho et al showed that flight attendants who frequently fly across different time zones show cognitive defects associated with the reduction in the temporal lobe volume [39]. Another classic example which illustrates that disruption of circadian regulation can cause disorder comes from studies done in shift workers [40–43]. These studies suggest that the incidence of cancer, psychological disorders, metabolic syndrome, diabetes, and cardio-vascular diseases is higher in these individuals. Studies on the role of molecular clock components in regulating physiological processes have shown that clock plays a critical role in coordinating many of important

processes including metabolism, cell cycle progression and immune regulation among many others. Furthermore, several valuable studies performed with genetic knockouts for the clock components have given an insight into the processes that are regulated by circadian rhythms. Table 1 provides some of the important functions, physiological target genes and characteristics of genetic knockouts for these clock components. In this section, we focus on selected disorders including metabolic syndrome, obesity, diabetes, inflammatory disorders and cancer that can be the cause or the result of disruption in circadian rhythms and discuss processes at the transcription level that are involved. In addition we discuss how understanding circadian rhythms in gene expression can be useful in optimizing dose timing to maximize the efficacy of drug action and at the same time reduce adverse effects and in the identification of new therapeutic targets.

2.1. Metabolic syndrome, obesity and diabetes

Maintaining a proper glucose concentration in plasma is very important, as neuronal cells in the brain require a constant supply of glucose as their primary energy source since they are not capable of oxidizing amino acids or fatty acids. An extensive systemic network of organs which includes liver, muscle, adipose, pancreas, and the brain itself functions in maintaining blood glucose homeostasis [44]. When the animal is fed, the elevated glucose level in the blood stimulates the pancreas to secrete insulin. Insulin promotes glucose uptake by peripheral tissues, glycogen synthesis in liver and muscle, and inhibits glucose production via gluconeogenesis in the liver and kidney. It also stimulates the production of fatty acids and lipids in the liver. Excess glucose taken up by the adipose tissue and not used for glycolysis is used for lipogenesis. In the fasting state, appropriate blood glucose concentrations are maintained by glycogen breakdown in the liver along with gluconeogenesis in the liver and the kidney so that there is a constant glucose supply for the brain. In addition, lipids stored in the adipose tissue can be used as an energy fuel by other tissues. Any imbalance in the timely coordination between these organs can affect glucose and energy homeostasis and may lead to disorders such as metabolic syndrome, obesity, and diabetes.

Several lines of evidence suggest that metabolic processes are under the control of circadian regulation. First, microarray studies in various organs demonstrate that genes involved in controlling metabolic processes show circadian rhythms [2,23,45,46]. Many of the genes involved in regulating key reactions in pathways like glycolysis, gluconeogenesis, lipid biosynthesis and metabolism, and oxidative phosphorylation are either directly or indirectly under the control of core clock genes. Studies using *Clock* gene mutants demonstrate that these transgenic rodents show characteristics of metabolic syndrome such as hyperleptinemia, hyperlipidemia, hepatic steatosis, hyperglycemia, and hypoinsulinemia [47]. In addition, *Clock* mutant rodents shows dysregulation in the circadian pattern of peptide hormones like ghrelin, orexin, and CART (Cocaine and amphetamine regulated transcript) which play important roles in regulating food intake and metabolic processes. These observations are consistent with the fact that *Clock* mutant rodents are hyperphagic and obese with excessive accumulation of adipose tissue. PPAR alpha is an important nuclear receptor transcription factor that is involved in regulating lipid metabolism. Studies suggest that PPAR alpha expression is regulated by CLOCK via an E-box rich region present in the *Ppar* alpha gene. The rhythmic expression of PPAR alpha is lost in the liver of *Clock* mutant rodents [48]. These results suggest that CLOCK plays an important role in regulating metabolic processes in peripheral tissues. Similarly, BMAL 1 which is the heterodimeric partner of CLOCK is also important in regulating metabolic processes. Lamia et al. 2008 performed a liver specific deletion of the *Bmal1* gene in rodents and found that these animals exhibited hypoglycemia restricted to the fasting period, increased glucose clearance and loss of rhythmic expression in the hepatic genes involved in glucose

metabolism [49]. The orphan nuclear receptor NR1D1 (REV-ERB alpha) which is an important core clock transcription factor is found to play an important role in regulating hepatic gluconeogenesis [50]. These results suggest that core clock genes present in peripheral tissues play an important role in regulating metabolic processes, and disruption of these genes results in metabolic disorder.

Evidence to support the fact that circadian rhythms in gene expression are affected in diabetes and metabolic syndrome comes from studies conducted using animal models of obesity and/or diabetes. Studies in the severely obese diabetic model, KK-A^y mice showed that the circadian expression of certain core clock genes and genes involved in metabolic processes were disrupted [51]. Also studies suggest that high fat fed animals show disruption in circadian rhythms in mRNA expression of clock genes [52]. Evidence for disruption of circadian rhythms is not just limited to obese animals, but is also found in non-obese diabetic animal models. The Goto-Kakizaki (GK) rat is a polygenic model of non-obese diabetes which was developed by multiple generational breeding of Wistar-Kyoto (WKY) rats selected for high glucose intolerance [53]. Studies suggest that serum melatonin (the hormone involved in circadian entrainment) concentrations are reduced in the GK diabetic rats because of the disruption in the expression of melatonin synthesizing enzymes present in the pineal gland of these animals [54,55]. It was further observed that expression of melatonin receptor in beta islet cells was up-regulated in the GK rats which might be a compensatory response to the decrease in melatonin. In another study Frese et al. 2007 found that the rhythmic expression of both GLUT2 and glucokinase is altered in the beta cells of these rats [56]. It was also reported that the circadian rhythm of glucose uptake by cultured muscle cells and adipocytes from GK rats were altered [57]. These results suggest that the circadian rhythm in gene expression in diabetic animals is disrupted and this dysregulation can be causally related to the development of diabetes.

Although it has been well established that the core circadian mechanism regulates the expression of genes involved in metabolic processes, recent evidence suggests that the converse is also true. Zang et al. 2009 performed a genome wide siRNA screen in order to identify the genes that can potentially regulate the internal clock mechanism [58]. This is a high throughput plate-based in vitro screen where U2OS (human osteosarcoma) cells were treated with 90,000 different individual siRNA and the effect of knock down on the circadian clock was studied by analyzing the changes in rhythm amplitude, phase, or period length of the luciferase reporter gene expression which was under the control of *Bmal1* or *Per* gene promoter. The result from this study showed that many genes that are involved in metabolic pathways such as insulin signaling, folate metabolism and others were found to regulate the core clock components.

The significance of understanding the circadian regulation of gene expression and how it is affected in disease states may be useful in optimizing dosing time for drugs in order to get maximum therapeutic efficacy and minimum toxicity. From microarray studies in liver tissue of Wistar rats we found that HMG-CoA reductase (3-Hydroxy-3-methylglutarylcoenzyme A reductase), Sqle (Squalene epoxidase) and Cyp7a1 (Cytochrome p450 (cholesterol hydrolase 7 alpha)) all had circadian rhythms with peak expression occurring at the middle of the active (dark) period [22]. All of these proteins are involved in the biosynthesis of cholesterol and are the therapeutic targets for cholesterol-lowering drugs. For example statins are potent inhibitors of HMG-CoA reductase and are prescribed to patients with hypercholesterolemia. The current practice is that patients take the drug in the night before going to bed (inactive period) which would seem inappropriate as the HMG-CoA reductase peaks during the active period (which is day time in humans). Human studies have also confirmed the observation that HMG-CoA reductase peaks during the active day

time period [59]. This result suggests that for humans taking statins in the morning may either increase efficacy or decrease muscle toxicity.

Therapeutic targets for two important drugs (metformin and thiazolidinediones) used to treat diabetes regulate and/or show circadian rhythms. Metformin, a biguanide class drug used to treat T2DM, reduces blood glucose by activating AMPK (AMP- activated protein kinase) which in turn inhibits the expression of PEPCK, an important enzyme in regulating gluconeogenesis. Um et al. 2007 showed that the administration of metformin can lead to a phase advancement in the circadian expression of clock genes in rodents through AMPK by targeting the CK ξ protein which phosphorylates and destabilizes PER proteins [60]. Furthermore, the gluconeogenic enzyme PEPCK which is inhibited by AMPK also shows circadian rhythms in gene expression in peripheral tissues [61,62]. The other class of drugs, thiazolidinediones, which are PPAR gamma agonists, bind to the nuclear receptor PPAR gamma and activate the PPAR gamma mediated transcription of a number of specific genes that regulate adipogenesis and energy metabolism. Thiazolidinediones act as insulin sensitizers by increasing the uptake of glucose by organs and reduce plasma free fatty acids by increasing their uptake by adipose tissue. PPAR gamma is found to be rhythmically expressed in adipose tissue [63]. Studies reported by Wang et al. 2008 show that vascular PPAR gamma also exhibits rhythmic expression and is important in controlling the circadian regulation of blood pressure and heart rate [64]. In this study thiazolidinedione treatment resulted in the up-regulation of aortic BMAL1, while CHIP and promoter assays showed that Bmal1 is a direct PPAR gamma target gene.

2.2. Inflammatory diseases and autoimmune disorders

Immune responses and inflammation are very important biological processes which serve to protect the body from invading pathogens and cancer cells. Such processes are not confined to a single organ or cell type. Rather, they are regulated by a plethora of cell types present in different organs along with small molecule or protein mediators that are secreted by these cells. Some examples include white blood cells (monocytes and lymphocytes) in circulation, macrophages infiltrated into adipose tissues and lungs, Kupffer cells in liver, mast cells and macrophages in muscle, and microglial cells in the brain. Circadian regulation of these processes in these cell types occurs at different levels of organization. Studies in both humans and rodents demonstrate that the core clock genes are rhythmically expressed in cells regulating inflammation and immune response processes [65–67]. Additionally, these studies indicate that inflammatory mediators also show circadian rhythms. The mRNA expression profiles and plasma protein profiles or cytokines such as IL-6, IL-2, IL-12, TNF alpha, GM-CSF, Interferon gamma, and others show circadian rhythmicity in both humans and rodents. For example, plasma concentrations of IL-6 and TNF alpha which are important pro-inflammatory cytokines peak at the beginning of the active cycle (early morning in humans and just after lights off in rodents) [68]. Rhythms are also observed in cell counts and trafficking of monocytes, neutrophils, natural killer (NK) cells, T-lymphocytes and B-lymphocytes [69]. Numbers of monocytes and T and B lymphocytes in blood peak during the inactive period of the organism, whereas neutrophils and natural killer cells peak during the active period. Hormones like melatonin, glucocorticoids and prolactin which show robust circadian rhythms in their secretion are also found to regulate immune response processes [29,30].

