06 - 498 The Role of Circadian Biology in Health and Disease

498 The Role of Circadian Biology in Health and Disease

Millán-Zambrano G et al: Histone post-translational modifica tions—cause and consequence of genome function. Nat Rev Genet 23:563, 2022. Sendinc E, Shi Y: RNA m6A methylation across the transcriptome. Mol Cell 83:428, 2023. Vandereyken K et al: Methods and applications for single-cell and spatial multi-omics. Nat Rev Genet 24:494, 2023. Zhang W et al: The ageing epigenome and its rejuvenation. Nat Rev Mol Cell Biol 21:137, 2020.

The Role of Circadian

Biology in Health

and Disease Jonathan Cedernaes, Kathryn Moynihan Ramsey, Joseph Bass Circadian rhythms are anticipatory, circa 24-h, autonomous cycles of physiology and behavior. These evolutionarily conserved rhythms have evolved at both the cell and tissue level to synchronize organismal function in anticipation of the 24-h rotation of the Earth. A common feature of modern “24/7” life is the routine disruption of these endog enous circadian cycles due to the rise in shift work, jet travel across time zones, exposure to blue light–emitting devices at night, and dis rupted sleep-wake behavior. In-depth characterization of the molecular basis of circadian disorders has generated novel avenues for research on how sleep-wake disruption has been associated with aging, metabolic disease, inflammation, and cancer. This chapter provides an overview of (1) the basic biology of the circadian system; (2) primary circadian rhythm and interrelated sleep disorders; and (3) the role of the circa dian system in both normal human physiology and disease states. We also include an overview of how the emerging field of chronobiology may impact drug action. A glossary of terms used in circadian biology is summarized in Table 498-1. ■ ■BASIC EVOLUTION AND STRUCTURE

OF THE CIRCADIAN SYSTEM Long before the emergence of multicellular life, the Earth's constant rotation around its axis gave rise to a daily cycle of light and darkness. At the emergence of the first prototypal gene involved in biological clock regulation—3.4 billion years ago in photosynthetic cyanobacteria— the period of Earth's rotation along its own axis was only 8 h. The co-occurrence in molecular evolution of the biological clock and photosynthesis hints at an interrelated and selective advantage of the clock in the regulation of energetic processes. Indeed, biological clocks coordinate oxygenic reactions with periods of sunlight each day, and perturbation of clock cycles reduces fitness, reproduction, and survival. Additionally, clocks protect photosynthetic organisms from the DNAdamaging effects of sunlight by timing the production of DNA repair processes, such as photolyase-mediated repair, to the nighttime. Across billions of years of evolution, as day length has gradually extended to today's circa 24 h, highly conserved circadian clocks (from circa diem, meaning "about a day") have been found in all photosensitive organisms, governing a wide range of biochemical, physiologic, and behavioral processes. A defining property of the circadian clock system is that it enables organisms to anticipate, rather than simply react to, daily changes in the external environment that are tied to the day-night cycle. In mammals, circadian systems are organized hierarchically with a light-responsive "master" circadian pacemaker located within the suprachiasmatic nucleus (SCN) of the anterior hypothalamus, which in

turn presides over a network of both extra-SCN and peripheral clocks (see "Anatomic Organization of the Circadian Clock Network" below). Daily light exposure signals to the SCN and entrains the circadian system to the 24-h day (see "Entrainment and Measurement of the Circadian System," below). In turn, the SCN maintains synchrony of a diverse network of both central and peripheral clocks via a variety of signals that have as of yet to be fully identified. These signals involve direct physiologic rhythms (core body temperature), the autonomic nervous system, and neuroendocrine signals, such as cortisol, which is part of the hypothalamic-pituitary-adrenal (HPA) axis.

■ ■MOLECULAR ORGANIZATION OF THE MAMMALIAN CIRCADIAN CLOCK At the molecular level, mammalian circadian rhythms are generated by a transcription-translation autoregulatory feedback loop. The forward limb of the clock is composed of the basic helix-loop-helix transcrip tion factors (TFs) CLOCK (or its paralogue, NPAS2) and BMAL1. These drive expression of their own repressors (PERs and CRYs) in the negative limb in a cycle that repeats itself every 24 h (Fig. 498-1). A second short feedback loop involves CLOCK/BMAL1-mediated tran scription of the retinoic acid–related orphan nuclear receptor families ROR and REV-ERB, which activate and repress Bmal1 transcription, respectively. Rhythmic posttranslational regulation of the stability and degradation of core clock TFs occurs via events such as phos phorylation by casein kinase 1 epsilon (CK1ε) and casein kinase 1 delta (CK1δ) and ubiquitination by FBXL3 and FBXL21. In addition to the circa 24-h oscillation of core clock genes, a wide array of downstream clock-controlled genes (CCGs) exhibit broad rhythmic amplitude in expression, ultimately giving rise to rhythmic physiologic processes. CHAPTER 498 The importance of localized clock gene expression has been dem onstrated by genetic animal studies, such as with targeted ablation of Bmal1, the only clock gene that lacks a known functional paralog. Deletion of Bmal1 either in the whole brain or in regions that span the brain region that coordinates circadian rhythms—the SCN—causes behavioral arrhythmicity, even when genetic ablation occurs in adult life. Conversely, restoring Bmal1 expression specifically in brain in global adult Bmal1 mutant mice rescues behavioral locomotor rhythms. Of note, whereas the protein CLOCK normally heterodimer izes with BMAL1, the paralogous protein NPAS2 can functionally substitute for CLOCK within the pacemaker neurons. Thus, while mice lacking either

Clock or Npas2 genes maintain rhythmicity, mutants lacking both CLOCK and NPAS2 lack circadian rhythms in locomotor activity. Further, mutations in many of the clock genes are associated with impaired circadian rhythms and physiology in both experimental animal models and humans (see “Primary Pathologies of the Circadian System” below). The Role of Circadian Biology in Health and Disease

A major transformation in our understanding of circadian biology came with the discovery that the molecular clock network is present not only in the SCN but also within most peripheral tissues, as well as in extra-SCN neurons in the brain. In primates, ~82% of all proteincoding transcripts exhibit daily 24-h rhythms in some tissue or other. In rodents studied under constant conditions, ~3–16% of the transcrip tome in each tissue exhibits 24-h rhythms in mRNA expression levels, even though the repertoire of such genes varies substantially between tissues, in accordance with tissue-specific functions. The core clock feedback loop and the induction of transcriptional CCG rhythms also involves epigenetic mechanisms such as conformational chromatin dynamics, histone acetylation, and DNA methylation. Conversely, posttranscriptional events such as RNA polyadenylation, nucleocy toplasmic shuttling, alternative splicing, and mRNA translation also exhibit circadian variation, further increasing the repertoire of rhyth mic regulation at a cellular level. ■ ■ANATOMIC ORGANIZATION OF THE

CIRCADIAN CLOCK NETWORK The molecular circadian feedback loop is synchronized with sunrise each day by photosensitive melanopsin-expressing neurons within the retina. These neurons provide input to the SCN via the retino hypothalamic tract (RHT), allowing mammals to maintain coherent

TABLE 498-1 Glossary of Terms Used in Discussion of the Circadian System TERM DESCRIPTION ASPD Advanced sleep phase disorder (see text for description). CBT Core body temperature. Often used as an indicator of the circadian rhythm but can be masked by sleep and exercise. CCGs Clock-controlled genes; output of the molecular clock. Chronotype Internal circadian rhythm of an individual determined by phase of entrainment, determining sleep propensity and timing of maximum alertness over a 24-h period. Circadian period Time required for one complete cycle or oscillation. Calculated by the time distance between two consecutive peaks or troughs of a circadian variable. Circadian phase Timing of the circadian rhythm. Defined by comparing, e.g., the peak (acrophase) or trough (bathyphase) to a fixed event, e.g., to a point in time. Synonymous with phase angle. Circadian rhythm A biological process that exhibits an endogenous, entrainable oscillation of ~24 h. Circadian rhythm sleep disorders Disorders of multiple etiology that have in common that they result in maladjustment of the biological clock with respect to the environment. Constant routine An experimental paradigm designed to study endogenous circadian rhythms in humans, by keeping behavioral and environmental factors constant. These paradigms thereby typically entail a combination of constant dim lighting, evenly distributed isocaloric energy intake, semirecumbent posture, and forced extended wakefulness. Desynchrony Loss of synchrony occurring either between a rhythm and its zeitgeber (external, “time giver” signal) or between two or more rhythms within an organism (internal). Diurnal rhythm An oscillation synchronized with the day/night cycle that repeats itself with a 24-h period. The rhythm does not have to persist when time cues (e.g., light) are absent. DLMO Dim-light melatonin onset; a marker of melatonin rhythm. DSPD Delayed sleep phase disorder (see text for description). Entrainment Synchronization of a circadian rhythm or other self-sustaining oscillation by a factor—zeitgeber—that enforces the oscillator. Constant entrainment between the zeitgeber and the oscillator results in a stable phase

