Chapter 4
Biomedical Effects of Circadian Rhythm Disturbances

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Abstract Circadian rhythms are biological processes that recur on a daily basis and exist to appropriately organize physiology, metabolism, and behavior relative to the 24-h light/dark cycle created by the rotation of the Earth. These rhythms are controlled by a genetically encoded molecular clock active in most, if not all, cells in the body. In mammals, these cell-autonomous oscillators are regulated and synchronized by the master clock in the suprachiasmatic nucleus (SCN) of the hypothalamus through a variety of direct and indirect pathways. The circadian timekeeping system imposes integrated temporal organization to ongoing biochemical and physiological processes throughout the body, ensuring optimal functioning in the context of repeated environmental changes driven by the solar cycle. It is well known that shift workers are at greater risk for development of a large number of chronic diseases and recent experimental evidence has shown that disruption of circadian organization leads to physiological impairments and dysfunction that are relevant for disease development and pathology. In particular, circadian disturbances yield metabolic derangements capable of predisposing individuals to diabetes, obesity, gastrointestinal and cardiovascular disease, and to disease states which have been linked to increases in risk for various cancers. In addition, the molecular circadian machinery has been linked to regulators of the cell cycle and other prominent pathways involved in cancer, including DNA repair and apoptosis. An understanding of the circadian timekeeping system and recognition of its fundamental role in temporal organization of biochemical pathways and physiological processes enables a framework

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upon which the concept of time on a 24-h basis can be applied to translational research and brought into the realm of clinical medicine in order to improve diagnostics, therapeutics and, ultimately, patient outcomes.

Keywords Circadian rhythms • Physiology • Metabolism • Obesity • Cancer • Circadian disturbances • Cell-autonomous oscillators • Master clock • Suprachiasmatic nucleus (SCN) • Cell-autonomous molecular pacemaker • Night eating syndrome (NES) • CLOCK • BMAL1 • Per1/Per2/ • Cry1/Cry2 • Clock-controlled genes (CCGs) • Chronotherapy

Introduction

Circadian rhythms, from the Latin *circa dies* ("about a day"), are biological rhythms with a length of about 24-h that persist in the absence of any external environmental timing signals. These innate and self-sustaining oscillations, nearly ubiquitous in living systems, exhibit several fundamental properties: they are temperature compensated, meaning that the rhythm length is consistent across a physiologically relevant range of ambient temperatures; they are entrained by, or synchronized to, specific periodic environmental signals such as light, humidity, and food availability; and they have a remarkably small variance in cycle length (i.e., the circadian system is characterized by extreme precision) [1]. Circadian rhythms are generated by a cell-autonomous molecular pacemaker, active in nearly all cells of the body [2]. In mammals, the master circadian pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus [3]. The SCN consists of a bilateral pair of neuronal clusters containing about 10,000 neurons, which are organized into coupled topographical networks that fire synchronously [3]. The circadian system integrates timing signals from the environment (cycles of light and dark, temperature, food availability, etc.) to organize internal rhythms in the appropriate phase relationships to one another and the external environment. The circadian system thus imposes temporal organization to behavior and ongoing physiological and biochemical processes, which results in enhanced fitness by enabling organisms to anticipate and prepare for predictable daily environmental changes, as well as to optimize functionality in the context of an environment shaped by the rotation of the Earth about its axis every 24-h.

Given the fundamental role of circadian clocks in biological processes, it is perhaps not surprising that disruption of overall circadian organization results in physiological aberrations, alterations, and dysfunctions that are relevant for the maintenance of health and development of disease. Indeed, there is a growing body of evidence that circadian misalignment is associated with, and possibly contributes to, numerous diseases and disorders affecting nearly all systems of the body. Much of this evidence comes from studies of shift workers, individuals who must perform both cognitively and physically at the wrong time of day according to their internal circadian clock. These individuals suffer from chronic circadian disruption and are

an important study population for many investigators in the circadian rhythms field. As discussed in Chap. 7, Shift Work and Cancer Risk, shift workers have an increased risk for the development of several cancers. In addition, shift workers are more likely to suffer from metabolic, cardiovascular, and gastrointestinal diseases, among others [4–12].

Epidemiological evidence also links sleep loss and sleep disruption with cancer (see Chap. 8, Sleep Disorders and Cancer Risk, and Chap. 9, "Sleep Deprivation/ Insomnia and Cancer Risk") and cardiometabolic disease (see Chap. 2, "Effects of Sleep Disorders on Cytokines, Hormones and Metabolism"). The regulation of the sleep-wake cycle is a major output of the circadian clock [13], and sleep-wake physiology has strong, bidirectional interactions with circadian clock genes [14–17]. Thus, sleep disorders and sleep disruption likely represent a form of chronic circadian disruption. In the laboratory setting, clinical studies of humans have revealed that short-term misalignment of internal circadian rhythms causes immediate and substantial dysfunction of metabolic and endocrine physiology at a magnitude consistent with increased disease risk [18]. The generation, characterization, and utilization of animal models of chronic circadian disorganization have enabled translational studies aimed at identifying the specific adverse consequences of circadian disruption. Taken together, these lines of evidence all support the emerging consensus that circadian misalignment has significant, sustained, and relevant biomedical effects that contribute to disease pathophysiology and warrants consideration for the promotion and maintenance of health.

This chapter will begin with a brief overview of the molecular machinery driving the circadian clock. This description will highlight the role of metabolic genes in the functioning and regulation of the circadian system, leading into a discussion of how metabolism and the circadian clock are intimately intertwined and bidirectionally interacting. In addition to these interactions at the molecular and biochemical levels, environmental feeding-fasting cycles have recently been demonstrated to be critically important links between the circadian clock and metabolism. Studies exploring the role of time-restricted food availability on metabolism will be reviewed and presented alongside a description of night eating syndrome (NES), a disorder in humans that may potentially represent an extreme and pathologic example of mistimed feeding rhythms. Next, this chapter will discuss evidence linking circadian disruption to cardiovascular disease and gastrointestinal disease. We have focused on metabolic, cardiovascular, and gastrointestinal diseases and circadian misalignment in this chapter because of the links between these and cancer. A separate section will examine the role of circadian misalignment and circadian clock genes in cancer. For all of these sections, emphasis will be placed on translational studies using animal models and clinical experiments with humans. Epidemiological studies will be mentioned and discussed where appropriate, but for comprehensive reviews and in-depth analysis on the association between shift work and cancer, the reader is referred to Chap. 7, "Shift Work and Cancer Risk." This chapter will conclude with the argument that the incorporation of biological timing on a 24-h basis into clinical medicine and our understanding of disease pathogenesis has transformative potential across a broad spectrum of disease states, including cancer.

The Molecular Pacemaker

The molecular circadian clock is composed of interlocked autoregulatory feedback loops that give rise to characteristic 24-h cycles of gene expression and protein activity (see [2, 19] for detailed recent reviews of the core molecular mechanism of the circadian clock). Briefly, the proteins CLOCK and BMAL1 form a heterodimer that drives the expression of the circadian clock components *Per1/Per2* and *Cry1/Cry2*. The products of these genes form a repressor complex, regulated by *casein kinase 1ε/δ* and E3 ubiquitin ligase complexes, which colocalizes to the nucleus and inhibits CLOCK-BMAL1-mediated expression. This negative limb of the circadian pacemaker thus reduces the expression of *Per1/Per2* and *Cry1/Cry2*, resulting in reduced PER-CRY-mediated repression and subsequent reactivation of the positive CLOCK-BMAL1 limb of the pacemaker. CLOCK-BMAL1 also promotes the expression of *RORα* and *Rev-erbα*, which primarily modulate rhythmic *Bmal1* expression, which exhibits a peak about 12 h out of phase with the *Pers* and *Crys*.