Evidence from recent studies suggests that the core molecular clock components can directly modulate the expression of genes regulating immune response and inflammation. Liu et al. 2006 showed that in rodents PER2 is a very important regulator of interferon gamma production, and hence plays an important role in the normal functioning of NK cells [69]. *Per2* mutant mice showed defects in interferon gamma production and impaired NK cell function. Migita et al. 2004 showed that orphan nuclear receptor REV-ERB alpha, which is

also a component of the molecular clock, regulates the expression of IL-6 and cyclooxygenase-2 genes in vascular smooth muscle cells [70]. It was also reported that REV-ERB alpha increased the transactivation of nuclear factor kappa B (NFκB) which is an important transcription factor involved in regulating the expression of a large number of immune response genes. A study by Wang et al. 2006 demonstrated that in liver, *Rev-erb* alpha and beta regulate the expression of plasminogen activator inhibitor type 1, a critical regulator of the fibrinolytic system and of the inflammatory response in the vascular wall [71]. Furthermore, the orphan receptors RORα and RORγ were also found to play an important role in the development and functioning of immune cells both under normal conditions and in inflammatory or autoimmune disorders [17]. Mutation of RORγ in rodent models resulted in abnormalities in lymphoid organ development and these animals failed to develop lymphoid nodes and Payer's patches [16,17]. These mutants also showed reduced survival rates of thymocytes, the precursor cells of T lymphocytes and NK cells. Animal models for *RORα* or *RORγ* mutants show reduced susceptibility to autoimmune and inflammatory disorders suggesting that both RORα and RORγ might be attractive pharmacological targets for both inflammatory and autoimmune disorders including asthma, chronic colitis, and multiple sclerosis [17]. For example, both *RORα* and *RORγ* mutant rodents are less susceptible to ovalbumin-induced lung inflammation and showed reduced eosinophil and lymphocytes levels, attenuated pulmonary inflammation, and lower levels of protein and mRNA expression of Th2 cytokines in lungs [72,73]. Both these orphan receptors are critical for the differentiation and functioning of TH17 cells which plays a key role in the above mentioned inflammatory and autoimmune diseases. Together, these studies suggest that core molecular clock components can regulate inflammatory and immune responses and can be potential therapeutic targets for inflammation and autoimmune-related disorders.

Circadian rhythms in gene expression and/or protein production/secretion of immune modulating agents are relevant to clinical observations found in chronic inflammatory conditions. For example in rheumatoid arthritis (RA), a systemic chronic inflammatory disorder, symptoms like joint pain, morning stiffness and functional disability peak in the early active period (morning in the case of humans) [68]. This is consistent with gene expression and plasma protein profiles of pro-inflammatory cytokines. Both IL-6 and TNF alpha are elevated in the disease and peak early in the morning (active period) in RA patients. It should be noted that circadian rhythms in cytokine expression are preserved even in the disease state, although the mean level of expression is elevated when compared to healthy controls.

Synthetic glucocorticoids (corticosteroids) are potent anti-inflammatory agents used to treat symptoms of RA. Although they are widely used for the treatment of a plethora of inflammatory and autoimmune disorders, long-term therapy can cause severe metabolic disorders leading to Cushing's syndrome, hypertension, hyperglycemia, muscle atrophy, and dyslipidemia [35]. Optimizing dose and timing of administration are necessary to obtain maximum therapeutic use and minimize adverse effects. It is a general practice for RA patients to take immediate-release corticosteroids in the morning at around 6 to 8 AM [74]. However, it has been suggested that this dose schedule is inappropriate as the pro-inflammatory stimulus that causes the irritation and swelling of synovial tissue has already been triggered by the increase in the pro-inflammatory cytokines which peak at early morning. Studies performed in RA patients showed that administration of immediate-release corticosteroids during the night (2 AM) before the rise in the pro-inflammatory cytokines improved the efficacy of the drug by inhibiting proinflammatory cytokine production [75]. It was also observed that the blood concentration of the drug resembled the normal circadian rhythm of the endogenous corticosteroid. This treatment regimen requires lower doses of the drug and hence reduces the magnitude of adverse effects. A problem with this treatment

schedule is that it is impractical to wake the patients in the middle of the night to give the drugs regularly. This problem is circumvented by the development of a modified release oral tablet that contains a coating that can burst open 4 h after intake because of the penetration of water [74].

Another inflammatory disease that shows rhythmicity in clinical symptoms is asthma. It is a common observation that asthma in humans worsens during the night. The increased occurrence of night-time asthmatic attacks is related to a number of factors that show circadian rhythms. The first and foremost factor is lung functioning. In normal humans, lung function has a modest circadian rhythm, but in asthmatic patients the amplitude of the circadian rhythm is very high with minimum functioning during the night [76,77]. In humans, levels of cortisol, epinephrine, and beta-adrenergic receptors are low during the night [77,78]. Cortisol (endogenous glucocorticoid) is a potent anti-inflammatory agent and epinephrine helps in bronchodilation through the beta-adrenergic receptor. That all these factors are low at night results in the worsening of symptoms. Further worsening the condition, both cellular markers of inflammation like alveolar eosinophils, neutrophils, lymphocytes, and chemical mediators like histamine and their responsiveness to inhaled bronchoconstrictors are also found to be high during the night period [77,79,80]. This circadian rhythm in the complex interaction between these factors results in the worsening of nocturnal asthma and drugs like theophylline and β_2 -adrenergic receptor agonists that are used for the treatment of asthma are recommended to be given to patients at higher dose in the evening than in the morning [79].

2.3. Cancer

Cell cycle and DNA repair processes are highly regulated and any disruption can lead to pathological disorders such as cancer. The stages of the cell cycle are divided into G1 (gap 1), S (DNA and other macromolecule synthesis phase), M (mitosis) and G2 (gap 2) phases. Cells can exit the cell cycle and enter the G0 phase which is a stage of cell cycle arrest. The transitions between these different stages are controlled by phase specific cyclins, cyclin dependent kinases (CDKs), and cyclin dependent kinase inhibitors (CKIs). CyclinB1 and its associated kinase regulate the transition from G2 to M phase and cyclins A, D and E and their associated kinases regulate the G1 to S phase transition [81,82]. DNA damage and repair process are also closely connected with regulation of the cell cycle.

Several *in vivo* and *in vitro* studies show that DNA synthesis processes, mitotic indices, and DNA repair processes all show circadian rhythms in different cell types in various organs. Studies demonstrate that many of the cyclins, CDKs, CKIs, tumor suppressor genes, and oncogenes that are involved in regulating these processes show circadian rhythmicity in their expression and are under the control of core clock genes [82].

2.3.1. Relationship between genetic components of the clock mechanism and cancer

Circadian clock bHLH-PAS transcription factors and cancer: The transcription factor WEE1 inactivates cyclin B1-CDK1 complexes by phosphorylating CDK1 and hence is a key regulator of G2 to M transition. CLOCK/BMAL1 and CLOCK/NPAS2 heterodimers regulate the circadian expression of Wee1 through the three E-box elements found in the promoter region of the gene [82]. Evidence that CLOCK/BMAL complexes regulate the circadian expression of *Wee1* comes from liver studies by Matsuo et al. 2003 in which *Clock* mutant mice showed a considerable decrease in *Wee1* gene expression [83] and by Grechez-Cassiau et al. 2008 in which *Bmal1* deficient mice lost circadian rhythmicity in the expression of *Wee1* transcripts [84]. BMAL1 is also a critical regulator of CDKN1a and p53 expression [84,85]. CDKN1a, also called p21, negatively regulates the G1 to S phase transition by

inhibiting the activity of cyclin- CDK2/4 complex. p53 is a well known tumor suppressor protein that is involved in the regulation of a myriad of processes ranging from cell cycle checkpoints to DNA damage and repair. A recent study by Taniguchi et al. 2009 suggests that BMAL1 epigenetic inactivation can possibly contribute to the development of some hematologic malignancies, such as diffuse large B-cell lymphoma and acute lymphocytic and myeloid leukemias [86]. In this study, they found that the promoter CpG islands of BMAL1 genes were hypermethylated in the cells with hematologic malignancies which resulted in the transcriptional silencing of the gene. This was observed both in cultured cell lines and from samples obtained from patients. It was further shown that the restoration of *Bmal1* expression in these hypermethylated cells results in the reduction of tumor growth, and knockdown of *Bmal1* expression using siRNA in unmethylated cells resulted in an enhanced tumor growth. These results suggest that BMAL1 plays an important role in regulating cell cycle processes and any disruption in its expression can result in abnormal cell growth and cancer.

Period transcription factors and cancer: Results from several studies suggest that the PER transcription factors can potentially act as tumor suppressor genes [87]. *Per1* and *Per2* expression are found to be deregulated in different types of cancers including breast cancer, lung cell carcinoma, prostate cancer, and others [87–90]. Rodents deficient in the expression of *Per* genes are more cancer-prone, show abnormal response to gamma irradiation, and the thymocytes of these animals have a slower p53-mediated apoptotic response to ionizing radiation [87,91]. Furthermore, over-expression of *Per1* or *Per2* genes in cancer cells can inhibit the growth of tumors and sensitize cancer cells to DNA damage induced cell apoptosis. A study by Gery et al. 2006 shows that *Per1* over-expression induced cell apoptosis by inducing c-Myc expression and suppressing CDKN1a expression in cancer cells [92]. Also co-immunoprecipitation shows that PER1 interacted with Ataxia telangiectasia mutated (ATM) kinase and its downstream CHK2 which play an important role in regulating cell cycle checkpoints. The results also indicate that PER down-regulates the expression of cyclin B and its kinase CDK1 by p53-dependent mechanism and suppresses the expression of *Wee1* by direct circadian control. Furthermore, *Per* mutant rodents exhibit shortened cell cycles and increased cell proliferation.

Cryptochrome genes and cancer: The results from mutation studies on Cry1 and Cry2 genes in rodent models also provide insightful information. A study by Gorger et al. 2005 showed that fibroblast cells from Cry1/Cry2 mutant rodents grow at a rate that is indistinguishable from wild-type controls, and the cell arrest response at the G2 to M transition as well as radiation-induced DNA damage checkpoint response after ionizing radiation is similar to that of wild-type controls [93]. In addition, the mutant rodents did not show any difference with respect to radiation-induced morbidity and mortality. A recent study by Ozturk et al. 2009 suggests that loss of *Cry* in fact reduces the risk of developing tumors in rodent models. In this study the effect of Cry1/Cry2 mutation in the p53 mutated rodent was studied (p53 is found to be mutated in nearly 50% of human cancers) [94]. These triple knockout animals show a lower age adjusted incidence of cancer and longer lifespan when compared to the p53 only mutant animals. Genotoxic stress induced by UV irradiation of p53 knockout controls and the triple knockout skin fibroblastic cells show that the triple mutant cells are much more susceptible to UV-induced apoptosis which may explain the lower incidence of cancer occurrence in these animals. Another study by Matsuo et al. 2003 showed that Cry mutant rodents have higher antimitotic Wee1 expression which reduced CDK1 activity and hence resulted in diminished cell division and proliferation activity [83]. These results suggest that down-regulation of Cry gene expression or interference with its function may potentially have therapeutic value that can be used to treat certain types of cancer.