relationship between these entities. Infradian rhythm A recurrent cycle or period with a period length significantly greater than 24 h. Melatonin Hormone produced primarily by the pineal gland (chemical name N-acetyl-5-methoxytryptamine); derived from L-tryptophan. Various forms of melatonin can be prescribed for circadian rhythm sleep disorders or sleep disorders. PART 20 Emerging Topics in Clinical Medicine Non-24-h rhythm disorder A syndrome in which there typically are chronic 1- to 2-h daily delays in sleep onset and wake times in an individual living in society, e.g., due to complete blindness. Peripheral clocks Clocks presiding outside of the suprachiasmatic nucleus, the circadian system's master pacemaker. PRC Phase response curve; visual representation of how a particular manipulation (e.g., light) produces phase shifts as a function of the phase (i.e., circadian time) at which the manipulation occurs. Defining the PRC to light has enabled researchers to understand and predict how entrainment to light cycles is accomplished. SCN The suprachiasmatic nucleus or nuclei, also known as the master pacemaker in mammalian species. A bilateral set of nuclei positioned in the anterior ventral hypothalamus. Essential for entraining extra-SCN central and peripheral oscillators to the prevailing light-dark cycle via photic input from the retina. Shift work Work scheduled so that it occurs outside of the traditional work schedule of 9:00 a.m. to 5:00 p.m., or 7:00 a.m. to 6:00 p.m., depending on definition. Various forms of shift work exist, such as early morning, evening, or night shifts, as well as rotating shifts. Ultradian rhythm A recurrent cycle or period with a period significantly shorter than 24 h—e.g., a 2-h rhythm would exhibit 12 cycles within a circadian (24-h) rhythm. RRE Bmal1 CK1ε/δ PERs P CRYs P CLOCK BMAL1 E-box RORα/γ FIGURE 498-1 Central clock molecular mechanism. The core molecular clock machinery in mammals is encoded by interlocking transcription-translation feedback loops that oscillate with ~24-h periodicity. The transcription factors CLOCK and BMAL1 heterodimerize to drive transcription of downstream clock-controlled target genes containing E-box enhancer elements. Among these, the PER and CRY proteins multimerize and inhibit CLOCK/BMAL1, while RORs and REV-ERBs activate and inhibit, respectively, Bmal1 transcription, resulting in rhythmic oscillations of clock-controlled and downstream target genes.

Stabilization FBXL21 CRYs CRYs P FBXL3 Degradation Clock-controlled genes Rev-erbα/β

Environmental inputs and internal circadian organization Brain Clocks SCN Environmental light/dark cycle SCN Extra-SCN LHA PVN ARC PIT Non-autonomous circadian control Peripheral Clocks Vasculature Liver Adrenals Pancreas Muscle Environmental nutrient cycle fasting/feeding Fibroblasts Intestine Hematopoetic Adipose Autonomous circadian control FIGURE 498-2 Central and peripheral clocks coordinate environmental cues with behavior and physiologic outputs. Light entrains the master pacemaker neurons in the suprachiasmatic nucleus (SCN), which subsequently synchronizes extra-SCN and peripheral clocks. Brain clock output includes sleep-wake, fasting-feeding, and energy expenditure cycles, while peripheral clock output includes a wide range of physiologic processes, including glucose homeostasis, oxidative metabolism, cytokine production, and stress response. The right column indicates different ways that circadian disruptors, such as diet, shift work, or other circadian rhythm sleep disorders, may impact the clock—i.e., by changing circadian period, phase, or amplitude. organismal rhythms in line with the external light/dark cycle. Under standing the circuit organization of the circadian clock within the brain is increasingly relevant in understanding how the master circadian pacemaker center within the SCN regulates feeding, sleep-wake activ ity, endocrine processes, energy expenditure, and metabolism (Fig. 498-2). Identification of the SCN as the master pacemaker was first established by the observation that SCN lesioning induced complete loss of rhythms of locomotor activity, drinking behavior, and endo

crine hormone secretion. The ventral “core” region of the SCN, which is composed of neurons producing vasoactive intestinal polypeptide (VIP), receives photic information directly from the retina through the RHT. At the molecular level, circadian gene transcription is induced within the SCN through the initial activation of immediate early genes, such as Per1, Per2, c-fos, and jun. Cells within the “core” region of the SCN then signal primarily via γ-aminobutyric acid (GABA)-ergic neu rotransmitter release to synchronize the cells within the “shell” region of the SCN, which produce arginine vasopressin (AVP), the most important neuropeptide for maintaining intra-SCN synchronicity. The SCN communicates to extra-SCN and peripheral clocks through both secreted factors and neuronal projections. The former was elegantly proven by the ability of SCN grafts to partially restore locomotor rhythms in SCN-lesioned animals. Efferent nerve outputs arise both from the AVP-producing shell region of the SCN and the VIP-predominated core. The SCN projects to several hypothalamic and thalamic regions, including the median preoptic nucleus (MPO), the subparaventricular zone (SPZ), the dorsomedial hypothalamus (DMH), the paraventricular nucleus of the hypothalamus (PVH), and the paraventricular nucleus of the thalamus (PVT). Some of these

Behavioral and physiologic outputs Circadian disruptors Sleep/wake Feeding/fasting Energy expenditure Glucose homeostasis Δ Period (High fat) Original phase New phase period amplitude Environmental light cycle Internal circadian time phase Δ Phase (Shift work) Δ Amplitude (Night eating, insulin resistance) Glucose homeostasis Lipogenesis Oxidative metabolism Mitochondrial respiration Xenobiotic detoxifixation Cytokine production Vascular tone Hemostasis Stress response Thermogenesis Incretin production DNA damage/repair CHAPTER 498 The Role of Circadian Biology in Health and Disease
regions, in turn, regulate output to both sleep- and wake-promoting regions, as well as to regions involved in regulation of autonomic, body temperature, and hormonal rhythms, as well as feeding. The SCN is thereby thought to promote sleep in part through the transmission of neural signals that terminate in the sleep-promoting ventrolateral preoptic nucleus (VLPO), i.e., one of the brain regions that is active during sleep. In contrast, the SCN promotes wakefulness during the active phase by transmission of neural signals that—by passing through regions such as the SPZ and the DMH—terminate in wake-promoting regions, including the locus coeruleus, lateral hypothalamic nucleus, ventral tegmental area, and dorsal raphe nucleus. The SCN also signals via noradrenergic fibers to the pineal gland to regulate the circadian production of the hormone melatonin. SCN con trol of the nighttime rise in pineal melatonin release (in both diurnal and nocturnal animals) is mediated through a pathway involving the PVH. Of note, artificial light at night delays the secretion of melatonin, ultimately affecting sleep (see “Endocrine Systems Regulated by the Circadian Clock” below). Melatonin plays a complex role in the circa dian system since the MT1 and MT2 melatonin receptors are expressed in the SCN itself; thus, melatonin feeds back to modulate circadian outputs to other cells in the brain and body. Neuronal output from the SCN also reaches peripheral tissues such as the adrenal glands, liver, and pancreas. The SCN produces rhythmic variation in multiple neuroendocrine axes, producing daily rhythms of gonadotropin, thyrotropin, and somatotropin. Prominent HPA axis rhythms ultimately give rise to daily variation in diverse pathways essential for hemodynamic stability, metabolism, and inflammation. These rhythms originate with SCN control of corticotropin-releasing

hormone (CRH)–producing cells in the PVH, which may regulate sleep as well as induce daily oscillations of both pituitary adrenocor ticotropic hormone (ACTH) and adrenal cortisol. Highlighting the importance of SCN output for peripheral rhythms, there is a dramatic reduction in the number

of transcripts that exhibit circadian rhythms in the liver following SCN ablation in mice. Nonetheless, when the autonomous clock in the liver is ablated in mice, some key clock transcripts such as Per2 still cycle provided the core body temperature rhythm persists. Whereas the SCN is exclusively entrained by light, meal timing can signal circadian time directly to peripheral tissues such as the liver. Thus, shifted meal timing as occurs during shift work or jetlag can uncouple peripheral clocks from the central pacemaker. Temperature can also phase shift peripheral tissue clocks, but not the SCN clock. This is an important phenomenon because, at the organis mal level, the SCN generates the core body temperature rhythm as one of the major mechanisms to signal circadian time to peripheral clocks.

■ ■ENTRAINMENT AND MEASUREMENT

OF THE CIRCADIAN SYSTEM Under normal light-dark cycles, the circadian system is corrected or “entrained” on a daily basis, producing diurnal rhythms of 24 h. Such signals of entrainment are called zeitgebers (German for “time-giver” signals) and include light exposure, meal timing, and activity patterns. Light serves as the dominant zeitgeber for the circadian system, and a breakthrough in understanding photoentrainment in mammals came with the discovery of the melanopsin system, which is composed of a specialized class of photosensitive retinal ganglion cells that expresses the blue light–sensitive photopigment melanopsin in the inner retina, separate from the photoreceptive rods and cones. Blue light around this wavelength (~480 nm) suppresses melatonin, such that melatonin levels are normally low during the day, promoting subjective and objec tive (electroencephalography assessed) wakefulness. PART 20 Emerging Topics in Clinical Medicine The ability of light to entrain the circadian system functions accord ing to a so-called phase response curve (PRC). When light exposure occurs prior to the critical phase of the core body temperature (CBT), defined by the CBT’s minimum, light produces a phase delay in the circadian rhythm. Conversely, light exposure after this critical period causes phase advances. The circadian system can respond even to small changes in light intensity (e.g., dim light at ~100 lux can produce half of the phase delay compared with an almost 100-fold greater light exposure). This responsiveness has been found to be highly individual and varies widely. This is in part due to genetic variation, as variants in clock genes can modulate the responsiveness of the human circadian system to light. When an organism is placed in an environment without zeitgebers, the circadian rhythm is said to free-run, as it relies on the endogenous rhythm of the circadian system. In humans, the study of endogenous circadian rhythms can be achieved by using a so-called constant rou tine that eliminates the risk of masking by factors such as sleep. In these paradigms, subjects are kept awake in a constant semi-recumbent posture, meals are provided on an hourly basis, and light is constantly kept below the level that can phase shift the SCN. Concurrently, circa dian rhythms are assessed by frequently measuring CBT, melatonin, or peptidergic hormone rhythms over the course of more than 24 h. In ani mals, endogenous circadian rhythms are instead studied by examining behavior, physiologic responses, and voluntary locomotor activity fol lowing 30–36 h of complete darkness. From these measurements, key properties of the circadian system can be ascertained, such as period length (peak-to-peak or trough-to-trough time), amplitude (peak-totrough difference), and phase (timing of peak or trough in relation to a reference point) (Fig. 498-2). These studies have revealed that the endogenous human circadian clock runs with a period length of ~24.2 h, while that of mice runs at ~23.5 h, with some variability across strains. In humans, evidence further indicates that females may have a slightly shorter circadian clock than males (24.1 vs 24.2 h), and many circadian parameters have been found to exhibit differences that