Taken together, this self-sustaining feedback cycle takes about 24 h and is active in nearly all cells of the body [20–22]. CLOCK-BMAL1 also drives the expression of numerous additional genes, termed *Clock-controlled genes* (CCGs), which are ultimately responsible for establishing overt rhythms. Microarray studies have revealed that approximately 3–10 % of genes in any given tissue are expressed on a rhythmic basis under constant environmental conditions [23–28]. Interestingly, there is little overlap in the circadian transcriptome between different tissues [19], suggesting that the sets of CCGs have tissue-specific roles critical for the function of the organ and, more broadly, that diverse physiological processes in different cells and tissues are rhythmic. The identification and characterization of the core circadian clock genes was originally achieved primarily through conventional molecular genetics and biochemical techniques, such as mutagenesis and mapping. More recently, the advent and widespread adoption of high-throughput screening and advanced computational techniques have enabled a rapid expansion in the list of molecules known to influence circadian rhythmicity [29–31]. In our view, the circadian clock system can be considered an integrator and organizer that imposes temporal structure and coordination to ongoing physiological processes spatially separated within the body, thus ensuring optimal functioning of the organism in the context of behavior and predictable daily environmental change.

Another integral lesson of recent studies examining the molecular circadian clock is the profound degree of interconnection and bidirectional interaction between core circadian components and metabolism (discussed in detail in sections "Circadian Clock Components and the Regulation of Metabolism" and "Environmental Metabolic Input Affects Circadian Rhythms and Energy Balance"). At the molecular level, perhaps the first indication that the molecular clock system is linked to metabolism came from the observation that the activity of the core clock transcription factors is sensitive to the redox state of the cell [32]. Since then, the recognition of the tight association between circadian clocks and metabolism at the molecular level has grown appreciably (Fig. 4.1, see [33] and [34] for excellent recent reviews).

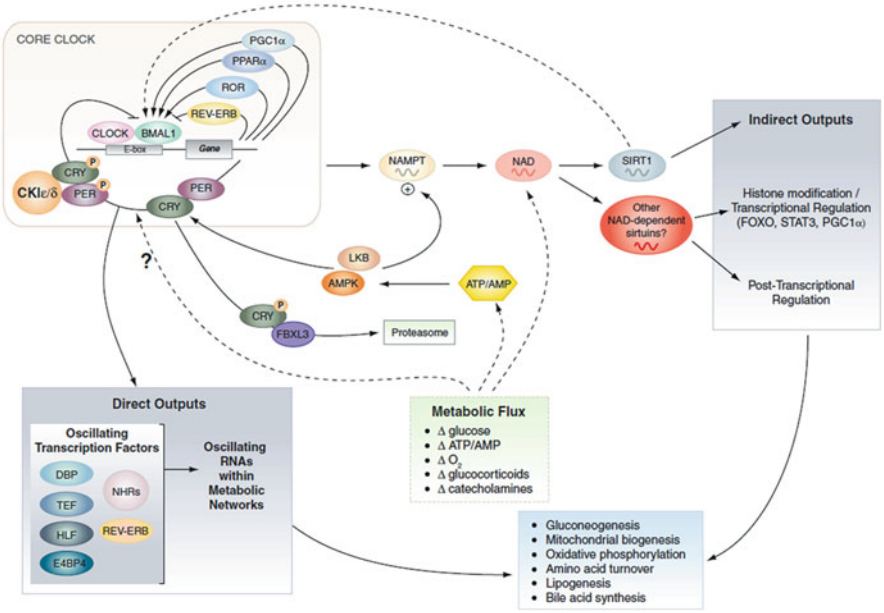


Fig. 4.1 The molecular circadian clockwork integrates and bidirectionally interacts with cellular metabolism. The core circadian clock mechanism consists of a positive limb, the CLOCK-BMAL1 transactivating complex; a negative limb, the PER-CRY repressor complex; and an accessory limb, the REV-ERB and ROR loop. Together, these integrated, autoregulatory, and self-sustaining feedback loops generate cycles of approximately 24 h that persist under constant environmental conditions. The CLOCK-BMAL1 heterodimer activates transcription of a number of target genes, including *Pers* and *Crys*. Translation of these gene products precedes complex formation in the cytoplasm, which then translocates to the nucleus where it inhibits CLOCK-BMAL1 (thus, repressing the expression of the *Per* and *Cry* genes, among others). Removal of the PER-CRY complex (via several mechanisms) derepresses transcription, enabling activation of the positive limb and initiation of another cycle. The core clock mechanism regulates metabolic processes both directly by regulating the expression of critical genes in metabolic pathways in a rhythmic manner—essentially controlling the timing of activity of those pathways—and indirectly by generating rhythms in NAD+ availability, which drive rhythmic deacetylase activity by SIRT1 and possibly other enzymes, as well as rhythms in other cellular processes, including translation and histone modification. Metabolic activity and flux within the cell reciprocally regulates the circadian clock through nutrient sensors, such as AMPK, LKB, and other rhythmically transcribed nuclear hormone receptors that can bind metabolites and other relevant ligands. The intimate interactions between the circadian clock system and metabolism at the molecular and biochemical levels highlight the key role of circadian organization in orchestrating cellular metabolism. Disruption of circadian organization thus has the potential to perturb many critically important cellular pathways, which may contribute to disease states (Reprinted, with permission from [33])

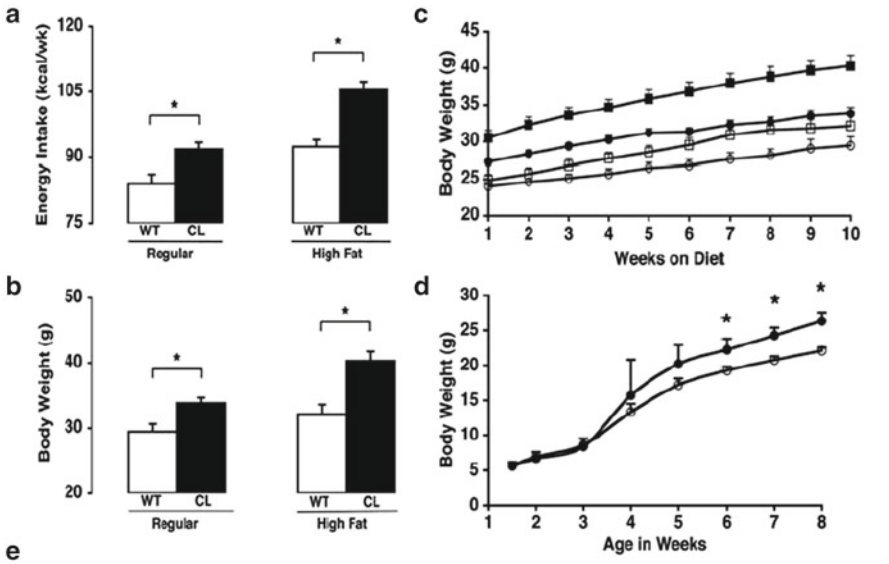
Indeed, a rhythm of peroxiredoxin oxidation was recently reported in the absence of transcription in eukaryotes [35, 36] and shown to be highly conserved across diverse phyla, even Archaea [37]. This apparently universal rhythm presumably represents the output of a non-transcription-based metabolic oscillator (i.e., normally coupled to

the genetically encoded pacemaker). This exciting finding has led to the hypothesis that circadian rhythms ultimately arose and evolved in the context of oxidative respiration and metabolism. Although the clinical implications of these exciting findings remain to be uncovered and worked out, the growing evidence that the circadian clock system serves an absolutely fundamental role in cellular metabolism underscores the relevance of circadian biology in regulating key signaling pathways, the activity of which are crucial in determining the balance between health and disease.