Other clock genes and cancer: Other clock regulating genes including REV-ERB, ROR alpha, ROR gamma and CK1 ξ are also involved in regulating cell cycle processes, and disruption of their expression is associated with tumor malignancies [82,95]. Nuclear receptor REV-ERB alpha can negatively regulate the expression of CDKN1a while ROR alpha positively regulates its expression, thereby controlling the G1 to S phase transition [82]. ROR alpha is often down-regulated in several tumor types including breast, lung, and gastric cancers, possibly due to epigenetic factors like methylation-mediated gene silencing [17]. Studies using rodent models deficient in ROR gamma expression show dysregulation in thymocyte differentiation and proliferation as well as a higher incidence of thymic lymphoma development [17]. CK1 ξ which phosphorylates and tags PER for degradation plays an important role in regulating cellular differentiation, proliferation, apoptosis, and chromosomal segregation. CK1 ξ is dysregulated (primarily overexpressed but sometimes mutated) in various cancer types including breast cancer, leukemia, pancreatic ductal adenocarcinoma, mammary ductal carcinoma, and others [95–97]. The anti-apoptotic and growth stimulatory property of CK1 ξ accounts for part of its oncogenic effect in tumor cells [98]. However, it has also been suggested that CK1 ξ exerts its oncogenic properties by reducing the stability of PER which acts as a tumor suppressor protein and also by positively regulating the Akt pathway which plays an important role in cell proliferation [96,98]. In addition, inhibition of CK1 ξ using specific competitive inhibitors in tumor cells can sensitize cancer cells to CD95-mediated apoptosis and inhibit tumor growth [99]. Therefore, inhibition of CK1 ξ is yet another potential therapeutic area for the treatment and prevention of tumor growth.

2.3.2. Effect of circadian rhythms in gene expression on cancer therapeutics

—In the case of cancer therapeutics the most important consideration is outcomes, which are based on the balance between the toxicity of the drug and its therapeutic value. Drugs used as cancer therapeutics usually have a very low therapeutic index, as most chemotherapeutic agents also affect normal healthy cells. Therefore, optimizing the dose timing could play an important role in maximizing therapeutic effects and at the same time reducing adverse effects. Many targets for chemotherapeutic drugs show circadian rhythms. For example in our microarray study in the liver from normal Wistar rats, 35 genes related to cell cycle and apoptosis show robust circadian rhythms [22]. Almost all of these genes are either targets for chemotherapeutic agents or biomarkers for the prognosis of the disease. Paclitaxel, which is a mitotic inhibitor, is used as a chemotherapeutic agent to treat ovarian, breast, skin, and certain lung cancers [100]. The therapeutic target of this drug, beta tubulin, shows a robust circadian rhythm which peaks during the early light (inactive) period in rats [22]. Fluropyrimidines like flurouracil are effective chemotherapeutic agents which target thymidylate synthetase (TS). TS is a rate limiting enzyme in the synthesis of thymidine triphosphate, and plays an important role in DNA repair and synthesis. Both mRNA expression and the activity of TS show circadian rhythms in a cell type specific manner [101,102]. These results suggest that both the efficacy and the adverse effects of the drug may depend on the dose timing. To further complicate the problem the enzymes involved in metabolizing flurouracil also show circadian rhythms, which in turn can affect the therapeutic efficacy and toxicity of the drug [103]. Ornithine decarboxylase is yet another enzyme that is a potential target for cancer treatment, and specific inhibitors for this enzyme and gene silencing approaches were successful in inhibiting tumor growth and metastasis. Our microarray studies as well as studies from other laboratories show that the mRNA expression of this enzyme has a strong circadian rhythm which peaks during the middle dark (active) period in rodents [22,104,105]. Other potential therapeutic targets of cancer like Nucleolin (involved in synthesis and maturation of ribosomes), ELF4A (a RNA helicase protein involved in translation initiation), CD20 antigen (drug target of the monoclonal antibody Ibritumomab), and others also show circadian rhythms in their expression [22].

Many biomarkers including Proline rich 13 (PRR13), Death associated protein kinase 1 (DAPK1), Reprimo (RPRM), and others show circadian rhythms in mRNA expression [22]. A recent study by Iurisci et al. 2009 demonstrated that the cyclin dependent kinase inhibitor Seliciclib can disrupt the circadian molecular clock in the liver based on the time of administration of the drug [106]. This disruption could account for the liver toxicity of the drug through the resulting disruption of clock-controlled detoxification pathways, suggesting that the timing of administration of Seliciclib could be critical in reducing its toxicity.

Although the circadian rhythm in many of the cancer therapeutic targets is well established, the exploitation of these observations may not be straightforward. If circadian rhythms of gene expression in both cancer and normal cells are entrained in the same way, then attaining an optimum balance between efficacy and toxicity could be complicated. However, evidence suggests that cancer cells have altered circadian rhythms in cell division and cell cycle regulation and other physiological processes that are potential therapeutic targets which can be exploited in optimizing the dosing time of chemotherapeutics [22,107]. A thorough understanding of circadian regulation in gene expression in both tumor cells and normal cells will be necessary to achieve maximum therapeutic efficacy and at the same time decrease the toxicity of chemotherapeutic agents.

3. Circadian rhythms in gene expression and drug disposition kinetics

It is well accepted that the time of administration of a drug can play an important role in not only the pharmacodynamic effects of the drug but also in determining the pharmacokinetic parameters including AUC, CL, C_{max}, T_{max} and t_{1/2} [108]. All four processes (absorption, distribution, metabolism and elimination) that govern disposition of drugs show circadian rhythms and at least some are under the control of molecular clocks. In this section we discuss the effect of circadian rhythms on these four processes and how circadian rhythms in gene expression can directly or indirectly control these processes.

3.1. Absorption

Absorption reflects the passive or active transport from the site of administration to the systemic circulation. Oral dosing is the most common method of drug delivery, and the absorption of the drug from the stomach or intestine depends on various factors including the physico-chemical properties of the drug, gastric pH, gastric emptying rate, gastrointestinal blood flow and drug transporters which are involved in both absorption and efflux of the drug [109]. Studies show that almost all of these processes show circadian rhythms. The components of the core molecular clock are present in the stomach and the intestine and play an important role in regulating the expression of genes involved in various physiological functions [110–112]. For example, nutrient and drug transporters present in the GI tract show a robust circadian rhythm in their expression and some of these transporters are under the direct control of core clock genes [113,114].

Lipophilic drugs like cyclosporin, nifedipine, digoxin, and others show greater oral absorption during the day (active period) when compared to night in humans [115–117]. This is consistent with the fact that the absorption of lipids from the intestine also shows a circadian rhythm, with the absorption rate maximal during the active period of the organism [118]. The rhythm in lipid absorption is mediated by circadian variation in the expression of genes such as microsomal triglyceride transfer protein (MTP), apolipoprotein B (ApoB), apolipoprotein A4 (ApoA4), and others that are involved in the absorption and transport of lipids from the GI tract to the systemic circulation. Furthermore, the rhythmic expression of these genes and lipid absorption are disrupted in *Clock* mutant rodents. Stearns et. al 2008 found that *Mdr1*, *Mct1*, *Mrp2*, *Pept1* and *Bcrp1* show circadian patterns in their mRNA

expression [113]. MDR1, MCT1 and BCRP1 are all members of ATP-binding cassette (ABC) transporters which play an important role as efflux proteins, transporting the drug from inside the cells back to the intestinal lumen. MDR1 acts on small hydrophobic drugs like digoxin. Chemotherapeutic drugs such as doxorubicin and certain cephalosporin antibiotics are substrates for MCT1, while BCRP is effective in effluxing purine analogs like metotrexate (an important chemotherapeutic agent used to treat various types of cancer). MCT1 is a mono-carboxylate transporter and aspirin is one of the substrates for this transporter. PEPT1 is a proton-coupled peptide transporter which has a wide range of substrates including oral beta-lactam antibiotics, anticancer agents like bestatin, and angiotensin-converting enzyme inhibitors.

Further evidence suggests that core clock components can directly regulate expression of genes that control drug absorption. Murakami et al. 2008 show that the promoter of the gene for MDR1A (a member of ABC transporter proteins) contained a PAR bZIP protein response element which could potentially bind clock-controlled output transcription factors such as DBP, TEF and HLF as well as an E-box element which is a consensus binding site for the CLOCK/BMAL heterodimer [114]. Using luciferase activity they show that DBP, TEF and HLF proteins up-regulated the expression of *Mdr1a*-Luc reporter transcriptional activity by binding to the consensus region present in the promoter of the gene. Furthermore, the circadian rhythm in expression of the *Mdr1a* gene in the intestine is lost in *Clock* mutant mice. In these mutant animals, *Mdr1a* shows a very low expression which results in the accumulation of digoxin in the intestine. In another study, Saito et al. 2008 reports that expression of PEPT1 transporter is directly controlled by the clock-output transcription factor DBP [119]. The expression of *Pept1* is in phase with that of DBP, and gel shift assays indicate that DBP can physically bind to the promoter region of the *Pept1* gene. Together, these results suggest that circadian rhythms in gene expression controlled by the molecular clock play an important role in regulating the absorption of many drugs.

3.2. Distribution

Factors affecting the distribution of a drug between the systemic circulation and different tissues include physical volumes, blood flow rates to the organ/tissue, presence of drug transporters or efflux pumps, and protein binding both in plasma and tissues. As of now there is no evidence for any kind of circadian variation in the actual physical volume of plasma or organs. However, the other three processes can show circadian variations which can affect the rate and extent of drug distribution. Circadian variation is seen in cardiac output and the fraction of blood flow received by different organs based on metabolic need. In rodents, cardiac output is increased during the active (night) period when compared to the inactive period [120]. It is also observed that blood flows to muscles and the digestive system are higher during the active period. This increased blood flow supports the enhanced metabolic needs of muscle and increased digestion and nutrient absorption during the active feeding period. In addition, diurnal changes in blood flow to liver, adipose tissue, and skin are also observed in animal experiments. As a result, the distribution of drugs with high diffusion rates are limited by circadian variations in cardiac output and blood flow rates to different organs. It is well documented that various ion channels, transporters, and efflux pumps show circadian variations in their expression in a tissue specific manner [2,22,121]. Many of these transporter proteins play important roles in both the transport and efflux of drug molecules and the circadian variation in these proteins could potentially affect the tissue specific distribution of these drugs.

Protein binding in both plasma and tissue is another major determinant of drug distribution.

According to the Gillette equation $V = V_p + V_t \frac{f_{u,p}}{f_{u,t}}$ (where V is the volume of distribution of

the drug, V_p and V_t are the plasma and tissue volume and $f_{u,p}$ and $f_{u,t}$ are the fraction unbound of the drug in plasma and tissue) [122]. In this equation, $f_{u,p}$ and $f_{u,t}$ depend on the protein binding of the drug in plasma and tissue components. Any change in protein binding affects these parameters and therefore affects the volume of distribution. Albumin and other plasma binding proteins show circadian rhythms in the systemic circulation, which can affect the free fraction of the drug in plasma and therefore the distribution of the drug. For example, the plasma protein binding and free fraction of cisplatin, which is a platinum-based chemotherapeutic agent, shows circadian rhythms in studies on humans [123]. The peak plasma binding for this drug occurs at around 4 PM which coincides well with the acrophase of the albumin rhythm in plasma [124,125]. Similar observations are available for carbamazepine, where the circadian rhythm of plasma protein binding of this drug is in phase with that of the albumin rhythm [108]. Other plasma binding proteins such as transcortin, which binds to both endogenous and some exogenous corticosteroids also exhibits a circadian rhythm with peak binding to corticosteroids occurring at around 4 PM and lowest binding occurring at around 4 AM [126]. Other plasma carriers for endogenous proteins such as soluble leptin receptor which binds to the adipokine leptin also shows a circadian rhythm which is inverse to that of leptin, and may regulate circadian changes in the biological activity of the hormone [127].