are dependent on biological sex. Notably, interindividual variability in the endogenous circadian period length is further diversified by the existence of genetic polymorphisms in

clock genes (see below). These gene variants can confer extremes in the endogenous circadian period as well as phase; the latter can be advanced or delayed by ~3–4 h in each direction. This is due both to altered cir cadian rhythms at the cellular level and to altered SCN responsiveness to entrainment by light. For instance, PER3 gene contains a variablenumber, tandem-repeat polymorphism. Individuals homozygous for a PER3 5/5 genotype have been reported to be more responsive than PER3 4/4 homozygous individuals to the melatonin-suppressing effect of eve ning blue light exposure. By analyzing genetic variation across ~700,000 individuals, the number of genetic loci that have been identified that contribute to variability in chronotype is in the hundreds. Using specifically developed questionnaires to establish preferred sleep-wake timing, individuals can be categorized into so-called morningness-eveningness types or chronotypes. The most commonly used questionnaires are the Horne-Östberg Morningness-Eveningness Questionnaire (MEQ) and the Munich ChronoType Questionnaire (MCTQ). A composite MEQ score allows grouping into five categories that range from definite morning-type to evening-type individuals based on preferred waking time. In contrast, the MCTQ centers on the midpoint of sleep as a circadian marker, queries age and sex across a range of geographical locations, and can be used to ascertain differ ences between socially imposed sleep patterns (e.g., on working days) and sleep patterns on free days (the difference constituting so-called social jetlag). According to MCTQs obtained from primarily European populations, ~1% of the general population goes to bed before 10:00 p.m. and ~8% after 3:00 a.m. Differences in chronotype are linked to altered circadian timing, including peak levels of melatonin, which can vary by up to 4 h between extreme morning and evening types. Extreme chronotypes have also been shown to be linked to various traits; i.e., low morningness scores have been associated with greater tolerance to night shift work. Melatonin is one of the most commonly used peripheral markers of an individual’s circadian rhythm, reflecting the rhythmic function of the SCN. Circadian rhythms of melatonin can be measured in saliva or plasma, whereas 6-sulphatoxymelatonin (aMT6S), a metabolite generated from the breakdown of melatonin, can also be measured in urine. Accurate estimations of melatonin rhythms are often obtained by analyzing the dim light melatonin onset (DLMO). As the name implies, this involves evening/nighttime sampling of melatonin as opposed to 24-h sampling. This makes DLMO quantification useful in both the clinical and research settings. In normally entrained indi viduals, the DLMO can be used to ascertain whether an individual’s circadian rhythm is phase advanced or delayed, and this onset typically occurs ~2 h before the onset of sleep. The midpoint of sleep—the main marker used by the MCTQ—correlates more strongly with melatonin onset than the MEQ score. In the morning hours, the offset of mela tonin (“DLMoff”) can be used as a marker of circadian alignment or misalignment with the light-dark cycle. When individuals are exposed only to the natural light-dark cycle dictated by the sun, such as in an outdoor natural lighting environment, DLMO and DLMoff occur earlier. In contrast, exposure to artificial light has the overall effect of delaying the biological night and contributes to widening differences between chronotypes in modern society. The CBT is also often utilized as an indicator of the circadian rhythm. Even though CBT is more variable than DLMO, it usually correlates well with the phase obtained using the melatonin rhythm. The CBT, however, can be masked by factors such as sleep, food intake, and activity. CBT can be recorded and registered wirelessly with rela tive ease. In humans, CBT can be recorded via rectal thermometers or probes that are swallowed to pass through the gastrointestinal tract. When humans are studied under normal conditions with normal light ing and sleep duration

from 2300 to 0700 h, the CBT reaches around 37.2°C by 0900 h, and from there, it continues to rise slowly until it reaches 37.4°C around 11 h later. The CBT then drops to the daily low of 36.5°C in the early morning (0400 h). The minimum in body tem perature also corresponds to the trough in the 24-h rhythm in resting energy expenditure. Given the interrelationship between the circadian system and sleepwake systems, researchers have developed paradigms that uncouple

the circadian system from sleep-wake states, enabling the study of the contribution of the circadian system to investigated parameters across the entire sleep-wake cycle. These paradigms are known as “forced desynchrony” protocols and involve enforcing a significantly shortened (e.g., 20 h) or prolonged (e.g., 28 h) day length upon individuals. These protocols thus attempt to approximate what occurs during rotating shift work or “jetlag,” e.g., when travel across several time zones sud denly shifts the light-dark and behavioral cycles drastically away from the entrained 24-h rhythm. As described below, forced desynchrony protocols have contributed to uncovering how the circadian system regulates parameters such as cognitive performance, subjective alert ness, and metabolic and cardiovascular health. ■ ■PRIMARY PATHOLOGIES OF THE

CIRCADIAN SYSTEM An overarching term for disorders of the circadian system is circadian rhythm sleep disorders (CRSDs), where there is a mismatch between subjective behavioral and physiologic rhythms with the environmental light-dark or social activity-rest cycles (i.e., the body clock is out of sync with the external light-dark cycle). CRSDs can arise either due to misalignment of an exogenous environmental factor, such as light, or misalignment of the activity-rest cycle, such as occurs with shift work or jetlag, in relation to endogenous circadian timing. Mutations in the core clock genes themselves can also alter intrinsic circadian timing in relation to the external environment, which makes it difficult for these individuals to properly realign themselves. These disorders often result in adverse effects such as excessive sleepiness or depressed mood, often causing individuals to be unable to maintain a job or attend school at regular hours. The criteria for CRSDs based on the International Clas sification of Sleep Disorders (ICSD) are shown in Table 498-2. Animal models have greatly advanced our understanding of how core molecular clock components contribute to maintaining normal sleep-wake/rest-activity cycles (Table 498-3). For example, ClockΔ19/Δ19 mice have reduced total sleep duration and less induction of rapid eye movement (REM) sleep in response to sleep deprivation. Further, TABLE 498-3 Animal Models of Genetic Circadian Disruption AVERAGE CIRCADIAN TIME OF PEAK TRANSCRIPT LEVEL GENE MUTANT PHENOTYPE SCN PERIPHERY Bmal1 (Arntl) 15–21 22–2 Bmal1−/− Arrhythmic CK1δ (Csnk1δ) No rhythm No rhythm Csnk1δ+/– 0 to 0.5-h shorter period CK1ε (Csnk1ε) No rhythm No rhythm CK1εtau 4-h shorter period CK1ε — — CK1ε−/− 0.2- to 0.4-h longer period Clock No rhythm 21–3 Clock−/− 0.5-h shorter period — — — ClockD19/D19 4-h longer period/arrhythmic Clock/Npas2 — — Clock−/−/NPAS2−/− Arrhythmic Cry1 8–14 14–18 Cry1−/− 1-h shorter period Cry2 8–14 8–12 Cry2−/− 1-h longer period — — — Cry2A260T 0.2-h shorter period Dbp — — Dbp−/− 0.5-h shorter period Npas2 N/A 0–4 Npas2−/− 0.2-h shorter period Per1 4–8 10–16 Per1−/− 0.7-h shorter period — — — Per1brdm1 1-h shorter period — — — Per1ldc 0.5-h shorter period/arrhythmic Per2 6–12 14–18 Per2brdm1 1.5-h shorter period/arrhythmic — — — Per2ldc Arrhythmic Per3 4–9 10–14 Per3−/− 0 to 0.5-h shorter period Rev-erbα (Nr1d1) 2–6 4–10 Rev-erbα−/− 0.5-h shorter period/disrupted photic entrainment Rorα 6–10 Arrhythmic/various staggerer 0.5-h shorter period/disrupted photic entrainment Rorβ 4–8 18–22 Rorβ−/− 0.5-h longer period Rorγ N/A 16–20/various Rorγ−/− Normal behavior Note: Normal circadian rhythms of circadian clock and related genes, with description of circadian phenotype in mutant mice. Abbreviation: N/A,

not applicable. Source: Adapted from Hum Mol Genet 15:R271, 2006, and Adv Genet 74:175, 2011.