Circadian Clock Components and the Regulation of Metabolism

The first circadian gene identified in mammals, termed *Clock*, was initially discovered in a screen of mutagenized mice nearly 20 years ago [38]. Characterization of the $Clock^{\Delta 19}$ mutation [39–41] ushered in a rapid and profound revolution in our understanding of the molecular basis of circadian rhythms [30]. It is perhaps fitting, therefore, that examination of $Clock^{\Delta 19/\Delta 19}$ mutant mice has contributed substantially to the development of widespread interest in the connection between circadian rhythms and metabolic disease. $Clock^{\Delta 19/\Delta 19}$ mutant mice gain significantly more weight than wild-type littermates on a high-fat diet and exhibit broad metabolic dysfunction (Fig. 4.2, adapted from [42]). Mutant mice have blunted diurnal feeding and activity rhythms and consume a greater proportion of their daily calorie intake during the light phase [42]. Furthermore, mutants have altered expression of energy-regulating genes in the hypothalamus [42]. These findings linked the circadian clock to energy balance and systemic metabolism regulation at the genetic level for the first time, and paved the way for additional studies exploring the role of specific circadian clock components in different peripheral tissues involved in metabolic regulation. These initial studies in $Clock^{\Delta 19/\Delta 19}$ mutant mice included animals harboring the mutation in every cell of the body from birth onward. Due to the potential for developmental compensation and/or pleiotropic effects, a critical next step involved the generation and characterization of tissue-specific circadian clock mutants and knockouts in order to study the role of the molecular pacemaker in different tissues.

In contrast to the master clock in the SCN, clocks in peripheral tissues rapidly and stably entrain to feeding cycles [43, 44], supporting an important role in metabolic regulation. Deletion of the molecular clock in the liver (achieved via liver-specific deletion of the critical clock component *Bmal1*) resulted in hypoglycemia during the fasted state of the daily cycle (i.e., in the light phase of the light/dark (LD) cycle when mice normally are not consuming much food [45]), indicating that the molecular clock in the liver works to maintain normoglycemia by promoting hepatic glucose export during the extended daily fasting phase. This counterbalances the rhythm of circulating glucose induced by feeding patterns (in ad libitum-fed mice, about 75–80 % of caloric intake occurs during the dark, or active, phase of the LD cycle [46, 47]). The loss of the liver clock also dampens the expression rhythms of hepatic transcripts, including glucoregulatory genes [45, 48], further supporting the



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Metabolic parameter	WT	*Clock*	*P* value
Triglyceride (mg/dl)	136 ± 8	164 ± 8	<0.05
Cholesterol (mg/dl)	141 ± 9	163 ± 6	<0.05
Glucose (mg/dl)	130 ± 5	161 ± 7	<0.01
Insulin (ng/ml)	1.7 ± 0.3	1.1 ± 0.1	n.s.
Leptin (ng/ml)	3.4 ± 0.4	4.6 ± 0.3	<0.05

Fig. 4.2 The *Clock*$^{\Delta 19/\Delta 19}$ mutant mouse, a model of genetic disruption of the circadian pacemaker, develops obesity and metabolic syndrome. (**a**) *Clock*$^{\Delta 19/\Delta 19}$ mutant mice (CL, n = 10) have significantly elevated food intake compared to wild-type littermates (WT, n = 8) on both regular chow and high-fat diets. (**b**) *Clock*$^{\Delta 19/\Delta 19}$ mutant mice (CL) weigh significantly greater than wild-type littermates (WT) after 10 weeks on either a high-fat or regular chow diet. (**c**) Body weight gain is greater in *Clock*$^{\Delta 19/\Delta 19}$ mutant mice on both the regular chow (*closed circles*) and high-fat diet (*closed squares*) than in wild-type littermates (*open circles* and *open squares*, respectively). (**d**) Growth trajectories after weaning are significantly higher in *Clock*$^{\Delta 19/\Delta 19}$ mutant mice compared to controls. (**e**) *Clock*$^{\Delta 19/\Delta 19}$ mutant mice exhibit significant alterations in metabolic parameters, including higher triglycerides, cholesterol, glucose, and leptin, as well as a trend for reduced insulin compared to wild-type littermates (Adapted, with permission, from [42])

hypothesis that the liver clock is critical for proper hepatic function and maintenance of circulating glucose levels. More recently, it has been demonstrated that the core circadian clock gene *Rev-erbα* directs a rhythm of HDAC3 (histone deacetylase 3) recruitment to the genome in the liver in mice. These dramatic genome-wide patterns of HDAC3 binding and release across the day are associated with rhythmic histone acetylation and marked rhythms in expression of genes involved in lipid metabolism [49]. Importantly, loss of the interaction between *Rev-erbα* and HDAC3 abrogates rhythmic HDAC DNA binding and results in hepatic steatosis [49], indicating that the proper circadian and temporal organization of gene expression networks is necessary to protect against the development of fatty liver.

The characterization of global HDAC3 binding and subsequent acetylation rhythms in the mouse liver stemmed from previous work examining epigenetic changes at the intersection of circadian and metabolic physiology [50]. Indeed, there is much excitement in linking the circadian clock, metabolism, and epigenome [51, 52]. Although metabolic diseases remain a primary research target, it seems likely that such fundamental molecular findings linking the circadian clock and epigenetic regulation may pave the way to a deeper and more fundamental understanding of many diseases from various biomedical disciplines, including cancer, neurodegenerative disease, and psychiatric disease, among others [53]. The molecular clock system has been linked to critical cellular processes and biochemical pathways, including chromatin remodeling, NAD+ regulation and SIRT1 [54–57], and the expression of nutrient-sensitive nuclear hormone receptors has been shown to be regulated by the clock [58]. These and other dramatic advances in our understanding of the molecular mechanisms of the circadian clock and their intimate, bidirectional links to crucial metabolic processes underscore the fundamental impact of the circadian clock system in pathways critically important for cell function and, therefore, the maintenance of health and disease. Although the specific clinical implications of some of these findings may not be perfectly clear at present, increasing our understanding of the ways in which the circadian clock is linked to metabolism opens up a novel way of thinking about, and potentially treating, metabolic diseases associated with circadian disruption. For example, two recent studies in mice suggested that pharmacological approaches may be used to consolidate and increase the robustness of circadian rhythms (by activating the core circadian clock genes *Rev-erbα* and/or *Rev-erbβ*), as well as potentially alleviate metabolic dysfunction [59, 60].