3.3. Metabolism

Liver is the major organ that is involved in the metabolism of drugs. Factors that could affect the metabolism of a drug in liver include the hepatic blood flow rate, specific enzymatic activity and plasma protein binding (as only the free unbound drug can be metabolized) [128]. As discussed above, both the blood flow rate to the liver and plasma protein binding show circadian rhythms. It is well established that several enzymes involved in the metabolism of drugs also have circadian rhythms in expression and activity [23,129]. Evidence suggests that the transcription of these enzymes is at least in part, regulated by core clock genes.

Metabolism of drugs and endogenous compounds can undergo two phases of metabolism with phase one being the functionalization of the drug which includes oxidation, reduction or hydrolysis and phase two involving conjugation which includes glucuronidation, peptide conjugation, methylation, acetylation, sulfation, or glutathione conjugation. Cytochrome P450 enzymes are the most common and important group of enzymes involved in phase one processes. Many of the Cyp 450 genes in liver including *Cyp2b10*, *Cyp2e1*, *Cyp4a14*, *Cyp2a4*, *Cypa5*, *Cyp2c22*, *Cyp2e1*, *Cyp3a*, *Cyp4a3*, *Cyp7* and *Cyp17* show circadian rhythmicity in expression [22,129,130]. Other phase one enzymes that show circadian rhythmicity in liver include quinone oxidoreductases (reduction), paraoxonases (hydrolysis), and aldehyde dehydrogenases (oxidation) all of which play important roles in the metabolism of drugs [129]. Studies also show that the enzymes involved in phase two metabolism including glutathione S-transferase, carboxylesterase, cysteine dioxygenase, UDP-glucuronosyltransferase, and sulfotransferase show circadian rhythmicity in their expression [129]. In general, mRNA expression of the phase one enzymes peaked during the active period. The phase two enzyme glutathione S-transferase peaks during the early inactive period, UDP-glucuronosyltransferase in the late inactive period, and sulfotransferase peaks in the early active period. Because of the circadian rhythmicity in these drug metabolizing enzymes in the liver, the metabolism of several drugs like Pnitroanisole, hexobarbital, aniline, paracetamol, and others show circadian variation [108].

There is evidence suggesting the direct involvement of core circadian clock genes in controlling the expression of drug metabolizing enzymes. Lavery et al. 1999 showed that the circadian expression of *Cyp2a4* and *Cyp2a5* mRNA and protein expression are controlled by the circadian expression of DBP (a member of PAR bZip protein), and that in DBP mutant

rodents the circadian expression of these genes is impaired [130]. Further evidence that PAR bZip proteins can regulate hepatic metabolism comes from studies on triple mutants for DBP, HLF and TEF [19,131]. These studies show that these mutants have increased liver mass which is associated with xenobiotic stress. In addition, the plasma concentration of alanine aminotransferase, which is a diagnostic marker of liver damage is elevated. Microarray analysis of liver mRNA expression from both wild type and triple mutant mice suggest that PAR bZip proteins can potentially control the expression of many metabolizing enzymes. It was further shown that phenobarbital-induced sleep duration was constitutively higher irrespective of time of injection in the triple mutant animals, while in wild type animals sleep duration depends on the time of injection. In addition, the plasma concentrations of chemotherapeutic agents such as mitoxantrone and cyclophosphamide after an intraperitoneal injection are elevated in the triple mutant mice when compared to wild-type, confirming that drug metabolism is impaired in these mutant animals. These studies provide a strong link between molecular clock output genes and hepatic drug metabolism.

Although circadian variations are observed in various factors controlling hepatic metabolism, the extent to which these factors contribute to the circadian variation in the metabolism of a drug varies. The equation for hepatic clearance according to the well stirred

model is $Cl_H = \frac{Q_H * f_{ub} * Cl_{int}}{Q_H + f_{ub} * Cl_{int}}$ where Cl_H is the hepatic clearance, f_{ub} is the unbound fraction of the drug, Cl_{int} is the intrinsic clearance which is a property of the enzymatic activity, and Q_H is the hepatic blood flow [128]. For a high extraction drug the intrinsic clearance is much higher when compared to the hepatic blood flow rate and hence the hepatic clearance depends primarily on the hepatic flow rate ($Cl_H = Q_H$). In this case the circadian variation in the hepatic blood flow rate is the major factor influencing the circadian rhythm in the metabolism of a drug showing high extraction. In contrast, for a low extraction drug the intrinsic clearance is very low when compared to the hepatic blood flow rate and the hepatic clearance is dependent on the product of unbound drug fraction and the intrinsic clearance ($Cl_H = f_{ub} * Cl_{int}$). Therefore, for drugs showing low extraction the circadian variation in both protein binding and enzymatic activity will be the major factors that determine the circadian rhythmicity in their metabolism.

3.4. Excretion

The elimination of the drug either in the unmodified form or as a metabolite is the last step in its disposition. Although the kidney is the most important organ for the excretion of most drugs, excretion from the liver into the bile occurs with some drugs which are finally lost with the feces. In addition, as discussed under drug absorption, the presences of efflux pumps in the intestine can efflux certain types of drugs from the body back into the intestinal lumen.

Glomerular filtration, tubular secretion and tubular reabsorption are the three processes that control the excretion of drugs by kidney. Studies using inulin as a marker for glomerular filtration rate (GFR) suggests that GFR shows circadian variation which peaks during the active period (dark period for rodents and day time for humans) and is lower during the inactive period [132]. The circadian variation in renal blood flow, blood pressure and the renin-angiotensin system controls the circadian rhythmicity of GFR [108]. For drugs which are primarily eliminated through glomerular filtration and show very low protein binding the circadian variation in renal excretion depends primarily on the circadian variation in GFR. For example, the aminoglycoside antibiotic amikacin which shows circadian variation in renal excretion is very similar to that of GFR [133]. However, for drugs with high plasma

binding, the circadian variation in protein binding (as discussed under drug distribution) can also affect the circadian changes in renal excretion of drugs.

Tubular secretion is an active transport process by which endogenous compounds and drugs are eliminated via specific transporters including P-glycoproteins, organic anion transporters, organic cation transporters, and proteins of solute carrier families. Any circadian rhythms in the expression and activity of these proteins can affect the elimination of drugs through renal secretion. A recent study by Zuber et al. 2009 shows that some of the solute carrier proteins that are involved in the secretion of a variety of compounds and drugs show circadian rhythms in their expression which peaks during the time of onset of the active feeding period in anticipation of intake of xenobiotics with food and water [134]. In addition, pharmacokinetic studies of several drugs that are primarily excreted by tubular secretion indicate circadian variation in this process. For example, the study by White et al. 1995 shows that ampicillin, a drug primarily excreted by tubular secretion, shows circadian variation in clearance which suggests a possible circadian rhythm in tubular secretion process [135].

Tubular reabsorption is the process by which most solvent and essential solutes are recovered back into the body. Many drugs are also reabsorbed by either an active process or simple passive diffusion. Active reabsorption is mediated by specific protein transporters and we found no evidence to suggest circadian variations in this process. However the passive reabsorption of a drug depends on the lipophilicity and pKa of the drug and the urinary pH and flow rate. Circadian variation is observed in both the urinary pH and urinary flow rate, with urinary pH being more acidic during the inactive period and urinary flow rate peaking during the active period [108]. Studies show different degrees of ionization of some drugs depending on the urinary pH and diurnal differences in passive reabsorption.

The kidney also possesses an intrinsic circadian timing system similar to other central and peripheral tissues, and shares the same circadian molecular mechanism of core clock genes and the clock-controlled output genes [134]. Many of the genes expressed in kidney including genes controlling sodium ion and water balance, and transporters. show circadian variations in their mRNA expression [134,136,137]. The circadian rhythm in gene expression is disrupted in *Clock* mutant and DBP, TEF and HLF triple mutant rodents suggesting a prominent role of the molecular clock in regulating and coordinating these functions [134].

4. Conclusion

Circadian rhythmicity is observed in virtually all aspects of mammalian function from the expression of genes to complex physiological processes. Disruption of circadian rhythms are both the cause and the consequence of many diseases. Circadian rhythms also affect the timedependent therapeutic efficacy and toxicity of drugs by influencing both the pharmacokinetics and pharmacodynamics. Understanding the circadian rhythmicity in gene expression is of central importance in developing mechanism-based pharmacokinetic/ pharmacodynamic/ pharmacogenomic (PK/PD/PG) models. For many years our laboratories have been involved in developing mechanistic models describing both the efficacy and the adverse effects of corticosteroids. More recently, these mechanistic models have incorporated the circadian rhythmicity in hormonal levels, mRNA expression, protein levels and sometimes activities necessary for the quantitative understanding of both the physiological and pharmacological mechanisms controlled by corticosteroids [138–140]. A wide variety of mathematical and statistical models are available to characterize circadian rhythmicity in experimental data. Chakraborty et al. 1999 compared commonly used mathematical models including Single Cosine, Dual Ramps, Two Rates, Dual Cosines,

Harmonics and L^2 -Norm Approximation (based on Fourier analysis) methods to characterize circadian rhythms in endogenous cortisol in placebo treated volunteers and after administration of synthetic glucocorticoids which suppress the endogenous production [141]. It was found that the Fourier based L^2 -Norm method with three harmonics was very successful in capturing the asymmetry present in the circadian profile in both the placebo treated and drug treated subjects. However, it should be noted that the selection of a mathematical model to characterize circadian rhythms will strictly be case-specific depending on the experimental data. The review by Refinetti et al 2007 discusses most of the relevant models that are used to characterize circadian rhythmicity [142]. Understanding and characterizing the circadian rhythmicity in gene expression in various tissues will play an important role in both optimizing the timing of drug administration and in the development of new therapeutics targeting the molecular clock. With the huge increase in the development of antibody and antisense oligonucleotide based therapeutics, characterizing the circadian rhythmicity in mRNA and protein expression will become more prominent in the future.

Acknowledgments

This work was supported by grant GM24211 from the National Institutes of Health.