TABLE 498-2 Criteria for Circadian Rhythm Sleep Disorders CRITERIA DESCRIPTION A A persistent or recurrent pattern of sleep disturbance due primarily to one of the following: • Alterations of the internal circadian timekeeping system. • Misalignment between endogenous circadian rhythms and exogenous factors that affect the timing or duration of sleep. B A circadian-related sleep disruption that leads to insomnia, excessive daytime sleepiness, or both. C A sleep disturbance that is associated with impairment of social, occupational, or other areas of functioning. mice that lack Bmal1 have increased total sleep time, but it is more fragmented and lacks clear 24-h sleep-wake rhythms, and mice lacking the repressors Cry1 and Cry2 are arrhythmic and spend more time in non-REM sleep. Finally, while ablation of the circadian gene Dbp does not alter the specific duration of sleep stages, it does lead to an altered circadian sleep-wake distribution, with more sleep during the normal wake period and vice versa. Consistent with a key role of clock genes in regulating sleep-wake behavior, human genetic studies of twins have found that up to half of the variation in diurnal preference is heritable. Established genetic variants associated with diurnal preference and cir cadian sleep disorders are listed in Table 498-4. The following briefly mentions the most common CRSDs, but readers should refer to the chapter on sleep disorders (Chap. 33) for a more detailed description. Delayed Sleep Phase Disorder Delayed sleep phase disorder (DSPD; or delayed sleep-wake phase disorder [DSWPD]) is one of the more common circadian rhythm sleep disorders, ranging from 0.2–16% of the population depending on definition used, and is most common in adolescence and early adulthood. DSPD is characterized by chronic and significant delays in both sleep onset and wake times compared to “socially acceptable” sleep-wake hours (i.e., “extreme night owls”). Rhythms of CBT and melatonin levels are also often CHAPTER 498 The Role of Circadian Biology in Health and Disease
ALLELE

TABLE 498-4 Mutations and Gene Variants Linked to Sleep-Wake Disorders and Diurnal Preference GENE POSITION POPULATION SYNDROME/SLEEP PREFERENCE hCKIε S408N Japanese Protection against DSPS hCKIγ T44A Pedigree FASPS hCKIΔ H46R Pedigree FASPS hCLOCK T3111C (3′-UTR) European Eveningness hCRY2 A260T Pedigree FASPS hPER2 S662G (missense mutations in CKIε binding region) Pedigree FASPS hPER2 C111G (5′-UTR) British Extreme morningness hPER3 P415A/H417R Pedigree FASPS and seasonal affective disorder hPER3 G647 Swedish/Finish/Austrian/German Morningness hPER3 G647, P864, 4-repeat, T1037, R1158 Japanese DSPS hPER3 Increased repeats (exon 18, 54 bp) Brazilian DSPS hVIP rs9479402 (gene variant 54 kb upstream of VIP) European (>97% European ancestry) Morningness Abbreviations: DSPS, delayed sleep phase syndrome; FASPS, familial advanced sleep phase syndrome. delayed. DSPD is associated with polymorphisms within the circadian clock genes CLOCK, PER3, and CRY1, and the circadian period (tau) of these individuals may be longer. The most effective treatment includes bright-light therapy after waking in the morning (and/or dark-room therapy in the evening) in combination with melatonin administra tion in the evening several hours prior to the onset of sleep. These approaches attempt to realign endogenous circadian rhythms with the desired sleep-wake schedule, though often face challenges because individuals suffering from DSPD also phase delay more rapidly. PART 20 Emerging Topics in Clinical Medicine Advanced Sleep Phase Disorder Another CRSD whereby one gets the correct amount and quality of sleep but at a shifted time is advanced sleep phase disorder (ASPD; or advanced sleep-wake phase disorder [ASWPD]). The prevalence of this disorder may be <1%, but the condition may be underreported, given that it

may cause fewer conflicts with societal demands (i.e., 9-to-5 schedules) compared with DSPD. Individuals with ASPD experience an advance in their major sleep episode in relation to the desired sleep-wake times. Thus, this disorder typically results both in very early evening bedtimes and morning awakenings (e.g., “extreme early birds”), resulting in reduced quality of life due to excessive sleepiness during the early evening, even in social situations. Individuals with ASPD also have phase-advanced temperature and melatonin rhythms. ASPD occurs more often in older individuals, although early-onset autosomal dominant familial variants (familial advanced sleep phase syndrome [FASPS]) have also been associated with mutations in either the PER2 or the casein kinase 1δ (CK1δ) gene. PER2 is critical for SCN resetting by light, and these PER2 mutations have been found to shorten the endogenous circadian period to ~23.3 h compared with the normal 24.2-h period length. Treatment includes bright light or blue-enriched phototherapy in the evening hours to delay the phase of the circadian clock to a later hour. Shift Work Sleep Disorder Given the increased prevalence of shift work in today’s 24/7 society and the accumulating evidence for increased incidence of sleep and metabolic disorders, including obesity, type 2 diabetes, cardiovascular disease, and cancer, in shift workers, the need to develop effective treatments for shift work sleep disorder (SWSD) is increasingly important. SWSD is at its core defined by the primary symptom of either insomnia or excessive sleepiness arising due to work scheduled during regular sleeping hours or at irregular times. The symptoms may arise because recovery sleep consumes a large proportion of the individual’s free time, potentially leading to negative social consequences such as difficulties maintaining social relationships. Older individuals are typically at an increased risk of SWSD due to the age-associated decline in the ability to maintain sleep during the time of day that would normally constitute the wake period. Therapeutic approaches include optimizing the sleep environment at home to minimize disruptions, melatonin prior to sleeping, and timed bright-light therapy. For example, for night workers, intermittent bright-light exposure during the night and avoidance of bright light

during the morning, even on days off, have been shown to improve sleep and feelings of alertness. Genetic screening combined with chronotype questionnaires may become useful tools for determining whether a given individual is suited for shiftwork. For instance, a twin study indicated that a genetic variant of the circadian gene DEC2 was associated with reduced sleep duration and shorter recovery sleep fol lowing extended sleep deprivation. More studies may reveal additional genetic variants that confer an advantage to repeated phase advances and phase delays as typically occurs in shift work. Irregular Sleep-Wake Rhythm Damage to the SCN can produce arrhythmicity in animals and is thought to be one of the possible underlying reasons for the temporally disorganized sleep-wake pattern that characterizes the disorder known as irregular sleep-wake rhythm (ISWR). Other contributing factors may be reduced responsiveness to entraining signals such as light and physical activity, as well as decreased exposure to such signals, as often occurs with increasing age. Despite normal total sleep time, there is a relative absence of a circadian pattern to the sleep-wake cycle such that sleep occurs in several distinct randomly distributed bouts. ISWR is often associated with neurologic impairment, foremost Alzheimer’s disease in older age; however, ISWR can also occur in individuals with poor sleep hygiene. Treatments involve multimodal interventions such as increased light exposure, improved sleep hygiene, and promotion of social and physi cal activities. Non-24-h Sleep-Wake Rhythm Disorder Individuals with non-24-h sleep-wake rhythm disorder (“non-24”), otherwise known as free-running disorder (FRD), have endogenous circadian rhythms that are not synchronized with the external 24-h day-night cycle due to an inability to readjust the circadian clock to the 24-h day

on a daily basis. This most commonly occurs in individuals who are completely blind (i.e., lacking all photoreceptors) since they are unable to respond to daily light cues that normally would reset the endogenous circa dian clock (although the condition has also been reported in sighted individuals). Instead, the sleep-wake period length corresponds to the individual's endogenous circadian rhythms, which are typically slightly longer than 24 h, thereby shifting sleep and wake cycles over time in relation to the light-dark cycle. Instead of sleeping at the same time each day, their sleep time would gradually be delayed each day until their sleep period literally goes "around the clock." Depending on the individual's endogenous rhythm, the individual will take a given number of days to realign their endogenous phase (in a 360° phase plot) with the zero time point in the exogenous 24-h light-dark cycle. Because of this chronic cycling, prominent symptoms of non-24 include sleep-wake cycle disruption (insomnia and daytime sleepi ness), impaired alertness and mood levels, and severe difficulties par taking in normally scheduled work, school, or social activities. Non-24 can be diagnosed following diurnal analysis of an individual's melato nin or cortisol rhythms, in combination with analyses of sleep diaries

where the sleep onset and offset can be visualized over time to identify the free-running period. Treatments for sighted non-24-h patients include a combination of bright-light therapy with appropriately timed melatonin administration, whereas melatonin and dual melatonin (MT1 and MT2) receptor agonist administration in completely blind non-24-h patients has been shown to entrain free-running rhythms and improve symptoms. Jetlag Most have experienced symptoms associated with jetlag, including insomnia, daytime sleepiness, and fatigue, when traveling from one time zone to another, as one's endogenous circadian rhythms are not yet aligned, or entrained, to the new external light-dark cycle. This is due to the slowness of the circadian system to adapt to the new time zone. Typically, the human circadian system can shift up to ~1.5 h a day in the westward direction (i.e., a phase delay), whereas it shifts more slowly (up to ~1 h daily) with eastward direction of travel (i.e., achieving a phase advance). Usually, symptoms of jetlag abate within the first couple of days after traveling and may present themselves after a first night of good sleep (which is more dependent on a high buildup of homeostatic sleep pressure). Older individuals (age >50) appear to be more at risk. While symptoms are transient, therapeutic approaches aim to hasten the synchronization of internal and external circadian cycles. Behavioral treatments include appropriately timed bright-light exposure and avoidance of bright light during the nighttime in the new destination, while pharmacologic approaches include timed melatonin administration before bedtime both prior to and following travel, resulting in improved sleep quality and decreased night waking. Social Jetlag Individuals with a late chronotype are prone to suffer from "social jetlag," a phenomenon in which individuals are forced to awaken at a point at which their bodies are entrained to be asleep due to discrepancy between alignment of social and biological time. Social SLEEP FASTING WAKE FEEDING CNS Inhibition of hunger Melatonin and GH secretion Neurotoxic substance clearance Memory consolidation Muscle Oxidative metabolism Adipose Lipid catabolism Leptin secretion Liver Gluconeogenesis Glycogenolysis Mitochondrial biogenesis Cholesterol synthesis Pancreas Glucagon secretion FIGURE 498-3 The circadian clock partitions behavioral, physiologic, and metabolic processes according to time of day. The partitioning of metabolic processes to appropriate times of day is critical for the maintenance of health from cellular to mammalian organisms. This figure highlights which processes peak within the central nervous system (CNS), muscle, adipose, liver, and pancreas during either the sleep/fasting or wake/feeding cycle in humans. GH, growth hormone.

jetlag can be estimated using questionnaires, such as the MCTQ, to compare sleep timing on working or school days compared with free days. This has established that a large proportion of the European population suffers from 2 or more hours of social jetlag. Chronic social jetlag is associated with an increased risk of developing obesity and metabolic syndrome, as well as with greater alcohol consumption, smoking, and poorer academic performance in students.