In the initial characterization of the metabolic phenotype of $Clock^{\Delta 19/\Delta 19}$ mutant mice, it was discovered that mutants exhibit hyperglycemia and hypoinsulinemia [42], suggestive of a defect along the insulin axis. Careful examination revealed that isolated murine pancreatic islets had self-sustaining oscillations of core circadian clock genes and proteins, including *Clock* and *Bmal1*, which were altered in mutants [61]. Circadian clock mutants also exhibited impaired glucose tolerance, hypoinsulinemia, and alterations in size, growth, and function of pancreatic islets. In order to test the hypothesis that the molecular clock in the pancreas itself underlies these metabolic abnormalities, conditional, pancreas-specific *Bmal1* knockout mice (i.e., animals lacking a circadian clock in the pancreas, with intact clocks elsewhere) were generated. These animals developed frank diabetes at an early age due to defective insulin secretion by pancreatic islets [61], indicating a critical role for the pancreatic clock in regulating and coordinating insulin release in the context of the sleep-wake and feeding-fasting cycles. Importantly, loss of the pancreatic cell clock was sufficient to cause diabetes, suggesting that circadian disruption within the pancreas may hasten the onset and/or progression of diabetes.

More recently, specific deletion of *Bmal1* in adipocytes was shown to cause obesity in mice [62]. Mice lacking an adipocyte clock shifted their diurnal food intake rhythm, consuming more food in the light phase. This effect on feeding rhythms was not observed in mice with hepatocyte-specific or pancreatic islet-specific

Bmal1 mutations and was associated with altered expression of hypothalamic energy-regulating neuropeptides [62], suggesting that the clock in the adipocyte works in conjunction with hypothalamic feeding centers to regulate the timing of energy intake, which in turn influences energy balance. Taken together, these findings support the hypothesis that the circadian clock system acts as an integrator of signals between different tissues to optimize physiology and behavior, in this case, leading to coordinated feeding behavior and hypothalamic energy regulation in order to maintain homeostasis.

The examples presented above demonstrate the impact that genetic disruption of circadian organization can have on metabolism and, in particular, on the function of peripheral organs critical for metabolic health and homeostasis. The utilization of genetic mouse models of circadian disruption has enabled great strides in our understanding of the role of the molecular clock in disease-relevant pathways. A major long-term goal of many investigators in the circadian research community is to translate these findings into the realm of clinical medicine in order to improve the diagnosis and/or treatment of disease. Thus, it is particularly important to continue studying humans at risk for chronic circadian disruption (see Chap. 7, "Shift Work and Cancer Risk"), as well as to supplement the studies in animal models with clinical studies in humans. Using a "forced desynchrony" protocol to separate the sleep-wake cycle from the internal circadian clock, it was recently shown that circadian misalignment (i.e., when the circadian clock is out of sync with the sleep-wake cycle) acutely induces substantial metabolic, endocrine, and cardiovascular abnormalities [18]. Maximal misalignment (i.e., when the sleep-wake cycle is completely out of phase with respect to the circadian clock) resulted in impaired postprandial glucose clearance consistent with a prediabetic state in 3 of 8 young, lean male subjects [18], indicating that circadian disruption may be sufficient to push susceptible individuals into the disease state.

Environmental Metabolic Input Affects Circadian Rhythms and Energy Balance

Under ad libitum conditions in the laboratory, the master pacemaker in the SCN entrains to the LD cycle and synchronizes rhythms in peripheral tissues throughout the body through a variety of direct and indirect mechanisms including neural neural projections, endocrine signaling, body temperature rhythms, and behavioral rhythms, such as feeding-fasting and sleep-wake cycles [19]. Loss of the SCN causes widespread desynchronization of rhythms in peripheral tissues [22]. Although experiments demonstrated that circadian clocks in peripheral tissues involved in metabolic regulation rapidly and stably entrain to windows of temporally restricted food availability over 10 years ago [43, 44], it has only recently become appreciated that the timing of food intake may exert a significant influence on metabolic physiology as well. Wild-type mice given access to a high-fat diet at the "wrong" time of day—exclusively during the light phase of a 12:12 LD cycle—gain significantly

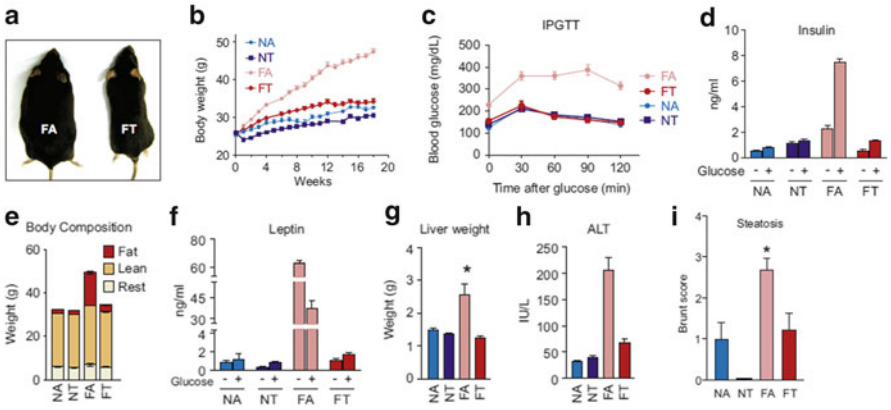

Fig. 4.3 Restricting feeding to 8-h of the dark phase prevents diet-induced obesity and metabolic syndrome without decreasing caloric intake. (**a**) Forced time (FT) feeding regimens prevent diet-induced obesity in mice. Mice were given access to high-fat diet (F) or a normal, regular chow diet (N) either ad libitum (A) or only during an 8-h window during the dark phase (T). Despite similar overall intake levels, the FT group failed to gain weight as the ad libitum did. FT feeding regimens prevented high-fat diet-induced obesity (**b**), glucose intolerance (**c**), hyperinsulinemia (**d**), fat mass gain (**e**), hyperleptinemia (**f**), and fatty liver disease (**g–i**) despite similar overall caloric intake between time-restricted and ad libitum feeding groups. With the exception of steatosis score (**i**), no differences were observed between ad libitum normal chow-fed (NA) and timerestricted normal chow-fed (NT) mice (Adapted, with permission, from [64])

more weight than mice given access to the same food at the "right" time of day (i.e., exclusively during the dark phase), despite similar overall caloric intake and physical activity levels [63]. Also, as discussed above, *Clock*$^{\Delta 19/\Delta 19}$ mutant mice and mice lacking an intact molecular clock in adipocytes have increased light-phase feeding and develop obesity [42, 62], further supporting a role for the timing of food intake in energy balance. More recently, mice given limited access to a high-fat diet for only 8-h of the dark phase (i.e., the "right" time of day, from 1-h after lights off until 3-h before lights on) were compared to mice on the same diet fed ad libitum [64]. The mice on the temporally restricted feeding regimen exhibited more robust circadian and metabolic cycles of a higher amplitude than the ad libitum-fed mice [64]. Most impressively, without any reduction in caloric intake, the temporally restricted feeding regimen prevented obesity, insulin resistance, fatty liver and inflammation, and improved motor coordination (Fig. 4.3, [64]). Presumably, the protection from diet-induced obesity and metabolic syndrome arose, at least in part, from increased activation of key metabolic pathways, including CREB, mTOR, and AMPK, and more robust and coordinated expression of circadian clock genes and clock-controlled genes [64]. These provocative findings highlight a potentially beneficial non-pharmacologic approach for the treatment of obesity and metabolic syndrome. In addition, they demonstrate the physiological benefit of synchronized, high-amplitude circadian cycles in peripheral target tissues related to metabolism and energy balance.