References

1. Duffield GE. DNA microarray analyses of circadian timing: the genomic basis of biological time. *J Neuroendocrinol.* 2003; 15:991–1002. [PubMed: 12969245]
2. Almon RR, Yang E, Lai W, Androulakis IP, Ghimbovski S, Hoffman EP, Jusko WJ, Dubois DC. Relationships between circadian rhythms and modulation of gene expression by glucocorticoids in skeletal muscle. *Am J Physiol Regul Integr Comp Physiol.* 2008; 295:R1031–R1047. [PubMed: 18667713]
3. Bartness TJ, Song CK, Demas GE. SCN efferents to peripheral tissues: implications for biological rhythms. *J Biol Rhythms.* 2001; 16:196–204. [PubMed: 11407779]
4. Rusak B, Zucker I. Neural regulation of circadian rhythms. *Physiol Rev.* 1979; 59:449–526. [PubMed: 379886]
5. Ralph MR, Foster RG, Davis FC, Menaker M. Transplanted suprachiasmatic nucleus determines circadian period. *Science.* 1990; 247:975–978. [PubMed: 2305266]
6. Buijs RM, Scheer FA, Kreier F, Yi C, Bos N, Goncharuk VD, Kalsbeek A. Organization of circadian functions: interaction with the body. *Prog Brain Res.* 2006; 153:341–360. [PubMed: 16876585]
7. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. *Nature.* 2002; 418:935–941. [PubMed: 12198538]
8. Gooley JJ, Lu J, Chou TC, Scammell TE, Saper CB. Melanopsin in cells of origin of the retinohypothalamic tract. *Nat Neurosci.* 2001; 4:1165. [PubMed: 11713469]
9. Kriegsfeld LJ, Silver R. The regulation of neuroendocrine function: Timing is everything. *Horm Behav.* 2006; 49:557–574. [PubMed: 16497305]
10. Legoratti-Sanchez MO, Guevara-Guzman R, Solano-Flores LP. Electrophysiological evidences of a bidirectional communication between the locus coeruleus and the suprachiasmatic nucleus. *Brain Res Bull.* 1989; 23:283–288. [PubMed: 2590841]
11. Schulz P, Steimer T. Neurobiology of circadian systems. *CNS Drugs.* 2009; 23 Suppl 2:3–13. [PubMed: 19708721]
12. Challet E. Minireview: Entrainment of the suprachiasmatic clockwork in diurnal and nocturnal mammals. *Endocrinology.* 2007; 148:5648–5655. [PubMed: 17901231]
13. Dunlap JC. Molecular bases for circadian clocks. *Cell.* 1999; 96:271–290. [PubMed: 9988221]

14. Mirsky HP, Liu AC, Welsh DK, Kay SA, Doyle FJ 3rd. A model of the cell-autonomous mammalian circadian clock. *Proc Natl Acad Sci U S A*. 2009; 106:11107–11112. [PubMed: 19549830]
15. Preitner N, Damiola F, Lopez-Molina L, Zakany J, Duboule D, Albrecht U, Schibler U. The orphan nuclear receptor REV-ERB α controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell*. 2002; 110:251–260. [PubMed: 12150932]
16. Liu AC, Tran HG, Zhang EE, Priest AA, Welsh DK, Kay SA. Redundant function of REV-ERB α and β and non-essential role for Bmal1 cycling in transcriptional regulation of intracellular circadian rhythms. *PLoS Genet*. 2008; 4:e1000023. [PubMed: 18454201]
17. Jetten AM. Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. *Nucl Recept Signal*. 2009; 7:e003. [PubMed: 19381306]
18. Eide EJ, Kang H, Crapo S, Gallego M, Virshup DM. Casein kinase I in the mammalian circadian clock. *Methods Enzymol*. 2005; 393:408–418. [PubMed: 15817302]
19. Gachon F. Physiological function of PARBZip circadian clock-controlled transcription factors. *Ann Med*. 2007; 39:562–571. [PubMed: 17852034]
20. Balsalobre A, Damiola F, Schibler U. A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell*. 1998; 93:929–937. [PubMed: 9635423]
21. Welsh DK, Yoo SH, Liu AC, Takahashi JS, Kay SA. Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. *Curr Biol*. 2004; 14:2289–2295. [PubMed: 15620658]
22. Almon RR, Yang E, Lai W, Androulakis IP, DuBois DC, Jusko WJ. Circadian variations in rat liver gene expression: relationships to drug actions. *J Pharmacol Exp Ther*. 2008; 326:700–716. [PubMed: 18562560]
23. Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB. Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell*. 2002; 109:307–320. [PubMed: 12015981]
24. Challet E, Caldelas I, Graff C, Pevet P. Synchronization of the molecular clockwork by light- and food-related cues in mammals. *Biological Chemistry*. 2003; 384:711–719. [PubMed: 12817467]
25. Schibler U, Ripperger J, Brown SA. Peripheral circadian oscillators in mammals: time and food. *J Biol Rhythms*. 2003; 18:250–260. [PubMed: 12828282]
26. Coogan AN, Wyse CA. Neuroimmunology of the circadian clock. *Brain Res*. 2008; 1232:104–112. [PubMed: 18703032]
27. Dickmeis T. Glucocorticoids and the circadian clock. *J Endocrinol*. 2009; 200:3–22. [PubMed: 18971218]
28. Cailotto C, Lei J, van der Vliet J, van Heijningen C, van Eden CG, Kalsbeek A, Pevet P, Buijs RM. Effects of nocturnal light on (clock) gene expression in peripheral organs: a role for the autonomic innervation of the liver. *PLoS ONE*. 2009; 4:e5650. [PubMed: 19478857]
29. Altun A, Ugur-Altun B. Melatonin: therapeutic and clinical utilization. *Int J Clin Pract*. 2007; 61:835–845. [PubMed: 17298593]
30. Haus E. Chronobiology in the endocrine system. *Adv Drug Deliv Rev*. 2007; 59:985–1014. [PubMed: 17804113]
31. Poirel VJ, Boggio V, Dardente H, Pevet P, Masson-Pevet M, Gauer F. Contrary to other non-photic cues, acute melatonin injection does not induce immediate changes of clock gene mRNA expression in the rat suprachiasmatic nuclei. *Neuroscience*. 2003; 120:745–755. [PubMed: 12895514]
32. Agez L, Laurent V, Pevet P, Masson-Pevet M, Gauer F. Melatonin affects nuclear orphan receptors mRNA in the rat suprachiasmatic nuclei. *Neuroscience*. 2007; 144:522–530. [PubMed: 17067745]
33. Picinato MC, Hirata AE, Cipolla-Neto J, Curi R, Carvalho CR, Anhe GF, Carpinelli AR. Activation of insulin and IGF-1 signaling pathways by melatonin through MT1 receptor in isolated rat pancreatic islets. *J Pineal Res*. 2008; 44:88–94. [PubMed: 18078453]
34. Schibler U, Brown SA. Enlightening the adrenal gland. *Cell Metab*. 2005; 2:278–281. [PubMed: 16271527]

35. Almon RR, DuBois DC, Yao Z, Hoffman EP, Ghimbovski S, Jusko WJ. Microarray analysis of the temporal response of skeletal muscle to methylprednisolone: comparative analysis of two dosing regimens. *Physiol Genomics*. 2007; 30:282–299. [PubMed: 17473217]
36. Almon RR, DuBois DC, Jusko WJ. A microarray analysis of the temporal response of liver to methylprednisolone: a comparative analysis of two dosing regimens. *Endocrinology*. 2007; 148:2209–2225. [PubMed: 17303664]
37. Waterhouse J, Reilly T, Atkinson G, Edwards B. Jet lag: trends and coping strategies. *Lancet*. 2007; 369:1117–1129. [PubMed: 17398311]
38. Nagano M, Adachi A, Nakahama K, Nakamura T, Tamada M, Meyer-Bernstein E, Sehgal A, Shigeyoshi Y. An abrupt shift in the day/night cycle causes desynchrony in the mammalian circadian center. *J Neurosci*. 2003; 23:6141–6151. [PubMed: 12853433]
39. Cho K. Chronic 'jet lag' produces temporal lobe atrophy and spatial cognitive deficits. *Nat Neurosci*. 2001; 4:567–568. [PubMed: 11369936]
40. Woo JM, Postolache TT. The impact of work environment on mood disorders and suicide: Evidence and implications. *Int J Disabil Hum Dev*. 2008; 7:185–200. [PubMed: 18836547]
41. Esquirol Y, Bongard V, Mabile L, Jonnier B, Soulat JM, Perret B. Shift work and metabolic syndrome: respective impacts of job strain, physical activity, and dietary rhythms. *Chronobiol Int*. 2009; 26:544–559. [PubMed: 19360495]
42. Biggi N, Consonni D, Galluzzo V, Sogliani M, Costa G. Metabolic syndrome in permanent night workers. *Chronobiol Int*. 2008; 25:443–454. [PubMed: 18484373]
43. Pietroiusti A, Neri A, Somma G, Coppeta L, Iavicoli I, Bergamaschi A, Magrini A. Incidence of metabolic syndrome among night shift health care workers. *Occup Environ Med*. 2009
44. Tirone TA, Brunicardi FC. Overview of glucose regulation. *World J Surg*. 2001; 25:461–467. [PubMed: 11344399]
45. Zvonic S, Ptitsyn AA, Kilroy G, Wu X, Conrad SA, Scott LK, Guilak F, Pelled G, Gazit D, Gimple JM. Circadian oscillation of gene expression in murine calvarial bone. *J Bone Miner Res*. 2007; 22:357–365. [PubMed: 17144790]
46. Storch KF, Lipan O, Leykin I, Viswanathan N, Davis FC, Wong WH, Weitz CJ. Extensive and divergent circadian gene expression in liver and heart. *Nature*. 2002; 417:78–83. [PubMed: 11967526]
47. Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, Eckel RH, Takahashi JS, Bass J. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science*. 2005; 308:1043–1045. [PubMed: 15845877]
48. Oishi K, Shirai H, Ishida N. CLOCK is involved in the circadian transactivation of peroxisome-proliferator-activated receptor alpha (PPARalpha) in mice. *Biochem J*. 2005; 386:575–581. [PubMed: 15500444]
49. Lamia KA, Storch KF, Weitz CJ. Physiological significance of a peripheral tissue circadian clock. *Proc Natl Acad Sci U S A*. 2008; 105:15172–15177. [PubMed: 18779586]
50. Yin L, Wu N, Curtin JC, Qatanani M, Szewergold NR, Reid RA, Waitt GM, Parks DJ, Pearce KH, Wisely GB, Lazar MA. Rev-erbalpha, a heme sensor that coordinates metabolic and circadian pathways. *Science*. 2007; 318:1786–1789. [PubMed: 18006707]
51. Ando H, Oshima Y, Yanagihara H, Hayashi Y, Takamura T, Kaneko S, Fujimura A. Profile of rhythmic gene expression in the livers of obese diabetic KK-A(y) mice. *Biochem Biophys Res Commun*. 2006; 346:1297–1302. [PubMed: 16793009]
52. Hsieh MC, Yang SC, Tseng HL, Hwang LL, Chen CT, Shieh KR. Abnormal expressions of circadian-clock and circadian clock-controlled genes in the livers and kidneys of long-term, high-fat-diet-treated mice. *Int J Obes (Lond)*. 2009
53. Goto Y, Suzuki K, Ono T, Sasaki M. T. Toyota, Development of diabetes in the non-obese NIDDM rat (GK rat). *Adv. Exptl. Med. Biol*. 1988; 246:29–31. [PubMed: 3074659]
54. Peschke E, Frese T, Chankiewicz E, Peschke D, Preiss U, Schneyer U, Spessert R, Muhlbauer E. Diabetic Goto Kakizaki rats as well as type 2 diabetic patients show a decreased diurnal serum melatonin level and an increased pancreatic melatonin-receptor status. *J Pineal Res*. 2006; 40:135–143. [PubMed: 16441550]