The aforementioned categories of defined clinical circadian disor ders have been traditionally established based on consideration of the endogenous behavioral and physiologic cycles (primarily of melatonin and temperature) with the external 24-h light-dark cycle. In the fol lowing sections, we build on the concepts of circadian behavioral dis orders to consider new and emerging insight into the role of circadian disruption in organismal homeostasis (Figs. 498-3 and 498-4) and the availability of genetic strategies to dissect the interrelationship between clock function, health, and disease. ■ ■ROLE OF THE CLOCK SYSTEM IN PHYSIOLOGY Endocrine Systems Regulated by the Circadian Clock In addition to regulation of behavioral rhythms such as sleep-wake and fasting-feeding cycles, the circadian clock also regulates rhythms of the endocrine system. Cortisol rhythms are regulated through a feedback loop known as the HPA axis. Hypothalamic secretion of CRH and AVP promotes secretion of pituitary ACTH, which in turn regulates rhythmic cortisol secretion from the adrenal cortex. Cortisol release increases toward the morning, and this increase is believed to prepare the brain and peripheral tissues for daytime activity and food intake. AVP secretion in mice occurs prior to sleep to promote water intake, thereby preventing dehydration during the sleep period. Several hor mones, such as growth hormone (GH), cortisol, and melatonin, are influenced not only via circadian regulation, but also by sleep. For CHAPTER 498 The Role of Circadian Biology in Health and Disease
CNS Hunger signals Foraging behavior Cortisol secretion Neuronal activity Muscle Fatty acid uptake Glycolytic metabolism Adipose Lipogenesis Adiponectin production Liver Glycogen synthesis Bile acid synthesis Pancreas Insulin secretion

Circadian desynchrony CNS Depression Cognitive decline Pancreas Hypoinsulinemia Muscle Insulin resistance Sarcopenia Vasculature Adrenals Hematopoetic Chronic stress Disrupted HPA axis Autoimmunity FIGURE 498-4 Pathologies resulting from circadian desynchrony. Circadian rhythm sleep disorders, including advanced/delayed sleep phase disorder, jet lag, social jet lag, and shift work, result in a desynchrony between the environmental light-dark cycle “time” and the endogenous clock “time.” Pathologies can thus arise through misalignment imposed by exogenous (e.g., altered light cycle and/or feeding rhythm) and endogenous factors (e.g., mutations in core clock genes). Such desynchrony results in a host of wide-ranging pathologies across multiple tissues, including hypoinsulinemia (pancreas), disrupted hypothalamic-pituitary-adrenal (HPA) axis, autoimmunity, hypertension, obesity, and metabolic syndrome. CNS, central nervous system; IBD, inflammatory bowel disease. PART 20 Emerging Topics in Clinical Medicine instance, both GH secretion and the cortisol awakening response (CAR, i.e., the peak in cortisol soon after waking) are profoundly blunted by acute overnight wakefulness. GH secretion is primarily dependent on the occurrence of slow-wave sleep, which is a homeo statically driven sleep stage that occurs primarily in the first part of the sleep period. Both the CAR and daytime cortisol levels are also modulated by light exposure levels. Sleep also influences melatonin amplitude, such that sleep deprivation can increase melatonin levels. As sleep deprivation is often accompanied by artificial nighttime light exposure, the effect on melatonin can be combinatorial. In working environments, the effects of curtailed sleep are often confounded by mistimed exposure to light. The sensitivity to light levels

that suppress melatonin can vary by an order of magnitude or more in individuals. This partly explains how even low levels of light can potently suppress melatonin secretion. Together with altered timing in light exposure, perturbed hormonal levels likely represent a mechanism through which altered timing and duration of sleep can impact central and peripheral circadian oscillators. Centrally controlled rhythms of melatonin and cortisol are con sidered key regulators of extra-SCN and peripheral oscillators. Glu cocorticoid receptors exist in both the central nervous system and in peripheral tissues such as skeletal muscle, liver, and adipose tissue. Upon acute shifts in light-dark or feeding cycles, rhythmic levels in cortisol appear to modulate the rate at which behavioral and physi ological rhythms phase shift. Indeed, glucocorticoids regulate clock gene expression in muscle, kidney, and lung, and the powerful syn thetic glucocorticoid dexamethasone is often employed in vitro for its ability to synchronize (e.g., reset) circadian rhythms of cells, including liver cells. Consistent with a role for glucocorticoid regulation of the clock, both adrenalectomy, which results in a lack of cortisol, and exogenous corticosteroid supplementation significantly disrupt the circadian clock system. Several peripherally produced hormones and peptides are not only produced rhythmically but can also feed back to central clocks, includ ing within the SCN. For instance, both cortisol and thyroid hormones regulate their own rhythmic synthesis by feedback to central brain regions, i.e., the hypothalamus (for cortisol) and pituitary (for both hormones). Several other peripherally produced factors have been

Liver Circadian rhythm sleep disorders Dyslipidemia Steatosis Metabolic syndrome Jet lag Shiftwork Advanced/delayed sleep disorder Adipose Obesity Intestine Steatorrhea IBD flare Circadian dysbiosis Fibroblasts Thromboembolic events Hypertension Increased circulation of inflammatory cytokines Tumorigenesis proposed to influence the central clock, including fatty acids produced by the adipose tissue and fibroblast growth factor 21, a hormone pri marily produced by the liver. Peripheral hormones that signal energy state and hunger also exhibit circadian rhythms that are regulated by local tissue clocks. The most extensively studied of these hormones are leptin, which is released from white adipose tissue cells, and ghrelin, which is released from specific endocrine cells in the upper fundus region of the stomach. Ghrelin also exhibits significant peaks related to anticipated meal timing, which persist for several days of fasting in humans. Circulating rhythms of leptin and ghrelin are disrupted in circadian mutant mice and in humans experiencing circadian misalignment, with evidence for sex-specific effects. Per and Cry mutant mice exhibit severely blunted leptin rhythms, and wild-type mice exposed to jetlag (through repeatedly altered light-dark cycles) show a reduced wake-associated decrease in leptin. Similarly, humans forced to live 28-h days exhibit increased 24-h profiles of ghrelin and decreased levels of leptin. Ghrelin and leptin signal to several regions of the brain, including integrative appetitive regions of the hypothalamus such as the arcuate and paraventricular region. The response to these hormones is rhythmically regulated by the molecular clock within sev eral such central sites, effectively gating how these hormones influence rhythms of food intake and energy homeostasis in a time-of-day– and nutrient-dependent manner. Role for the Clock in Metabolic Homeostasis Circadian con trol of glucose homeostasis has long been recognized, as early studies demonstrated variation in glucose tolerance and insulin action across the day. For example, due to a combination of circadian control of both peripheral insulin sensitivity and pancreatic β-cell insulin secretion, oral glucose tolerance is lower in the evening and afternoon compared with the morning. Another example is the “dawn phenomenon,” whereby glucose levels peak prior to the onset of activity. Further, destruction of the SCN has been shown to abolish circadian regulation of glucose metabolism in rats, and daily cycles of insulin secretion and glucose tolerance are often perturbed in patients with type 2

diabetes, who also exhibit changes in gene expression rhythms in peripheral tissues such as adipose tissue. Changes in rhythmic parameters such as insulin secretion have also been observed in first-degree relatives