More generally, the finding that restricting food intake to the "right" time of day prevents weight gain and metabolic disease lends credence to the dictum that one should

eat "breakfast like a king, lunch like a prince, and dinner like a pauper." These findings invite clinical studies comparing food intake at different times of day in individuals in order to determine whether manipulation of feeding time can impact body weight regulation and energy balance. Such studies are necessary to provide evidence-based recommendations for strategies to prevent and/or alleviate the adverse sequelae of diet-induced obesity and metabolic dysfunction based on the principles and properties of the circadian clock system and its profound association with metabolic regulation.

These findings that link the timing of food intake to body weight regulation may have relevance for a clinical condition called night eating syndrome (NES), in which affected individuals consume a large portion of their daily calories during the night (often in binges), as well as frequently suffer from insomnia, anxiety, emotional distress and, occasionally, depression [65, 66]. Although NES lacks official diagnostic criteria and is not presently listed in the *Diagnostic and Statistical Manual of Mental Disorders*, it warrants consideration in a discussion of diurnal feeding rhythms and energy balance. It is associated with obesity clinically: NES is more prevalent in obese individuals than the general population, and individuals with NES frequently experience significant weight gain [65]. Although much remains to be learned about NES, one hypothesis is that it represents a primary disorder of circadian organization, in particular the feeding rhythm. When appropriate feeding-fasting cycles are not observed, genes regulating metabolism may become dysregulated and contribute to positive energy balance and increased weight gain.

Although the impact of feeding time and feeding rhythms in metabolic regulation is clear, it is important to recognize that the interaction is bidirectional. It has been demonstrated that a high-fat diet leads to altered circadian rhythms and disrupted circadian organization at both behavioral and molecular levels in mice [46]. Although the mechanism(s) of the effects of specific dietary components on the molecular clock are not understood, the ability of constituents of food to alter and impair overall circadian organization illustrates how a vicious cycle could easily be established, leading to worsened metabolic outcomes and even greater circadian desynchrony. Given that many nutrient-sensing nuclear hormones and energy regulators are controlled by the circadian clock or feed back to impact the core clock itself (or both) [33, 34], it is perhaps not surprising that specific dietary components can elicit significant effects on the properties of the circadian clock. Further work needs to be done to characterize the impact of different dietary components on the circadian clock system and to elucidate the resulting effects on disease-relevant molecular pathways.

Circadian Disruption and Cardiovascular Disease

For many years, it has been known that the timing of onset of severe adverse cardiovascular events, such as myocardial infarction, sudden cardiac death, cardiac arrest, angina, stroke, and arrhythmias, exhibits a diurnal rhythm with peak levels occurring between 6 am and noon (see [67] and references therein). It is clear that many

variables and parameters within the cardiovascular system are under substantial regulation by the circadian clock, highlighting the relevance of circadian organization for cardiovascular disease. Shift work has consistently been associated with increased cardiovascular disease risk [68–71]. The use of animal models to test the hypothesis that chronic circadian disruption exacerbates or augments cardiovascular disease began with a study of hamsters carrying a genetic predisposition to develop cardiomyopathy [72]. In that study, hamsters were randomized into one of two groups, the first of which was maintained on a constant LD cycle and the other was subjected to weekly phase shifts of the LD cycle. The animals exposed to chronic circadian disruption exhibited a significant increase in mortality, with an 11 % reduction in median lifespan [72]. Exposure to repeated phase shifts of the LD cycle has become a widely used model by researchers in the circadian rhythms field to examine the impact of environmental disruption of circadian rhythms, such as might occur with shift work or chronic jet lag. Studies utilizing variations of this model have shown that circadian misalignment increases mortality in aged mice [73], reduces reproductive success in mice [74], and alters immune responses [75]. Studies such as these and others support a model in which the adverse effects of circadian disruption become evident or apparent in the context of a physiological "challenge," such as genetic predisposition to disease, aging, pregnancy, immune challenge, or a high-fat diet. In this scenario, circadian disruption acts as a "second hit" to push susceptible individuals into the diseased or pathological state, or to exacerbate the severity of existing disease.

Recently, a study of hamsters carrying mutant alleles of the circadian clock gene *casein kinase 1ε* (termed *tau* mutants) provided an elegant demonstration of the role that global circadian dysregulation can have in the development of cardiovascular and renal disease [76]. Hamsters with one mutant *tau* allele have a faster circadian clock with a free-running period of about 22-h. Homozygous *tau* mutants have an even faster clock, with a free-running period of about 20-h. The investigators took advantage of the observation that hamsters with one mutant allele will try (unsuccessfully) to entrain to a 24-h LD cycle, whereas homozygous mutants do not and simply free-run throughout the LD cycle (i.e., the clock runs at its endogenous speed without attempting to synchronize to the LD cycle). Thus, heterozygotes experience chronic circadian disruption as they continually try to entrain, whereas wild-type and homozygous *tau* mutant hamsters do not experience such internal circadian disorganization. Heterozygotes develop significant age-related cardiomyopathy, cardiac fibrosis, and renal disease presumably due to chronic disorganization between the internal circadian clock and external LD cycle [76]. Importantly, heterozygotes on a 22-h LD cycle (that matches their endogenous circadian clock length) and SCN-lesioned heterozygotes (that cannot entrain and thus do not experience internal circadian desynchronization) on a 24-h LD cycle fail to develop cardiomyopathy and renal disease, strongly implicating chronic circadian disruption, as opposed to pleiotropic effects of the mutation, as the underlying cause of the pathology. These results complement previous work examining disruptions of the clock in specific tissues by showing that disorganization between tissues (and the environment) also contributes to pathologic transformation, at least in the cardiovascular and renal systems.

In addition to these studies of circadian disorganization at organismal level, rodent models have also been used to scrutinize the impact of genetic circadian disruption on cardiovascular tissue function and pathology. Circadian mutant mice exhibit increased pathological remodeling of vascular tissue and experience greater pathological damage in models of vascular injury. Isolated aortas from circadian mutant mice are characterized by endothelial dysfunction and reduced signaling in the Akt- and nitric oxide pathways, which are critical for normal arterial function [77]. The use of a vascular transplant model has enabled the study of wild-type mice with arterial-specific circadian clock mutations (and vice versa—circadian mutant mice with wild-type aortas) in order to assess the role of the molecular pacemaker in vascular function. When aortic grafts from circadian mutant mice (*Bmal1* knockout or *Per1/2* double knockout) were transplanted to wild-type hosts, severe arteriosclerotic disease and inflammation developed (Fig. 4.4, [78]), highlighting the role of intrinsic clocks in vascular tissue for immune regulation and proper physiological function. In addition to clinical relevance for classical cardiovascular diseases, including arteriosclerosis and atherosclerosis, these findings suggest that circadian clocks in transplanted tissues (particularly the endothelium) may also contribute to graft acceptance/rejection.

Although the diurnal variation in sudden cardiac death has been known for many years (see [67]), until recently the molecular mechanism for this rhythm was unclear. Sudden cardiac death is often precipitated by sustained ventricular arrhythmias, the risk of which is heavily influenced by the rate and pattern of cardiac repolarization. A recently published comprehensive study in mice demonstrated that the rhythmic expression of a clock-controlled transcription factor, *Klf15*, endogenously imparts a rhythm in the expression of a potassium channel subunit that controls the transient outward potassium current. This series of regulatory steps confers circadian rhythmicity to the QT interval duration on the electrocardiogram (the QT interval measures cardiac repolarization) [79]. Thus, circadian organization in cardiomyocytes generates rhythmic variation in the QT segment duration due to temporal regulation of critical ion channel components. Alteration of *Klf15* impacts cardiac repolarization, leading to dampened rhythms in QT segment variation and increased susceptibility to arrhythmias [79]. Taken together, these findings indicate that circadian disruption at the level of cardiomyocytes may contribute to arrhythmogenesis in humans. With an understanding of the molecular mechanisms involved, it becomes possible to design and deploy preventative and therapeutic strategies based upon the principles and properties of the circadian clock, which has an instrumental role in the physiology and function of the organs involved in disease pathogenesis.