55. Frese T, Bach AG, Muhlbauer E, Ponick K, Bromme HJ, Welp A, Peschke E. Pineal melatonin synthesis is decreased in type 2 diabetic Goto-Kakizaki rats. *Life Sci.* 2009; 85:526–533. [PubMed: 19695268]
56. Frese T, Bazwinsky I, Muhlbauer E, Peschke E. Circadian and age-dependent expression patterns of GLUT2 and glucokinase in the pancreatic beta-cell of diabetic and nondiabetic rats. *Horm Metab Res.* 2007; 39:567–574. [PubMed: 17712721]
57. Feneberg R, Lemmer B. Circadian rhythm of glucose uptake in cultures of skeletal muscle cells and adipocytes in Wistar-Kyoto, Wistar, Goto-Kakizaki, and spontaneously hypertensive rats. *Chronobiol Int.* 2004; 21:521–538. [PubMed: 15470952]
58. Zhang EE, Liu AC, Hirota T, Miraglia LJ, Welch G, Pongsawakul PY, Liu X, Atwood A, Huss JW 3rd, Janes J, Su AI, Hogenesch JB, Kay SA. A genome-wide RNAi screen for modifiers of the circadian clock in human cells. *Cell.* 2009; 139:199–210. [PubMed: 19765810]
59. Harwood HJ Jr, Bridge DM, Stacpoole PW. In vivo regulation of human mononuclear leukocyte 3-hydroxy-3-methylglutaryl coenzyme A reductase. Studies in normal subjects. *J Clin Invest.* 1987; 79:1125–1132. [PubMed: 3558818]
60. Um JH, Yang S, Yamazaki S, Kang H, Viollet B, Foretz M, Chung JH. Activation of 5'-AMP-activated kinase with diabetes drug metformin induces casein kinase Iepsilon (CKIepsilon)-dependent degradation of clock protein mPer2. *J Biol Chem.* 2007; 282:20794–20798. [PubMed: 17525164]
61. Tiedgen M, Seitz HJ. Dietary control of circadian variations in serum insulin, glucagon and hepatic cyclic AMP. *J Nutr.* 1980; 110:876–882. [PubMed: 6246227]
62. Kennaway DJ, Owens JA, Voultsios A, Boden MJ, Varcoe TJ. Metabolic homeostasis in mice with disrupted Clock gene expression in peripheral tissues. *Am J Physiol Regul Integr Comp Physiol.* 2007; 293:R1528–R1537. [PubMed: 17686888]
63. Gomez-Santos C, Gomez-Abellan P, Madrid JA, Hernandez-Morante JJ, Lujan JA, Ordovas JM, Garaulet M. Circadian rhythm of clock genes in human adipose explants. *Obesity (Silver Spring).* 2009; 17:1481–1485. [PubMed: 19478785]
64. Wang N, Yang G, Jia Z, Zhang H, Aoyagi T, Soodvilai S, Symons JD, Schnermann JB, Gonzalez FJ, Litwin SE, Yang T. Vascular PPARgamma controls circadian variation in blood pressure and heart rate through Bmal1. *Cell Metab.* 2008; 8:482–491. [PubMed: 19041764]
65. Hayashi M, Shimba S, Tezuka M. Characterization of the molecular clock in mouse peritoneal macrophages. *Biol Pharm Bull.* 2007; 30:621–626. [PubMed: 17409491]
66. Arjona A, Sarkar DK. Circadian oscillations of clock genes, cytolytic factors, and cytokines in rat NK cells. *J Immunol.* 2005; 174:7618–7624. [PubMed: 15944262]
67. Boivin DB, James FO, Wu A, Cho-Park PF, Xiong H, Sun ZS. Circadian clock genes oscillate in human peripheral blood mononuclear cells. *Blood.* 2003; 102:4143–4145. [PubMed: 12893774]
68. Cutolo M, Straub RH. Circadian rhythms in arthritis: hormonal effects on the immune/inflammatory reaction. *Autoimmun Rev.* 2008; 7:223–228. [PubMed: 18190882]
69. Liu J, Malkani G, Shi X, Meyer M, Cunningham-Runddles S, Ma X, Sun ZS. The circadian clock Period 2 gene regulates gamma interferon production of NK cells in host response to lipopolysaccharide-induced endotoxic shock. *Infect Immun.* 2006; 74:4750–4756. [PubMed: 16861663]
70. Migita H, Morser J, Kawai K. Rev-erbalpha upregulates NF-kappaB-responsive genes in vascular smooth muscle cells. *FEBS Lett.* 2004; 561:69–74. [PubMed: 15013753]
71. Wang J, Yin L, Lazar MA. The orphan nuclear receptor Rev-erb alpha regulates circadian expression of plasminogen activator inhibitor type 1. *J Biol Chem.* 2006; 281:33842–33848. [PubMed: 16968709]
72. Jaradat M, Stapleton C, Tilley SL, Dixon D, Erikson CJ, McCaskill JG, Kang HS, Angers M, Liao G, Collins J, Grissom S, Jetten AM. Modulatory role for retinoid-related orphan receptor alpha in allergen-induced lung inflammation. *Am J Respir Crit Care Med.* 2006; 174:1299–1309. [PubMed: 16973978]
73. Tilley SL, Jaradat M, Stapleton C, Dixon D, Hua X, Erikson CJ, McCaskill JG, Chason KD, Liao G, Jania L, Koller BH, Jetten AM. Retinoid-related orphan receptor gamma controls

- immunoglobulin production and Th1/Th2 cytokine balance in the adaptive immune response to allergen. *J Immunol.* 2007; 178:3208–3218. [PubMed: 17312169]
74. Buttgereit F, Doering G, Schaeffler A, Witte S, Sierakowski S, Gromnica-Ihle E, Jeka S, Krueger K, Szechinski J, Alten R. Efficacy of modified-release versus standard prednisone to reduce duration of morning stiffness of the joints in rheumatoid arthritis (CAPRA-1): a double-blind, randomised controlled trial. *Lancet.* 2008; 371:205–214. [PubMed: 18207016]
 75. Arvidson NG, Gudbjornsson B, Larsson A, Hallgren R. The timing of glucocorticoid administration in rheumatoid arthritis. *Ann Rheum Dis.* 1997; 56:27–31. [PubMed: 9059137]
 76. Hetzel MR, Clark TJ. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. *Thorax.* 1980; 35:732–738. [PubMed: 7466721]
 77. Martin RJ. Location of airway inflammation in asthma and the relationship to circadian change in lung function. *Chronobiol Int.* 1999; 16:623–630. [PubMed: 10513885]
 78. Dimitrov S, Benedict C, Heutling D, Westermann J, Born J, Lange T. Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. *Blood.* 2009; 113:5134–5143. [PubMed: 19293427]
 79. Lemmer B. The clinical relevance of chronopharmacology in therapeutics. *Pharmacol Res.* 1996; 33:107–115. [PubMed: 8870025]
 80. Gibbs JE, Beesley S, Plumb J, Singh D, Farrow S, Ray DW, Loudon AS. Circadian timing in the lung; a specific role for bronchiolar epithelial cells. *Endocrinology.* 2009; 150:268–276. [PubMed: 18787022]
 81. Murray AW. Creative blocks: cell-cycle checkpoints and feedback controls. *Nature.* 1992; 359:599–604. [PubMed: 1406993]
 82. Borgs L, Beukelaers P, Vandenbosch R, Belachew S, Nguyen L, Malgrange B. Cell "circadian" cycle: new role for mammalian core clock genes. *Cell Cycle.* 2009; 8:832–837. [PubMed: 19221497]
 83. Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F, Okamura H. Control mechanism of the circadian clock for timing of cell division in vivo. *Science.* 2003; 302:255–259. [PubMed: 12934012]
 84. Grechez-Cassiau A, Rayet B, Guillaumond F, Teboul M, Delaunay F. The circadian clock component BMAL1 is a critical regulator of p21WAF1/CIP1 expression and hepatocyte proliferation. *J Biol Chem.* 2008; 283:4535–4542. [PubMed: 18086663]
 85. Mullenders J, Fabius AW, Madiredjo M, Bernards R, Beijersbergen RL. A large scale shRNA barcode screen identifies the circadian clock component ARNTL as putative regulator of the p53 tumor suppressor pathway. *PLoS ONE.* 2009; 4:e4798. [PubMed: 19277210]
 86. Taniguchi H, Fernandez AF, Setien F, Ropero S, Ballestar E, Villanueva A, Yamamoto H, Imai K, Shinomura Y, Esteller M. Epigenetic inactivation of the circadian clock gene BMAL1 in hematologic malignancies. *Cancer Res.* 2009; 69:8447–8454. [PubMed: 19861541]
 87. Lee CC. Tumor suppression by the mammalian Period genes. *Cancer Causes Control.* 2006; 17:525–530. [PubMed: 16596306]
 88. Cao Q, Gery S, Dashti A, Yin D, Zhou Y, Gu J, Koeffler HP. A role for the clock gene per1 in prostate cancer. *Cancer Res.* 2009; 69:7619–7625. [PubMed: 19752089]
 89. Gery S, Komatsu N, Kawamata N, Miller CW, Desmond J, Virk RK, Marchevsky A, McKenna R, Taguchi H, Koeffler HP. Epigenetic silencing of the candidate tumor suppressor gene Per1 in non-small cell lung cancer. *Clin Cancer Res.* 2007; 13:1399–1404. [PubMed: 17332281]
 90. Winter SL, Bosnoyan-Collins L, Pinnaduwa D, Andrulis IL. Expression of the circadian clock genes Per1 and Per2 in sporadic and familial breast tumors. *Neoplasia.* 2007; 9:797–800. [PubMed: 17971899]
 91. Walisser JA, Bradfield CA. A time to divide: does the circadian clock control cell cycle? *Dev Cell.* 2006; 10:539–540. [PubMed: 16678767]
 92. Gery S, Komatsu N, Baldjyan L, Yu A, Koo D, Koeffler HP. The circadian gene per1 plays an important role in cell growth and DNA damage control in human cancer cells. *Mol Cell.* 2006; 22:375–382. [PubMed: 16678109]
 93. Gauger MA, Sancar A. Cryptochrome, circadian cycle, cell cycle checkpoints, and cancer. *Cancer Res.* 2005; 65:6828–6834. [PubMed: 16061665]