of patients with type 2 diabetes, possibly highlighting a key hereditary role for the circadian clock in the pathogenesis of metabolic disease. Ablating clock genes in mice has revealed a key function for both central and peripheral clocks in regulating energy homeostasis. The circadian system has been shown to regulate rhythmic insulin secre tion from the pancreas via both neural signals and hormonal levels (e.g., cortisol and norepinephrine), as well as via cell-autonomous clock regulation within the pancreatic β cell itself. An early observa tion was that whole-body ClockΔ19/Δ19 mutant mice developed obesity without displaying hyperinsulinemia, a phenomenon that indicated concurrent β-cell failure. This was later confirmed using pancreas- and β-cell–specific Bmal1-deficient mice, which exhibited glucose intolerance, hypoinsulinemia, and impaired glucose-stimulated insulin secretion. The molecular clock within other peripheral tissues such as liver, adipose tissue, and skeletal muscle also regulates circadian fluc tuations in insulin sensitivity and glucose disposal, which are highest in the morning and decline toward the evening in humans. Liver-specific ablation of Bmal1 in mice has revealed that the liver clock promotes gluconeogenesis, glycogenolysis, and mitochondrial oxidative metabo lism in the sleep/fasting period, while promoting glycogen synthesis in the wake/feeding period. Muscle-specific Bmal1-deficient mice display reduced glucose tolerance, concomitant with lower levels of proteins involved in glucose uptake by muscle cells (e.g., the glucose transporter GLUT4). Ablation of the Cry1 and Cry2 repressors in the negative limb of the clock alters glucagon and glucocorticoid signaling in the liver, contributing to hyperglycemia and impaired glucose tolerance in these mutant mice. Together, these genetic studies in mice suggest a role for tissue-specific clocks in the partitioning of energy utilization across the sleep-wake cycle. Importantly, peripheral clocks also interact with other environ mental factors such as diet and time of feeding. For example, high-fat feeding leads not only to obesity and metabolic syndrome in mice, but also to perturbed clock gene expression across multiple peripheral tissues and a disrupted sleep-wake/fasting-feeding cycle, as revealed by increased activity and feeding during the daytime, the normal rest period in mice. Furthermore, mice that are fed a high-fat diet exclu sively during their (inactive) light phase gain significantly more weight than mice that are fed the same diet during the dark period—the active period for mice. Additionally, the metabolic phenotypes arising from ad lib high-fat feeding can be significantly ameliorated by restricting the time of high-fat feeding exclusively to the dark period. Animals with disrupted clock throughout the hypothalamus and SCN exhibit mistimed eating and adverse metabolic rhythms that can be restored by dark-only feeding. Time-restricted feeding can also increase the activity of brown adipose tissue in mice and reduce hepatic glucose production to instead promote beta oxidation of fatty acids. The poten tial clinical utility of time-restricted eating (TRE) has been corrobo rated in human interventional studies. These have demonstrated that dietary interventions modulate transcriptional rhythms across tissues and that TRE can improve metabolic homeostasis as well as promote weight loss. Compared with calorie restriction, TRE has repeatedly been shown to promote weight loss by reducing calorie intake without the need to actively count calories. Time-restricted eating may also modulate the central regulation of sleep and hunger, as studies have found that humans who restrict their food intake to a shorter than ad lib period also consume fewer daily calories and report both lower hunger and improved sleep. In the setting of critical illness, nutrition is often provided at the incorrect phase of the light-dark cycle, and interventions to align feeding with environmental zeitgeibers may improve metabolic

health. There is also some evidence that consuming a greater proportion of daily calories early compared with later in the day confers metabolic advantages, including weight loss. One contrib uting mechanism may be that diet-induced thermogenesis (energy expenditure elicited by food intake) is higher in the morning compared with evening and that daytime hunger ratings are lower when calories are preferentially consumed earlier versus later in the day. Finally, animal studies have further shown that when the light-dark cycle is disrupted or when animals are subjected to conditions mimick ing “jetlag” by artificially advancing or delaying the daily light period,

there is desynchronization among circadian clocks and subsequent weight gain. Accumulating evidence in humans also finds that circa dian misalignment both disrupts and desynchronizes circadian clocks across tissues. Clinical studies that have sampled tissues such as blood, skeletal muscle, and adipose tissue at regular intervals have observed disruptions in the day-night rhythms in clock and metabolic genes following sleep-wake interventions. Prolonged circadian misalignment using forced desynchrony protocols reduces insulin sensitivity in the pre- and postprandial states. Under such conditions, insulin secretion fails to suppress glucose levels, suggesting inadequate β-cell compensa tion. Moreover, resting metabolic rate declines significantly both in the awake and sleeping state, altogether providing potential explanations why shift work can increase the risk of obesity, type 2 diabetes, and the metabolic syndrome.

Human genetic association studies also support a role for clock genes in metabolic homeostasis and β-cell function. Carriers of a certain BMAL1 polymorphism have a greater risk of developing type 2 diabetes, while CLOCK variants have been found to interact with diet, such that variants can have a protective effect on insulin sensitivity in individuals with high monounsaturated fat intake or in individuals provided a low-fat diet. In contrast, the minor allele of another CLOCK gene variant has been associated with increased waist circumference, but only in those with high saturated fat intake. Similarly, NPAS2 and BMAL1 variants have been associated with a greater risk of hyper tension. Melatonin receptor MTNR1B gene variants that result in increased expression of MTNR1B have been associated with elevated fasting blood glucose levels and reduced insulin secretion irrespective of their level of glycemic control, although how melatonin regulates glucose homeostasis remains incompletely understood. These associa tion studies highlight the role of the circadian system in metabolism, as well as the potential for interactions of external perturbations—such as circadian misalignment—with a protective or adverse genetic profile. CHAPTER 498 A large proportion of society recurrently shifts sleep-wake and eat ing times between working/nonfree days and free days. This social jetlag has been increasingly tied to metabolic disruptions, including a greater risk of obesity and type 2 diabetes. As this involves recurrent phase advances and phase delays—like shift work but often of smaller magnitude—it is possible that social jetlag, and often interlinked eat ing jetlag, also results in perturbed rhythms of energy expenditure in combination with disruptions to the circadian hunger drive, further increasing the risk of obesity. Repeated shifts in the food- and SCNdriven rhythm of insulin release may similarly over time increase the risk of type 2 diabetes. Shifted feeding rhythms in relation to the sleepwake cycle and the timing of SCN activity may be causally involved in this pathogenesis. This is exemplified by the disorders known as nighteating syndrome and sleep-related eating disorder. In the former syn drome, a large part of daily calorie consumption occurs in the evening and nighttime hours, and this shifted meal pattern has been associated with a delayed timing of the internal clock. Some evidence exists that these syndromes are associated with obesity. Individuals who report sleeping fewer hours or who are subjected to restricted sleep for a few consecutive

days have also been found to consume more calories, especially later in the evening, a period during which prolonged fast ing favors oxidative fuel utilization. As such, this may explain why sleep restriction increases the risk of obesity. These associations have also been observed in individuals with later onset of sleep, i.e., evening chronotypes. Night-eating syndrome and later chronotypes have also been linked to type 2 diabetes and may be more common than other eating disorders such as binge-eating disorder. Both conditions have also been found to be associated with impaired glycemic control—such as a greater likelihood of hemoglobin A1c values exceeding 7%—in patients already suffering from type 2 diabetes. This emphasizes how proper alignment of internal circadian rhythms with external factors are key contributing factors for long-term metabolic homeostasis. The Role of Circadian Biology in Health and Disease
Circadian Clocks in Relation to Brain Health and Cogni tion Molecular circadian clocks are present not only within the extra-SCN regions of the brain, but also in neurons, astrocytes, microg lia, and cells of the blood-brain barrier. Emphasizing the functional

significance of properly aligned clocks for brain health, shift work ers have been found to have decreased gray matter in brain regions involved in memory and executive functions, with more notable effects in individuals who had shorter recovery periods between the onset of each shift work cycle. Adults performing rotating shift work for many years have also been shown to exhibit signs of accelerated cognitive aging. Notably, evidence suggests that these effects may be reversible, as those who stopped carrying out shift work exhibited normal cognitive performance 5 or more years later.

Studies have also uncovered an important role for perturbed circa dian and sleep-wake rhythms in neurodegenerative conditions such as Alzheimer’s disease (AD), Huntington’s disease (HD), and Parkinson’s disease (PD). Amyloid beta (Aβ), a key pathognomonic component of AD, normally exhibits circadian fluctuations in the extracellular space in the brain, as well as in the cerebrospinal fluid and plasma in humans, peaking during the active period and falling during sleep. Of note, these daily rhythms of Aβ accumulation are dampened in mice that are prone to develop AD; reduced plasma Aβ fluctuations have also been noted in older compared with younger individuals. Animal studies indicate that removal of Aβ (and other neurotoxic substances) during the nighttime sleep period is facilitated by a lymphatic-like system that relies on glial cells (the “glymphatic” system). Relevance of this system to humans is suggested by the observation that non–rapid eye movement (NREM) sleep is accompanied by hemodynamic fluc tuations that alter the flow of cerebrospinal fluid, which can remove toxins such as Aβ. Consistent with a role for circadian rhythms in the pathogenesis of AD, ablation of core clock genes throughout the brain, within subregions of the brain or within glia, leads to pathology such as oxidative stress, neuronal cell death, and scarring of brain tissue (astro gliosis). Furthermore, perturbed light-dark cycles increased pathology associated with oxidative stress, and single nucleotide polymorphisms in CLOCK and BMAL1 have been associated with increased risk of developing AD. PART 20 Emerging Topics in Clinical Medicine Evidence also indicates that the relationship between the circadian/ sleep-wake system and AD is bidirectional. For example, patients suf fering from AD exhibit several signs of perturbed circadian rhythms, the most prominent of such phenomena being “sundowning,” whereby AD patients become more agitated and exhibit delirium-like symptoms in the afternoon or evening. Studies have furthermore indicated that in severe forms of AD, the circadian rhythm is phase delayed. Aged AD-prone mice also display perturbed sleep-wake patterns, which can be corrected by immunization against Aβ or by an orexin antagonist. Further research will help

uncover the primary role of the circadian system in disease pathology, independent of the contribution from perturbed sleep, in conditions like AD. Notably, evidence suggests that interventions that increase daytime light exposure and that include melatonin supplementation ameliorate symptoms of AD, presumably by counteracting disrupted circadian rhythms. Meta-analyses of cohort and longitudinal studies support an asso ciation between shift work and the risk of depression, with greater risk in women. This relationship is bidirectional, as disruption of sleep and circadian rhythms is a key feature of depression and multiple other neuropsychiatric conditions. Two important factors for why mis aligned sleep increases the risk of neuropsychiatric conditions may be mistimed light exposure and disrupted 24-h rest-activity rhythms. In cross-sectional and longitudinal analyses, greater time spent outdoors during the day has been associated with fewer symptoms of insomnia, lower risk of developing depression, and less need for antidepressive medication. Similarly, decreased rest-activity rhythms are associated with lower subjective happiness and reaction time and a greater lifetime risk of major depressive or bipolar disorder. This may be partly due to genetic variation, as clock genes have been implicated in depression and mood both in human and genetic animal studies. Polymorphisms in genes that regulate sleep and circadian rhythms—for instance, a long gene variant of PER3—have also been linked to bipolar disorder and schizophrenia, while CRY2 and CLOCK gene polymorphisms are asso ciated with seasonal affective disorder, a type of depression arising in the fall and winter months when the levels of sunlight are lowest. Bipo lar disorder is furthermore often triggered by circadian disruptions