Circadian Disruption and Gastrointestinal Disease

Circadian rhythms regulate many aspects of gastrointestinal physiology and function [80]. The signals controlling gut function originate from peripheral oscillators in cells throughout the GI tract as well as from CNS-mediated autonomic nervous

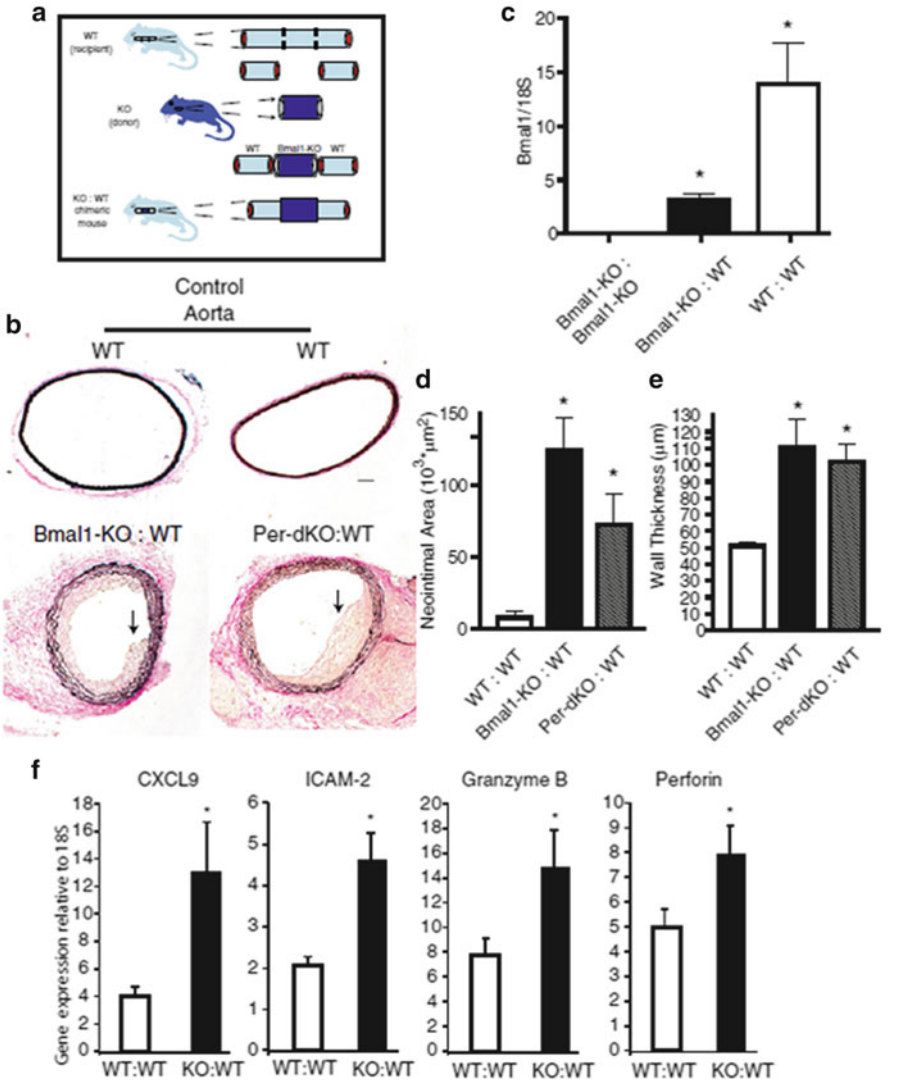

Fig. 4.4 Local genetic circadian disruption in transplanted arteries results in arteriosclerosis and inflammation. (**a**) Sections of aorta were transplanted between wild-type and circadian clock mutant mice (Bmal1-KO or Per1/Per2 double-KO, termed Per-dKO), generating chimeric mice used to determine whether local clock dysfunction in the transplanted artery influences the development of cardiovascular disease. (**b**) *Bmal1* expression is absent from Bmal1-KO host and recipient mice, markedly reduced in chimeras, and present at normal levels in wild-type mice. (**c**) Bmal1-KO:WT (Bmal1 KO donor tissue to WT recipient) and Per-dKO:WT (Per-dKO donor tissue to WT recipient) chimeras exhibit arteriosclerosis, consisting of neointimal hyperplasia (**d**) and increased vessel wall thickness (**e**). (**f**) Inflammatory responses are exaggerated in circadian mutant:WT chimeras, suggesting that the genetically disrupted local clock within the transplanted tissue contributes to inflammation and the development of vessel wall injury and arteriosclerosis (Adapted, with permission, from [78])

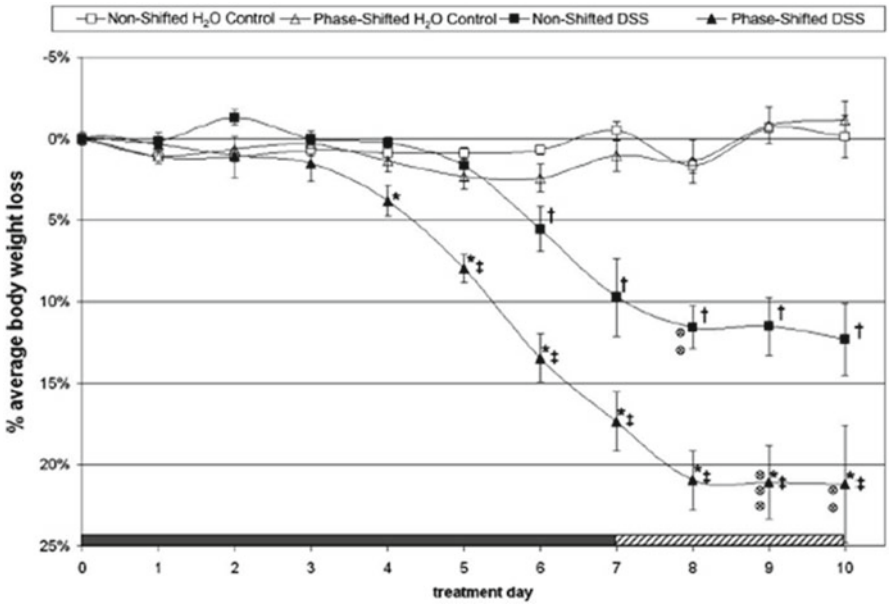

Fig. 4.5 Chronic disruption of circadian organization increased susceptibility to DSS-induced colitis in mice. Mice subjected to 3 months of weekly 12-h phase shifts of the light/dark (LD) cycle were administered with either dextran sodium sulfate (DSS), a compound toxic to the intestinal epithelial cells used to model colitis in mice, or a vehicle control in the drinking water. Two separate age-matched control groups were maintained on a constant 12:12 LD cycle and tested with either DSS or vehicle. To assess the severity of injury, body weight was monitored daily during and immediately following DSS administration. DSS-treated mice previously exposed to chronic circadian disruption (*filled triangles*) experienced an earlier onset and greater severity of the disease, including higher mortality, compared to non-shifted DSS-treated animals. In addition, DSS-treated mice that had been subjected to chronic circadian disruption exhibited greater histopathological damage and inflammation in the colon [83]. Taken together, these findings suggest that chronic circadian disorganization increases the sensitivity of intestinal epithelial cells to injury. In other words, circadian disruption may be a "second hit" that pushes certain individuals across a threshold into active disease (Reprinted, with permission, from [83])

system input [81]. One of the most common complaints associated with jet lag is gastrointestinal discomfort [82], presumably due to transient misalignment between rhythms in the GI tract and other internal clocks. Beyond jet lag, chronic circadian disruption, using the LD cycle phase shift model in mice, has been shown to sensitize intestinal epithelial cells to injury in a mouse model of colitis induced by dextran sodium sulfate (DSS) [83]. Animals exposed to phase shifts of the LD cycle experience an earlier onset of DSS-induced disease, with higher mortality, greater disease severity, and increased inflammation and histopathological damage (Fig. 4.5, [83]). These results support the "two hit" model wherein circadian disruption exacerbates the adverse effect of a physiological "challenge," in this case, DSS-induced intestinal injury and colitis.