94. Ozturk N, Lee JH, Gaddameedhi S, Sancar A. Loss of cryptochrome reduces cancer risk in p53 mutant mice. *Proc Natl Acad Sci U S A*. 2009; 106:2841–2846. [PubMed: 19188586]
95. Utz AC, Hirner H, Blatz A, Hillenbrand A, Schmidt B, Deppert W, Henne-Bruns D, Fischer D, Thal DR, Leithauser F, Knippschild U. Analysis of Cell-type Specific Expression of CK1 {varepsilon} in Various Tissues of Young Adult BALB/c Mice and in Mammary Tumors of SV40 T-Ag Transgenic Mice. *J Histochem Cytochem*. 2009
96. Modak C, Bryant P. Casein Kinase I epsilon positively regulates the Akt pathway in breast cancer cell lines. *Biochem Biophys Res Commun*. 2008; 368:801–807. [PubMed: 18262492]
97. Fuja TJ, Lin F, Osann KE, Bryant PJ. Somatic mutations and altered expression of the candidate tumor suppressors CSNK1 epsilon, DLG1, and EDD/hHYD in mammary ductal carcinoma. *Cancer Res*. 2004; 64:942–951. [PubMed: 14871824]
98. Yang WS, Stockwell BR. Inhibition of casein kinase 1-epsilon induces cancer-cell-selective, PERIOD2-dependent growth arrest. *Genome Biol*. 2008; 9:R92. [PubMed: 18518968]
99. Brockschmidt C, Hirner H, Huber N, Eismann T, Hillenbrand A, Giamas G, Radunsky B, Ammerpohl O, Bohm B, Henne-Bruns D, Kalthoff H, Leithauser F, Trauzold A, Knippschild U. Anti-apoptotic and growth-stimulatory functions of CK1 delta and epsilon in ductal adenocarcinoma of the pancreas are inhibited by IC261 in vitro and in vivo. *Gut*. 2008; 57:799–806. [PubMed: 18203806]
100. Tommasi S, Mangia A, Lacalamita R, Bellizzi A, Fedele V, Chiriatti A, Thomssen C, Kendzierski N, Latorre A, Lorusso V, Schittulli F, Zito F, Kavallaris M, Paradiso A. Cytoskeleton and paclitaxel sensitivity in breast cancer: the role of beta-tubulins. *Int J Cancer*. 2007; 120:2078–2085. [PubMed: 17285590]
101. Wood PA, Du-Quito J, You S, Hrushesky WJ. Circadian clock coordinates cancer cell cycle progression, thymidylate synthase, and 5-fluorouracil therapeutic index. *Mol Cancer Ther*. 2006; 5:2023–2033. [PubMed: 16928823]
102. Lincoln DW 2nd, Hrushesky WJ, Wood PA. Circadian organization of thymidylate synthase activity in normal tissues: a possible basis for 5-fluorouracil chronotherapeutic advantage. *Int J Cancer*. 2000; 88:479–485. [PubMed: 11054680]
103. Zhang R, Lu Z, Liu T, Soong SJ, Diasio RB. Relationship between circadian-dependent toxicity of 5-fluorodeoxyuridine and circadian rhythms of pyrimidine enzymes: possible relevance to fluoropyrimidine chemotherapy. *Cancer Res*. 1993; 53:2816–2822. [PubMed: 8504424]
104. Waldrop RD, Saydjari R, Arnold JR, Ford P, Rubin NH, Poston GJ, Lawrence J, Rayford PL, Townsend CM Jr, Thompson JC. Twenty-four-hour variations in ornithine decarboxylase and acid phosphatase in mice. *Proc Soc Exp Biol Med*. 1989; 191:420–424. [PubMed: 2771969]
105. Fujimoto M, Kanaya A, Nakabou Y, Hagiwara H. Circadian rhythm in the ornithine decarboxylase activity of rat small intestine. *J Biochem*. 1978; 83:237–242. [PubMed: 624705]
106. Iurisci I, Filipski E, Sallam H, Harper F, Guettier C, Maire I, Hassan M, Iacobelli S, Levi F. Liver circadian clock, a pharmacologic target of cyclin-dependent kinase inhibitor seliciclib. *Chronobiol Int*. 2009; 26:1169–1188. [PubMed: 19731111]
107. Canaple L, Kakizawa T, Laudet V. The days and nights of cancer cells. *Cancer Res*. 2003; 63:7545–7552. [PubMed: 14633665]
108. Labrecque G, Belanger PM. Biological rhythms in the absorption, distribution, metabolism and excretion of drugs. *Pharmacol Ther*. 1991; 52:95–107. [PubMed: 1805249]
109. Takano M, Yumoto R, Murakami T. Expression and function of efflux drug transporters in the intestine. *Pharmacol Ther*. 2006; 109:137–161. [PubMed: 16209890]
110. Scheving LA. Biological clocks and the digestive system. *Gastroenterology*. 2000; 119:536–549. [PubMed: 10930389]
111. Hoogerwerf WA, Hellmich HL, Cornelissen G, Halberg F, Shahinian VB, Bostwick J, Savidge TC, Cassone VM. Clock gene expression in the murine gastrointestinal tract: endogenous rhythmicity and effects of a feeding regimen. *Gastroenterology*. 2007; 133:1250–1260. [PubMed: 17919497]
112. Bron R, Furness JB. Rhythm of digestion: keeping time in the gastrointestinal tract. *Clin Exp Pharmacol Physiol*. 2009; 36:1041–1048. [PubMed: 19566817]

113. Stearns AT, Balakrishnan A, Rhoads DB, Ashley SW, Tavakkolizadeh A. Diurnal rhythmicity in the transcription of jejunal drug transporters. *J Pharmacol Sci.* 2008; 108:144–148. [PubMed: 18787306]
114. Murakami Y, Higashi Y, Matsunaga N, Koyanagi S, Ohdo S. Circadian clock-controlled intestinal expression of the multidrug-resistance gene *mdr1a* in mice. *Gastroenterology.* 2008; 135:1636–1644. e1633. [PubMed: 18773899]
115. Erol K, Kilic FS, Batu OS, Yildirim E. Morning-evening administration time differences in digoxin kinetics in healthy young subjects. *Chronobiol Int.* 2001; 18:841–849. [PubMed: 11763991]
116. Bruguerolle B. Chronopharmacokinetics. Current status. *Clin Pharmacokinet.* 1998; 35:83–94. [PubMed: 9739478]
117. Cao QR, Kim TW, Choi JS, Lee BJ. Circadian variations in the pharmacokinetics, tissue distribution and urinary excretion of nifedipine after a single oral administration to rats. *Biopharm Drug Dispos.* 2005; 26:427–437. [PubMed: 16217814]
118. Pan X, Hussain MM. Clock is important for food and circadian regulation of macronutrient absorption in mice. *J Lipid Res.* 2009
119. Saito H, Terada T, Shimakura J, Katsura T, Inui K. Regulatory mechanism governing the diurnal rhythm of intestinal H⁺/peptide cotransporter 1 (PEPT1). *Am J Physiol Gastrointest Liver Physiol.* 2008; 295:G395–G402. [PubMed: 18583459]
120. Delp MD, Manning RO, Bruckner JV, Armstrong RB. Distribution of cardiac output during diurnal changes of activity in rats. *Am J Physiol.* 1991; 261:H1487–H1493. [PubMed: 1951736]
121. Ko GY, Shi L, Ko ML. Circadian regulation of ion channels and their functions. *J Neurochem.* 2009; 110:1150–1169. [PubMed: 19549279]
122. Gibaldi M, McNamara PJ. Apparent volumes of distribution and drug binding to plasma proteins and tissues. *Eur J Clin Pharmacol.* 1978; 13:373–380. [PubMed: 668796]
123. Hecquet B, Meynadier J, Bonnetterre J, Adenis L, Demaille A. Time dependency in plasmatic protein binding of cisplatin. *Cancer Treat Rep.* 1985; 69:79–83. [PubMed: 4038472]
124. Dovio A, Generali D, Tampellini M, Berruti A, Tedoldi S, Torta M, Bonardi S, Tucci M, Allevi G, Aguggini S, Bottini A, Dogliotti L, Angeli A. Variations along the 24-hour cycle of circulating osteoprotegerin and soluble RANKL: a rhythmometric analysis. *Osteoporos Int.* 2008; 19:113–117. [PubMed: 17703272]
125. Peters T Jr, Peters JC. The biosynthesis of rat serum albumin. VI. Intracellular transport of albumin and rates of albumin and liver protein synthesis in vivo under various physiological conditions. *J Biol Chem.* 1972; 247:3858–3863. [PubMed: 5033393]
126. Baraldo M. The influence of circadian rhythms on the kinetics of drugs in humans. *Expert Opin Drug Metab Toxicol.* 2008; 4:175–192. [PubMed: 18248311]
127. Chan JL, Bluher S, Yiannakouris N, Suchard MA, Kratzsch J, Mantzoros CS. Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin: observational and interventional studies in humans. *Diabetes.* 2002; 51:2105–2112. [PubMed: 12086939]
128. Liu L, Pang KS. An integrated approach to model hepatic drug clearance. *Eur J Pharm Sci.* 2006; 29:215–230. [PubMed: 16806855]
129. Zhang YK, Yeager RL, Klaassen CD. Circadian expression profiles of drug-processing genes and transcription factors in mouse liver. *Drug Metab Dispos.* 2009; 37:106–115. [PubMed: 18838502]
130. Lavery DJ, Lopez-Molina L, Margueron R, Fleury-Olela F, Conquet F, Schibler U, Bonfils C. Circadian expression of the steroid 15 alpha-hydroxylase (*Cyp2a4*) and coumarin 7-hydroxylase (*Cyp2a5*) genes in mouse liver is regulated by the PAR leucine zipper transcription factor DBP. *Mol Cell Biol.* 1999; 19:6488–6499. [PubMed: 10490589]
131. Gachon F, Olela FF, Schaad O, Descombes P, Schibler U. The circadian PAR-domain basic leucine zipper transcription factors DBP, TEF, and HLF modulate basal and inducible xenobiotic detoxification. *Cell Metab.* 2006; 4:25–36. [PubMed: 16814730]