or curtailed sleep. Both bipolar disorder and schizophrenia have been linked to various forms of circadian disruption following disease onset, and a critical component of disease treatment often involves normal izing sleep and sleep-wake rhythms. Sleep deprivation by itself is known to reduce alertness, impair decision-making, and increase risk for accidents—after 18–24 h of con tinuous wakefulness, several skills exhibit the same degree of decline as following mild alcohol intoxication. However, cognitive abilities may suffer even further when sleep restriction is combined with circadian misalignment as in shift work. In one study, participants were subjected to ~43-h long days in parallel with reduced sleep (equivalent to 5.6 h of sleep in a 24-h period), yielding a forced desynchrony protocol coupled with sleep loss. When subjects were tested at the nadir of their circa dian period, the subjects’ reaction speed dropped almost by an order of magnitude compared with controls. In another study, researchers noted almost a 36% greater incidence of serious medical errors in resident interns who regularly worked 24-h or longer shifts compared with those who were randomly assigned to work up to 16-h-long shifts. Furthermore, errors that resulted in patient death were three times more likely to occur in residents working extended hours compared with those who only worked up to 16-h-long shifts. Circadian Regulation of Gastrointestinal Homeostasis and the Microbiota Physiologic aspects of the gastrointestinal (GI) tract exhibit day-night variations that anticipate and prepare for food intake and digestion during the active period. Gastric emptying and colonic motility are considerably greater during the active phase, as the phasic motor program supporting movement of digested mate rial along the intestine is approximately twice as fast during the day compared with night. Bile acid secretion also exhibits circadian rhyth micity in the intestine, as do absorption and the expression of many nutrient uptake transporters in the intestinal wall, including the main glucose transporter protein SGLT1. The permeability of the intestinal wall also varies throughout the sleep-wake cycle, and mice exposed to chronic sleep fragmentation exhibit increased intestinal permeability, which may enable inflammatory molecules from bacteria to reach the systemic circulation. The composition and

function of the fecal microbiome (i.e., the gut microbiota) also display circadian rhythmicity, orchestrated by both host circadian clock gene expression and food intake rhythms. Accord ingly, circadian disruption, either by environmental or genetic means, perturbs these microbial rhythms, disrupting both bacterial levels and the metabolic functions of the gut microbiota. For example, alterations in the expression and functions of the gut microbiota have been noted in humans exposed to acute jetlag, and evidence suggests that curtail ing sleep, which often accompanies shift work and jetlag, can alter the gut microbiota. Corroborating the importance of the daily timing of food intake, interventions with meals scheduled earlier versus later in the day, or that involve time-restricted eating, have been found to alter the composition of the gut microbiome in humans, although the causal relevance of this remains to be ascertained. By increasing local and systemic inflammation, circadian disrup tion of the gut microbiota may be causally involved in increased risk of inflammatory bowel disease (Crohn's disease and ulcerative colitis) and colon cancer in shift workers. Biological sex differences have also been reported, as female mice display more pronounced microbial rhythms. Interestingly, the gut microbiome has also been shown to influence the rhythms of host tissues, such as the intestine and liver, that also appear sex-specific. This relationship indicates that a bidirectional interaction exists between tissues that regulate metabolic processes and the gut microbiome across the sleep-wake cycle. These findings may further more have clinical implications, given that the gut microbiome may both directly (in the gut lumen) and indirectly (through host-microbi ota interactions such as through signaling molecules) impact metabolic responses and pharmacokinetic and pharmacodynamic properties of therapeutic drugs across the 24-h day-night cycle. Cardiovascular Health and the Circadian Clock An early epidemiologic observation was a greater incidence of myocardial infarction in the morning hours, with the lowest risk during the

period preceding sleep. Other cardiovascular outcomes such as sudden cardiac death and syncope also exhibit a daily peak in the morning. Blood pressure (BP) typically peaks around 2100 h and decreases later during sleep. The postexercise recovery response of BP is faster in the late afternoon compared with the morning, and the daily timing of physical activity has been found to modulate the risk of all-cause and cardiovascular disease mortality. The lowering of BP during sleep is partially due to a circadian nighttime dip of around 3–6 mmHg in systolic BP (SBP) and 2–3 mmHg in diastolic BP (DBP). A dip in BP of either <10% or >20% during normal sleep has been associated with worse cardiovascular prognosis and risk of dementia. Nighttime BP dipping is also often disrupted in sleep-wake disorders and correlates with increased cardiovascular disease risk in conditions such as insom nia and narcolepsy. Conversely, specifically lowering nighttime BP has been found to confer a lower prospective risk of cardiovascular disease. In addition to BP, heart rate also typically decreases during sleep, while mistimed sleep leads to higher heart rate during the sleep period. Studies also suggest that heart muscle may be more tolerant to hypoxia and thus fare better under surgery scheduled for the afternoon due to timing of cellular programs driven by the cell autonomous clock in car diomyocytes. Thus, a combination of factors—which may also involve altered glucocorticoid levels and increased platelet aggregation—may contribute to a greater risk of cardiovascular disease in the morning. Subsequent epidemiologic studies also have demonstrated that shift work increases the risk of dyslipidemia and hypertension, as well as the risk of coronary heart disease, including myocardial infarction. These findings are in line with interventional findings in which circadian misalignment has been induced either by inverting the sleep-wake cycle or by imposing days that are far outside what the endogenous circadian clock can adapt to (i.e., either too short [e.g., 20-h] or too long [e.g., 28-h]). These studies in healthy human

subjects have found that circadian misalignment elevates 24-h BP, particularly during sleep. These changes may be causally related to how the autonomic system is regulated during sleep, as evidenced by reduced vagal cardiac control when the sleep-wake cycle is inverted. Circadian Disruption and Cancer In 2007, the International Agency for Research on Cancer (part of the World Health Organiza tion) declared that shift work that involves circadian disruption is likely carcinogenic to humans. While evidence for an association between shift work and general cancer incidence is mixed, accruing evidence supports a link between shift work and increased risk of developing colon and breast cancer, as well as having a poorer cancer prognosis. Telomere shortening, a phenomenon in aging that destabilizes the genome, has also been observed in shift workers as well as in individu als suffering from short sleep. Such changes may reduce the ability of damaged or senescent cells to undergo apoptosis and, instead, lead to uninhibited cell growth and cancer. An indirect role for the circadian clock has also come from retrospective studies on how cancer risk is related to food timing and duration of the nighttime fast in humans. In combination with interventional studies on time-restricted feeding, these findings suggest that limiting food intake to a restricted period of the day, optimizes circadian processes thereby reducing the risk of potentially carcinogenic cell damage. Studies of recurring fasting have also shown that it lowers the risk and delays the onset of cancer. Experimental genetic evidence has also implicated clock disruption as a factor in tumorigenesis. Genetic loss of Per2 or Bmal1 has been shown to promote lung tumorigenesis, while studies in Per2 mutant mice have also revealed increased radiation-induced lymphoma asso ciated with dysregulation of the cell cycle. However, disruption of the Cry gene in mice has also been implicated in tumor protection due to increased susceptibility to cell death. In contrast, pharmacologic overactivation of REV-ERB may impair growth of glioblastomas. While epidemiologic, experimental, and chronotherapeutic evidence (see sec tion “Chronotherapy and Future Directions”) suggests a link between circadian disruption and cancer, the precise role of circadian systems in tumorigenesis remains to be determined. Circadian Regulation of the Immune System Circadian mis alignment and sleep restriction both alter population levels of immune

cells and decrease the ability of immune cells to produce reactive radi cals, in part likely through disruption of cytokine rhythms. Chronic circadian disruption may thereby impair the immune system’s ability to conduct immunosurveillance at the proper time of day. This may reduce the ability to mount an appropriate pathogen-induced effector (cytotoxic T-cell) response during the active period, as well as impair the more long-term adaptive immune response, which is favored by the cytokine milieu (e.g., surges in prolactin and GH) that accompanies the recovery/sleep phase. Instead, circadian misalignment increases a range of clinically used inflammatory markers (e.g., C-reactive protein, tumor necrosis factor α, and interleukin 6), and such changes have been noted even when the sleep-wake cycle is only prolonged to a slightly longer than normal 24.6-h day. While similar effects are also observed following acute total sleep deprivation or recurrent partial sleep restriction, circadian misalignment has been found to promote an even more pronounced elevation of such markers. Genetic clock disruption in peritoneal macrophages has also revealed clock control of Toll-like receptor 9, which is responsible for identifying molecules from foreign pathogens. Clock knockout mice also have reduced T-cell antigen response, and mice immunized during the day had a stronger T-cell response than mice immunized at night, supporting regulation of the immune system by the clock. Similar mechanisms likely take place in humans, as clinical studies have noted an impaired vaccine response following sleep disruption, and several studies show improved immunogenic response to various antigens when vaccinated in the morning

compared with afternoon.