A key function of the intestine is to maintain an epithelial barrier separating the proinflammatory contents of the gut lumen from circulation [84]. Disruption of the mucosal barrier permits translocation of bacterial endotoxin across the intestine wall into circulation and has been linked to numerous pathologies characterized by non-pathogen-mediated inflammation, including alcoholic steatohepatitis [85], metabolic syndrome [86, 87], cardiovascular disease [88], Parkinson's disease [89], and amyotrophic lateral sclerosis [90], among others. In an in vitro model of intestinal barrier function, using cultured human intestinal epithelial cells (i.e., Caco-2 monolayers), circadian clock genes were shown to be required for alcohol-induced increases in permeability across the monolayer [91], implicating the molecular clock in the development of barrier dysfunction in response to environmental insults such as alcohol. Further work is clearly warranted to characterize the role of circadian clock genes and circadian organization in maintaining intestinal epithelial barrier integrity, which is critical in promoting health and resisting disease.

Circadian Disruption and Cancer

Arguably, a field in which little progress has been made in linking circadian rhythms to pathology, disease pathogenesis, and/or clinical medicine at the molecular and genetic levels is cancer. This is unfortunate given that a diurnal rhythm in efficacy and sensitivity to chemotherapeutic agents was reported in mice over 40 years ago [92]. More recently, screening studies in rodents have demonstrated clear circadian rhythmicity in the antitumor activity and side effect profile of many anticancer agents, although at present, it is not possible to predict a priori at which time of day a given drug will be maximally effective (i.e., although rhythms are clearly present, little is known of their mechanistic underpinnings) [93]. Results such as these have given rise to the concept of "chronotherapeutics," in which the time of drug administration is taken into consideration in the treatment plan in order to maximize efficacy and minimize toxicity (Fig. 4.6, [93, 94]). Although some progress has been made, by and large, this approach has not made significant inroads into clinical oncology, especially in the United States. Increasing our understanding of chronotherapy and how it may be applied and used in clinical practice may represent a unique opportunity to improve patient outcomes by optimizing treatment strategies and modalities already in clinical use.

In addition to the impact of time of day on chemotherapy administration, there are indications that the molecular circadian clock directly interacts with and regulates pathways heavily implicated in many cancers. Perhaps most exciting are the lines of evidence that circadian clock proteins interact with the molecular regulators of the cell cycle [95]. Circadian clock gene targets include key molecules such as *p21*, *c-myc*, *Wee1,* and certain *cyclins*, which all contribute to the regulation of cell cycle phase transitions. Furthermore, disruption of the circadian clock and/or circadian clock genes induces dysfunction of the cell cycle (see [95] and references therein). It has also become evident in recent studies that the circadian clock

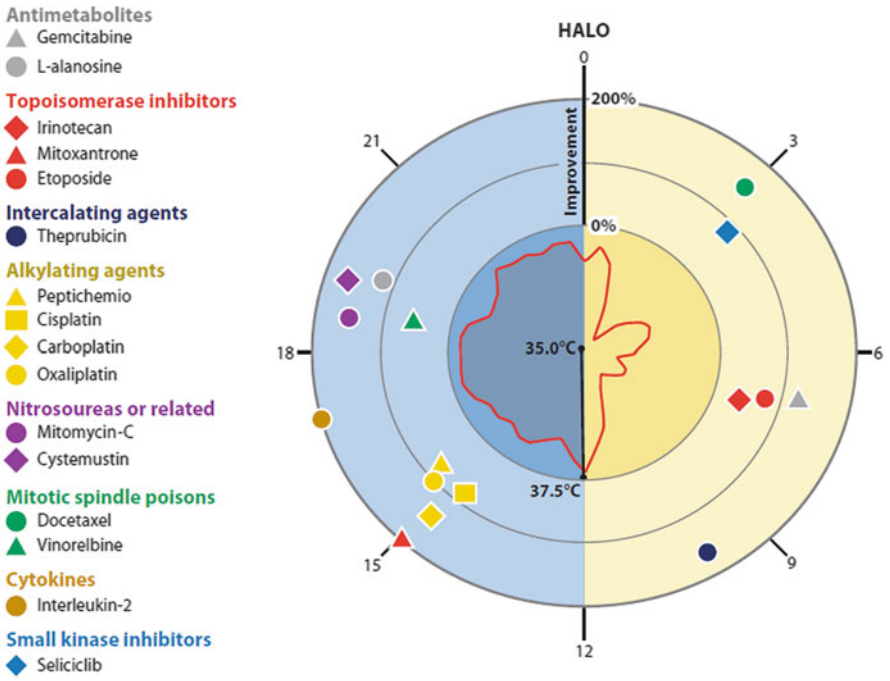

Fig. 4.6 Many commonly used chemotherapeutic agents exhibit significant rhythmicity in efficacy and tolerability. Sixteen anticancer drugs were tested across the diurnal cycle in male B6D2F1 mice housed in standard 12:12 LD conditions in the laboratory. The circadian time of maximal tolerability is plotted around the circle (HALO, hours after light onset). The distance outward from the center of the circle indicates the magnitude of the survival benefit provided by administration at the optimal time. Anticancer drugs from various classes exhibit circadian rhythms in tolerability and efficacy, and not all drugs from the same class have the same peak efficacy (Reprinted, with permission from [94])

machinery influences the rate and quality of DNA damage repair responses and pathways [96]. For example, the XPA protein, which plays a role in nucleotide excision repair, exhibits robust circadian rhythms of activity at both transcriptional and posttranslational levels in mouse hepatocytes, a finding that may have relevance for chemotherapeutic agents that induce damage amenable to nucleotide excision repair strategies [97]. Recently, mice were reported to have a diurnal variation in sensitivity to UVB radiation, with maximal vulnerability occurring during the night. Presumably this variation in sensitivity is due to coordinated progression through cell cycle states by groups of aligned skin cells throughout the day, with the most susceptible phase—S phase—occurring at night [98]. In addition, the circadian clock appears to contribute to the regulation of apoptosis [99, 100], which has obvious relevance for cancer. Despite these intriguing examples, it is important to point out that this field remains in its infancy and much detail remains to be worked out. For example, some effects may be due to functions of circadian genes that are

independent of their role in the clock mechanism [101], and it will be critically important to verify that observed effects in tissue samples and cultured cells are maintained in vivo [102].