132. Pons M, Forpomes O, Espagnet S, Cambar J. Relationship between circadian changes in renal hemodynamics and circadian changes in urinary glycosaminoglycan excretion in normal rats. *Chronobiol Int*. 1996; 13:349–358. [PubMed: 8957586]
133. Bleyzac N, Allard-Latour B, Laffont A, Mouret J, Jelliffe R, Maire P. Diurnal changes in the pharmacokinetic behavior of amikacin. *Ther Drug Monit*. 2000; 22:307–312. [PubMed: 10850398]
134. Zuber AM, Centeno G, Pradervand S, Nikolaeva S, Maquelin L, Cardinaux L, Bonny O, Firsov D. Molecular clock is involved in predictive circadian adjustment of renal function. *Proc Natl Acad Sci U S A*. 2009; 106:16523–16528. [PubMed: 19805330]
135. White CA, Pardue R, Huang C, Warren J. Chronobiological Evaluation of the Active Biliary and Renal Secretion of Ampicillin. *Chronobiology International*. 1995; 12:410–418.
136. Gumz ML, Stow LR, Lynch II, Greenlee MM, Rudin A, Cain BD, Weaver DR, Wingo CS. The circadian clock protein Period 1 regulates expression of the renal epithelial sodium channel in mice. *J Clin Invest*. 2009; 119:2423–2434. [PubMed: 19587447]
137. Pan X, Terada T, Irie M, Saito H, Inui K. Diurnal rhythm of H⁺-peptide cotransporter in rat small intestine. *Am J Physiol Gastrointest Liver Physiol*. 2002; 283:G57–G64. [PubMed: 12065292]
138. Hazra A, Pyszczyński N, DuBois DC, Almon RR, Jusko WJ. Modeling receptor/gene-mediated effects of corticosteroids on hepatic tyrosine aminotransferase dynamics in rats: dual regulation by endogenous and exogenous corticosteroids. *J Pharmacokinet Pharmacodyn*. 2007; 34:643–667. [PubMed: 17593325]
139. Yao Z, DuBois DC, Almon RR, Jusko WJ. Modeling circadian rhythms of glucocorticoid receptor and glutamine synthetase expression in rat skeletal muscle. *Pharm Res*. 2006; 23:670–679. [PubMed: 16673181]
140. Hazra A, Pyszczyński NA, DuBois DC, Almon RR, Jusko WJ. Modeling of corticosteroid effects on hepatic low-density lipoprotein receptors and plasma lipid dynamics in rats. *Pharm Res*. 2008; 25:769–780. [PubMed: 17674160]
141. Chakraborty A, Krzyzanski W, Jusko WJ. Mathematical modeling of circadian cortisol concentrations using indirect response models: comparison of several methods. *J Pharmacokinet Biopharm*. 1999; 27:23–43. [PubMed: 10533696]
142. Refinetti R, Cornelissen G, Halberg F. Procedures for numerical analysis of circadian rhythms. *Biological Rhythm Research*. 2007; 38:275–325.
143. Doi R, Oishi K, Ishida N. CLOCK regulates circadian rhythms of hepatic glycogen synthesis through transcriptional activation of Glycogen synthase 2. *J Biol Chem*. "in press".
144. Shimba S, Ishii N, Ohta Y, Ohno T, Watabe Y, Hayashi M, Wada T, Aoyagi T, Tezuka M. Brain and muscle Arnt-like protein-1 (BMAL1), a component of the molecular clock, regulates adipogenesis. *Proc Natl Acad Sci U S A*. 2005; 102:12071–12076. [PubMed: 16093318]
145. Kondratov RV, Kondratova AA, Gorbacheva VY, Vykhovanets OV, Antoch MP. Early aging and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock. *Genes Dev*. 2006; 20:1868–1873. [PubMed: 16847346]
146. Ratajczak CK, Boehle KL, Muglia LJ. Impaired steroidogenesis and implantation failure in *Bmal1*^{−/−} mice. *Endocrinology*. 2009; 150:1879–1885. [PubMed: 19056819]
147. Albrecht U, Bordon A, Schmutz I, Ripperger J. The multiple facets of Per2. *Cold Spring Harb Symp Quant Biol*. 2007; 72:95–104. [PubMed: 18419266]
148. Masuki S, Todo T, Nakano Y, Okamura H, Nose H. Reduced alpha-adrenoceptor responsiveness and enhanced baroreflex sensitivity in *Cry*-deficient mice lacking a biological clock. *J Physiol*. 2005; 566:213–224. [PubMed: 15860530]
149. Matsunaga N, Ikeda M, Takiguchi T, Koyanagi S, Ohdo S. The molecular mechanism regulating 24-hour rhythm of CYP2E1 expression in the mouse liver. *Hepatology*. 2008; 48:240–251. [PubMed: 18537186]
140. Doi M, Takahashi Y, Komatsu R, Yamazaki F, Yamada H, Haraguchi S, Emoto N, Okuno Y, Tsujimoto G, Kanematsu A, Ogawa O, Todo T, Tsutsui K, van der Horst GT, Okamura H. Salt-sensitive hypertension in circadian clock-deficient *Cry*-null mice involves dysregulated adrenal Hsd3b6. *Nat Med*. 2010; 16:67–74. [PubMed: 20023637]

151. Chomez P, Neveu I, Mansen A, Kiesler E, Larsson L, Vennstrom B, Arenas E. Increased cell death and delayed development in the cerebellum of mice lacking the rev-erbA(alpha) orphan receptor. *Development*. 2000; 127:1489–1498. [PubMed: 10704394]
152. Yin L, Wu N, Lazar MA. Nuclear receptor Rev-erbalpha: a heme receptor that coordinates circadian rhythm and metabolism. *Nucl Recept Signal*. 2010; 8:e001. [PubMed: 20414452]
153. Kang HS, Angers M, Beak JY, Wu X, Gimble JM, Wada T, Xie W, Collins JB, Grissom SF, Jetten AM. Gene expression profiling reveals a regulatory role for ROR alpha and ROR gamma in phase I and phase II metabolism. *Physiol Genomics*. 2007; 31:281–294. [PubMed: 17666523]
154. Wang Y, Solt LA, Burris TP. Regulation of FGF21 expression and secretion by the retinoic acid receptor-related orphan receptor{alpha}. *J Biol Chem*. "in press".

Figure 1.

Clustering of genes showing circadian regulation in their expression in rat skeletal muscle. Expression levels were obtained from microarray analyses. The figure represents 8 clusters of genes that show similar expression patterns within the daily light/dark cycle. The data was collected from a total of 54 animals across 18 time points throughout the 24 h cycle and the data is concatenated to form a 48 h time series for better visualization. The unshaded box represents the light period and the shaded box represents the dark (lights off) period.

Figure 2.

Schematic representation of the mammalian molecular clock oscillator. The central mechanism regulating the molecular clock consists of an auto-regulatory feedback loop which oscillates with a periodicity of approximately 24 h. The transcription factors CLOCK and BMAL1 heterodimerize to form the positive arm of the regulatory loop and up-regulate the expression of PER and CRY transcription factors by binding to the E-box element present in the upstream region of these genes. PER and CRY transcription factors form the negative arm of the regulatory loop. After reaching a critical concentration, PER and CRY form heterotypic complexes and repress the transcriptional activity of the CLOCK:BMAL1 complex. The nuclear receptor transcription factors ROR and REV-ERB are transcriptionally regulated by the CLOCK:BMAL1 complex through E-box elements present in the upstream region of these genes. In turn ROR up-regulates and REV-ERB down-regulates the expression of *Bmal1* by binding to the RORE element present in the upstream region of the *Bmal1* gene. Furthermore, Casein kinase 1 epsilon (CK1ξ) phosphorylates and tags PER for proteasome-mediated degradation and hence regulates the stability of the transcription factor. In the figure + represents up-regulation of the gene and | represents down-regulation.

Figure 3.

Circadian expression pattern of some of the core clock and the clock controlled transcription factors in rat skeletal muscle as a function of circadian time. Circles represent the original data and solid lines represent the curve fitted to a sine function. The unshaded box represents the light period and the shaded box represents the dark (lights off) period.

Table 1

Selected physiological functions, target genes, and knockout characteristics of molecular clock transcription factors

Molecular Clock Transcription Factors	Characteristics of Knockouts	Selected* Target Genes	Selected Functions	References
CLOCK	Obesity, metabolic syndrome, hyperphagia; hyperinsulinemia, hyperlipidemia; hepatic steatosis; dysregulation of ghrelin, orexin; CART	<i>Gys2, Ppara, Wee1</i>	Lipid and glucose metabolism; cell cycle regulation	[47,48,83, 143]
BMAL1	Hypoglycemia, impaired glucose clearance; reduced life span, premature aging; impaired steroidogenesis, reduced fertility; impaired adipogenesis	<i>Srebf1, Aebp1, Aacs, Fasn, Fabp4, Cebpa, Hmgcr, Pck1, Wee1, Cdkn1a</i>	Glucose homeostasis; adipogenesis; Cell cycle regulation	[49,84, 144–146]
PER	Cancer-prone, abnormal response to gamma irradiation, increased cellular proliferation; reduced muscular strength under stress; loss of food anticipatory activity	<i>Ccnd1, Ccna, Myc, Mdm2, Ifng, Vegf, Atm</i>	Tumor suppressor; cell cycle regulation	[69,87,91, 147]
CRY	Impaired liver regeneration; reduced α -adrenoceptor responsiveness, salt sensitive hypertension; reduced tumor development, lowered incidence of cancer	<i>Wee1, Cdk1, Cyp2e1, Hsd3b6</i>	Xenobiotic detoxification, cell proliferation	[83,94, 148–150]
REV-ERB	Elevated serum VLDL triglycerides; impaired Purkinje cells development, delayed proliferation and migration of granule cells; increased apoptosis of neurons in the internal granule cell layer; Aberrant expression of myosin isoforms in skeletal muscles	<i>Apoa1, Apoc3, G6p, Il6, Ptgs2, Pai-1, Cdkn1a</i>	Adipogenesis; fiber type switching; cerebellar development; hepatic gluconeogenesis	[50,70,71, 151,152]
ROR	Lower plasma triglycerides; abnormal lymphoid organ development, reduced survival rates of thymocytes, reduced susceptibility to autoimmune and inflammatory disorders; thin long bones; ataxia and severe cerebellar atrophy; fewer Purkinje cells, a loss of cerebellar granule cells	<i>Fgf21, Pcp2, Pcp4, Shh, Slc1a6, Apoc2, Apoa1, rebf1, Cyp7b1</i>	Bone formation; lipid homeostasis; several immune functions; steroids, bile acids and xenobiotics metabolism	[17,72, 153,154]
PAR bZIP Transcription Factors	Increased liver mass, xenobiotic stress; accelerated ageing/ premature death	<i>Cyp2a4, Cyp2a5, Car, Sult1d1, Gsta2, Gsta3, Aldh1a1, Aldh1a7, Ugt1a1, Slc15a1, Abcb1a</i>	Regulation of phase I, II and III detoxifying enzymes; hematopoietic cell differentiation	[19,130, 131]

Abbreviations: Aacs = acetoacetyl-CoA synthetase; Abcb1a = ATP-binding cassette, sub-family B (MDR/TAP), member 1; Aebp1 = AE binding protein 1; Aldh1a1&7 = aldehyde dehydrogenase 1 family, member A1&7; Apoa1 = apolipoprotein A-I; Apoc 2&3 = apolipoprotein C 2&3; Atm = ataxia telangiectasia mutated; Car = constitutive androstane receptor; Ccna = cyclin A; Ccnd1 = cyclin D1; Cdk1 = cyclin-dependent kinase 1; Cdkn1a = cyclin-dependent kinase inhibitor 1A; Cebpa = CCAAT/enhancer binding protein alpha; Cyp2a 4&5 = cytochrome P450, family 2, subfamily A, polypeptide 4&5; Cyp2e1 = cytochrome P450, family 2, subfamily E, polypeptide 1; Cyp7b1 = cytochrome P450, family 7, subfamily B, polypeptide 1; Fabp4 = fatty acid binding protein 4; Fasn = fatty acid synthase; Fgf21 = fibroblast growth factor 21; G6p = glucose-6-phosphatase; Gsta2&3 = glutathione S-transferase alpha 2&3; Gys2 = Glycogen synthase 2; Hmgcr = 3-hydroxy-3-methylglutaryl-Coenzyme A reductase; Hsd3b6 = hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 6; Ifng = interferon, gamma; Il6 = interleukin 6; Myc = myelocytomatosis oncogene; Pai-1 = Plasminogen activator inhibitor-1; Pck1 = phosphoenolpyruvate carboxykinase 1; Pcp 2& 4 = Purkinje cell protein 2&4; Ppara = peroxisome proliferator-activated receptor alpha; Ptgs2 = prostaglandin-endoperoxide synthase 2; Shh = sonic hedgehog homolog; Slc15a1 = solute carrier family 15 (oligopeptide transporter), member 1; Slc1a6 = solute carrier family 1 (high affinity aspartate/ glutamate transporter), member 6; Srebf1 = sterol regulatory element binding transcription factor 1; Sult1d1 = sulfotransferase family, cytosolic, 1D, member 1; Ugt1a1 = UDP glucuronosyltransferase 1 family, polypeptide A1; Vegf = vascular endothelial growth factor; Wee1 = WEE 1 homolog