Aging and the Circadian Clock Instability in the clock system is an often-overlooked hallmark of aging. Aging is associated with a decline in the robustness of intrinsic rhythmic processes at the behavioral, physiologic, and molecular levels in both human and animal models. At the behavioral level, aging leads to reduced and fragmented sleep, dampened locomotor activity and feeding rhythms, and a reduced ability to entrain to light, as old rodents are 20 times less sensitive to the entraining effects of light relative to young animals. Even middle-aged individuals exposed to jetlag exhibit more symp toms of circadian misalignment, such as increased time awake and reduced alertness, compared with young individuals. On a physiologic level, some of the hallmarks of aging are a reduction in amplitude (e.g., flattening of circadian pattern) of circadian processes, which can also be seen at the cellular level in peripheral cells isolated from older compared with younger individuals. This dampening of rhythms also impacts the circadian signal during the evening period (the wake maintenance zone). Epidemiologic evidence indicates that a dampened rest-activity amplitude is associated with an increased prospective risk of a range of common health conditions, such as dementia, CVD, can cer, and all-cause mortality. CHAPTER 498 The Role of Circadian Biology in Health and Disease
Aging also results in a phase advance (e.g., a shift in the timing of the peak or nadir) in rhythms of the endocrine and neuroendocrine sys tems, including sleep onset and offset. For example, cortisol, dehydro epiandrosterone (DHEA), and melatonin all have dampened rhythms and are phase advanced in aging; the combination of such changes may, for instance, contribute to more fragmented sleep and lower levels of restorative slow-wave sleep in aged individuals. Relatedly, aging results in reduced peptide expression in the SCN (VIP and AVP), cell loss in sleep-wake regions (including the SCN), and reduced amplitude of rhythms of SCN electrical activity. Further, while the SCN-dependent body temperature rhythm—a generally accepted marker for the integ rity of circadian rhythms—peaks in the evening and is lowest in the early morning in young individuals, aged healthy subjects display a phase advance and a decrease in circadian amplitude in body tem perature rhythms. Indeed, evidence suggests that internal desynchrony between core body temperature rhythms and the sleep-wake cycle may contribute to age-associated circadian alterations. On a molecular level, aging is associated with decreased expression and altered diurnal profiles of several of the core clock genes, includ ing Clock and Bmal1, within both SCN and peripheral tissues such as heart and liver. The acute induction of Per1 in response to light was markedly reduced in the SCN of aged mice compared with young mice,

potentially contributing to their delayed response to light entrainment. Mice lacking Bmal1 die prematurely compared with control mice, con sistent with premature accumulation of reactive oxygen species. These mice have an accelerated onset of numerous age-related pathologies, including cataracts, sarcopenia, reduced organ size, and decreased hair growth. Instead, deficiency of cryptochrome, a repressor of the core clock repressor, has been associated with alterations in liver regen eration, while BMAL1 and PER2 may be important for proper neuro genesis in the hippocampus, a brain region in which adult mammals normally exhibit continuous cell division. Altogether, this suggests that the highly conserved circadian clock is important for regulating a wide range of homeostatic processes, including cell-cycle pathways, which when properly phased to each other promote organismal fitness.

Shift workers have been found to exhibit molecular signs of accel erated aging, as measured by an accelerated DNA methylation clock. Measurements of altered circadian rhythms with age may serve as a useful biomarker for aging. An intriguing question is whether the decline in amplitude of rhythms correlates with a decline in func tion and, importantly, whether restoration of these rhythms with age, through either behavioral or pharmacologic intervention, would delay the aging process. Studies in mice indicate that behavioral and pharmacologic interventions (including exercise) can restore circadian oscillations in aging. Restoration of levels of the metabolite NAD+, which are reduced with aging, in old mice by supplementation with the NAD+ precursor nicotinamide riboside (NR) markedly restores rhythms of metabolic and stress response pathways, as well as late evening activity rhythms, that decline with aging through inhibition of the clock repressor PER2. Similarly, transplantation of the SCN from a young rat into an old rat “rescued” the rhythms of both locomotor activity and corticotropin hormone (CRH), suggesting that the SCN is an important target for age-related changes in clocks. Physical activity or targeted therapeutics may therefore ameliorate some of the circadian deterioration in aged humans. PART 20 Emerging Topics in Clinical Medicine ■ ■CHRONOTHERAPY AND FUTURE DIRECTIONS Chronopharmacology, also known as chronotherapy or circadian medicine, is a rapidly emerging field that studies how the timing of drug administration may impact its effectiveness. Since physi ologic processes vary across the day, the timing of administration of medication may help optimize patient care. For example, since endogenous cholesterol synthesis is rhythmic in liver and peaks dur ing the early morning hours, administration of statins (HMG-CoA reductase inhibitors) in the evening prior to bedtime has proven to be more effective than daytime administration at reducing low-density lipoprotein cholesterol (LDL-C) levels because the highest concentra tion of medication coincides with the peak in rhythmic endogenous cholesterol production. Given that BP exhibits a 24-h rhythm—being lowest during sleep—angiotensin-converting enzyme (ACE) inhibi tors have been shown to be most effective at night to normalize the BP rhythms, restoring the nighttime dip in BP that is foremost tied to the occurrence of sleep. Numerous studies have also demonstrated that administration of cancer treatments at specific times of the day can increase chemotherapy effectiveness while also decreasing toxicity for a wide range of drugs. For example, 5-fluorouracil works best to treat colorectal cancer when administered at night, a time when the cancerous cells are more vulnerable while normal cells are quiescent and therefore less sensitive. Doxorubicin administration early in the morning to treat ovarian cancer has also been shown to be less toxic, as white blood cells recover faster than if the drug is given in the evening. Finally, the more severe morning symptoms of rheumatoid arthritis are linked to increased inflammation toward the evening; therefore, prevention of the nighttime upregulation of the immune/inflammatory reaction is more effective when glucocorticoids are administered with a nighttime release formulation. Recognition of circadian rhythms is also critical for diagnoses and treatment of endocrine disorders. The diagnosis of Cushing’s syn drome, which is characterized by hypercortisolemia, might be missed if the patient’s cortisol levels are measured in the morning, when endogenous cortisol production peaks. Therefore, clinical diagnosis

requires cortisol to be measured in the late evening when the levels of this hormone should typically be low. On the other hand, adrenal insufficiency is diagnosed by measuring cortisol in the morning when at its physiologic peak, and glucocorticoid therapy for these patients aims to mimic the endogenous rhythms of cortisol, as short-acting synthetic glucocorticoids are usually given several times a day in taper ing doses, such that the largest amount is taken in the morning and the smallest in the evening. Diabetes is another endocrine disorder intimately tied to circadian rhythms. Oral glucose tolerance, which is commonly used to diagnose diabetes, is worse in the

afternoon and evening compared with the morning. This likely stems from greater daytime insulin sensitivity within peripheral tissues and reduced insulin secretion during the night. Similarly, due to a surge in hormone levels in the morning, diabetes patients may suffer from the dawn phenomenon (or dawn effect), an abnormally high morning increase in blood glucose due to impaired response in insulin secretion. A related phenomenon that can be tied to evening timing of insulin doses is the “rebound” or Somogyi effect. In this scenario, the initially noted clinical sign in the form of elevated glucose levels may be noted in the morning. However, the underlying cause is hypoglycemia occurring during the night, which produces a counterregulatory hormonal response that subsequently results in morn ing hyperglycemia. As patients with type 2 diabetes often have grossly impaired daily cycles of insulin secretion and glucose tolerance, this further highlights that time of day is an important consideration for the diagnosis and treatment of metabolic disorders such as type 2 diabetes. Another example of potential clinical relevance is how the pharmacoki netics of metformin—the most common treatment for type 2 diabetes— is significantly impacted by time of day due to rhythmicity in glomerular filtration rate and renal plasma flow. Notably, large interindividual vari ability in the pharmacokinetics seems to stem mostly from differences in chronotype, highlighting the need for patient-specific treatments dictated by circadian gene-environment interactions. Continuous measurements of 24-h glucose have provided insight into sleep-wake regulation of glucose metabolism. Compared with day time glucose levels, nighttime blood glucose levels have been found to more accurately predict a range of glucoregulatory parameters. Emerg ing evidence has also indicated that the daily timing of exercise may be an important determinant for more efficacious improvements in blood triglyceride and glucose levels. Furthermore, consideration of meal timing, particularly in the hospital setting, may impact patient health or responsiveness to treatments, as food in hospitals is often provided either continuously or just during the dark (rest) phase, with the latter being common in neonatal intensive care. As our knowledge of the complexity of how circadian processes mod ulate physiology deepens, further advances to rationally develop new strategies for treatments of disorders affected by circadian misalignment are essential. For example, novel compounds have begun to emerge from unbiased drug discovery screens that in cell- and animal-based assays impact circadian clock components, either shortening or lengthening the period. These compounds include CRY stabilizers and various inhibitors of CKIδ, CKIε, and GSK-3. Pharmacologic control of the circadian cycle may be useful in the treatment of circadian disorders and metabolic disturbances with a circadian component. Understanding how the cir cadian clock controls biological functions will shed new light onto the pathogenesis of metabolic disorders with a circadian component, such as type 2 diabetes and metabolic syndrome, and will yield insight into how timing of drug delivery will impact patient care.

Acknowledgment The authors would like to thank Billie Marcheva for her help with the figures and tables. ■ ■FURTHER READING

Allada R, Bass J: Circadian mechanisms in medicine. N Engl J Med 384:550, 2021. Buxton OM et al: Adverse metabolic consequences in humans of prolonged sleep restriction combined with circadian disruption. Sci Transl Med 4:129ra43, 2012.

Revision #1
Created 2026-01-06 16:36:19 UTC by Omar Ayman
Updated 2026-01-06 16:36:19 UTC by Omar Ayman