It is clear that more work needs to be done to link the molecular clock to cancer biology at the molecular and genetic levels. Although, at present, the field has largely struggled to take advantage of the rapid revolutions in our understanding of the molecular mechanism of the circadian pacemaker and the physiology of the circadian timekeeping system, there is tremendous potential for the future. As detailed in Chap. 7, "Shift Work and Cancer Risk," and Chaps. 8 and 9, "Sleep Disorders and Cancer Risk" and "Sleep Deprivation/Insomnia and Cancer Risk," respectively, a substantial body of epidemiological data now links disruption of circadian rhythms and sleep to cancer. It will be up to physician-scientists and researchers in the cancer field to address these results and incorporate the vast increases in our knowledge about molecular and organismal circadian biology into their research programs. The dramatic advances in computational power and sequencing ability have opened up new avenues to interrogate and mine large datasets in which gene expression is altered in, and possibly even causal to, cancer. It is now critically important to mine these datasets in the context of circadian clock genes and clock-controlled genes. It is clear that meaningful gains in patient outcomes for the seemingly intractable forms of cancer will require large, multidisciplinary, highly coordinated, and rapidly adapting teams of professionals that leverage vast group knowledge, experience, and technical abilities for the benefit of patients. In our view, circadian biologists have an important role in such teams by encouraging and facilitating the incorporation of timing and circadian biology in our understanding of cancer pathogenesis and in our approaches to treatment.

Conclusions

There is now substantial evidence that circadian misalignment results in clinically relevant physiological abnormalities. The epidemiological data continue to support the hypothesis that chronic circadian disruption contributes to the pathogenesis of numerous diseases and indicate that it may represent an important risk factor to be routinely taken under consideration. Clinical and translational studies have begun to elucidate the molecular mechanisms linking circadian clock genes to pathways involved in various diseases and disorders. Future work will refine and improve our understanding of how alterations in the circadian system result in downstream dysfunction, disease risk, pathogenesis, and progression. Although this chapter has focused on medical disorders, including metabolic, cardiovascular and gastrointestinal diseases, and cancer, there is also ample evidence that circadian disruption is involved in psychiatric disease as well [103]. Continued research and further insights will be instrumental for ushering in a new era in clinical medicine—one in which the concept of biological time on the circadian scale is incorporated to enrich our understanding of disease pathophysiology broadly across biomedical and clinical disciplines and exploited to improve clinical care.

Fig. 4.7 Composite view of the United States from space at night. The relatively recent advent and widespread dissemination of electrical lighting has fundamentally transformed our relationship with day and night. The biological implications of these drastic changes are only beginning to be recognized. Given that the powers of modernization and globalization are unlikely to reverse course, it will be important for clinical medicine to incorporate our understanding of circadian rhythms and their relevance for health and disease. The shift work population is no longer the only target patient population for those who hope to practice "Circadian Medicine." In reality, the immense pressure against maintaining natural cycles of light and darkness and sleep and wake posed by modern society creates a scenario in which many of us are chronically subjected to circadian disruption. One metric of this phenomenon is the concept of "social jetlag," which refers to the weekly shifts that arise from changing bed and wake times between work days and free days [105]. "Social jetlag" has been linked to obesity [106] and, yet, may represent only the tip of the iceberg with respect to the impact of widespread circadian disorganization on health and disease (Image from NASA (www.nasa.gov/images/content/712129main_8247975848_88635d38a1_o.jpg))

Although the shift work population will remain an important group to study, the application of the dramatic scientific improvements in our understanding of circadian biology to the realm of clinical medicine has much more broad and widespread implications. Given the increasing modernization of our world and environment (Fig. 4.7), circadian disruption and sleep loss are becoming hallmarks of society. We live in a world increasingly divorced from the natural cycles of light and darkness that have served as the primary, defining geophysical feature of life on this planet. With the flick of a switch, we can override the solar cycle and, in so doing, dramatically disrupt our internal clocks. Indeed, it was recently reported that exposure to dim light at night in mice caused increased weight gain by shifting feeding rhythms towards more consumption during the light phase of the LD cycle [104]. With the click of a mouse (i.e., a computer one), we can purchase airplane tickets capable of rapidly carrying us to the far corners of the globe, drastically divorcing our internal

clocks and rhythms from the environmental day. As a consequence of our work and school schedules, we frequently alter the timing and quantity of our sleep every week, creating repeated internal circadian desynchrony, termed "social jetlag" [105], which has been linked to obesity [106]. These evolutionarily recent changes to our environment and behavior are not likely to stop or reverse course, thus it will be important to fully understand the impact of these changes on our circadian clocks and subsequent disease risk. Only with an understanding of the underlying biological pathways involved will it be possible to design and deploy preventative and/or therapeutic strategies based on the principles and properties of the circadian clock system.

In considering the specific effects of circadian misalignment on biological pathways involved in various diseases and disorders, it is important to recognize the levels at which circadian disruption can occur. The circadian clock machinery within an individual cell can become altered and disorganized. Within a tissue, the rhythms of different cells can become disrupted and desynchronized from one another, resulting in tissue-level dysfunction. This may be the result of altered phase relationships and/or miscommunication between cells of the same type within a tissue or between different cell types (or both). At the systems level, rhythms of one tissue may become desynchronized from those of other tissues, resulting in higher order dysfunction. For example, this may occur in a condition of chronic jet lag when the cells of the SCN rapidly re-entrain to the new light schedule, whereas the cells in other tissues of the body respond more slowly and variably, leading to a prolonged period of internal misalignment. Another example is chronically mistimed feeding-fasting rhythms, which can entrain clocks in peripheral organs associated with digestion and metabolism at an altered phase relationship(s) to the SCN, which remains tightly linked to the LD cycle irrespective to feeding time [44]. Finally, there may be an abnormal relationship between the phase of the circadian clock and the external environment, leading to chronic circadian disruption. This appears to be the case in the advanced and delayed sleep phase syndromes (ASPS and DSPS, respectively): the circadian clock is permanently shifted relative to the range of expected times based on the solar day. Such a situation may have important social and emotional ramifications, in addition to the physiological and biochemical alterations associated with the circadian disruption. The complexities of the roles that circadian disruption can play in the development and/or progression of disease indicate that continued research is necessary to narrow down the specific pathological effects of circadian disruption, identify the affected pathways, and attempt to mitigate and/or alleviate the adverse effects.

The past 20 years have witnessed profound and dramatic improvements in our understanding of the circadian system from a scientific standpoint. Indeed, it is difficult to fully appreciate just how far the field has come in such a relatively short period of time. Despite these successes however, little of the new information has been applied to the benefit of patients suffering from the diseases linked to circadian disruption, which is particularly relevant for cancer since many of the diseases linked to circadian misalignment are comorbid with cancer, and cancer itself has been associated with circadian disorganization. The upcoming translation of these

scientific findings to the realm of clinical medicine yields significant transformative potential in opening up an entire new avenue for the conceptualization and understanding of disease risk and pathogenesis. The insights gleaned from the scientific world are expected to contribute to the development of novel, biologically based therapeutic strategies based on the principles and properties of the circadian system. Such an approach, which we propose to refer to as "Circadian Medicine," incorporates and fully appreciates the concept of biological time as a critical biological determinant of health and, conversely, disease. The dramatic scientific advances of the field offer a blueprint upon which a foundation of clinical understanding can be built. Whether the vast potential of "Circadian Medicine" is ultimately realized remains to be seen and depends upon the continued dedication of scientists and researchers at the forefront of integrating our understanding of circadian biology with that of disease pathophysiology and treatment.

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