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Circadian Rhythms and Their Impact on Aging

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Introduction

Aging is a function of the passage of chronological time, as the organism proceeds from birth to death. This simple statement does not account for the fact that individuals present differently as their chronological age progresses, and there is substantial heterogeneity among members of a birth cohort in the time of survival. For these reasons, the concept of biological age has been advanced, in recognition of this marked individual variation. Biological age takes into account the departure of any given individual from the population average in terms of time to death as well as function ability (Kim and Jazwinski 2015). Indeed, function ability, expressed in various ways, is used as a metric of biological age.

Given these considerations, biological aging can be defined as a progressive loss of function over time, in as much as such loss is characteristic of most individuals in the population. This loss of function occurs at many levels. The so-called 'hallmarks of aging' express this loss at the molecular and cellular levels, and they include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (Lopez-Otin et al. 2013). Except for the last, these hallmarks are entirely cell-autonomous. Intercellular communication, on the other hand, conjures up integrated function of the organism, to which the cell-autonomous hallmarks undoubtedly also contribute. Thus, we may consider biological aging to be the reflection of the loss of integrated function. At a clinical level, loss of integrated function leads to a degradation of robustness and resilience, such that biological aging is associated with an increase in the incidence of chronic disorders and susceptibility to acute diseases. These are often called the diseases and disorders of aging.

The circadian system has a period of about 24 hours, by definition. It assures that physiological processes are performed at the appropriate time of day or night. The circadian system is composed of a central clock in the suprachiasmatic nucleus (SCN) of the brain and peripheral oscillators in individual cells and tissues that operate in an autonomous fashion using similar core molecular components (Panda et al. 2002; Reppert and Weaver 2002; Froy and Miskin 2010). The central clock responds to light and dark (photoperiod), temperature, and nutritional status

(feeding behavior). The resulting entrainment is signaled by autonomic connections or circulating hormones to the peripheral oscillators. Entrainment synchronizes central and peripheral clocks, without which the endogenous rhythms free run with a period of approximately 24 h. The circadian clock through peripheral circadian oscillators affects virtually every aspect of physiology and behavior, including metabolic activity and gene expression (Panda et al. 2002; Reppert and Weaver 2002; Froy and Miskin 2010). Expression of clock genes oscillates in almost all mammalian cells, and they regulate more than 10% of the human transcriptome (Peirson et al. 2006; Storch et al. 2002).

Forty years ago, it was shown that departure from a natural 12-h light/12-h dark cycle caused a significant decrease in longevity of the fruit fly, *Drosophila melanogaster* (Pittendrigh and Minis 1972). Recently, these observations were extended to the laboratory mouse (Dubrovsky et al. 2010; Libert et al. 2012). Furthermore, there is an inverse association between the departure of the free-running period of circadian rhythm from 24 hours and lifespan in various rodent and primate species (Wyse et al. 2010). Transplantation of fetal SCN to old rodents restores the amplitude of their rhythm and extends their lifespans (Hurd and Ralph 1998), implicating central clock dysfunction in aging. However, age-related decline in the peripheral circadian machinery also occurs (Hill et al. 2013), and it may be at least partially mitigated by enhanced central clock signaling. As in animals, there is a shift in phase and decrease in amplitude of circadian rhythms with normal aging in humans (Hofman and Swaab 2006). This impairment is associated with a decline in nocturnal melatonin peak in elderly humans, contributing to the dampening of circadian rhythms (Hardeland 2010). A strong, albeit circumstantial, case can be made for an association of changes in circadian rhythm with a decline in health and age-related disease in human aging (Hardeland 2013).

It is abundantly clear that disruption of the circadian system has dire consequences for longevity (Dubrovsky 2010; Libert et al. 2012). It also has serious implications for health (Hardeland 2013), and during the course of the lifespan, clock activity becomes less and less robust (Hofman and Swaab 2006; Hardeland 2010). Virtually, every cell in every tissue marches to the time of its own molecular circadian clock. These peripheral oscillators determine the ebb and flow of metabolic processes, and they dictate the periodicity with which tissues communicate, setting and resetting physiologic thresholds throughout the organism (Panda et al. 2002; Reppert and Weaver 2002; Froy and Miskin 2010). It is obvious that these peripheral oscillators must be closely coordinated to assure the integrated function of the organism. Interestingly, integrated function of the organism decays with age, which can be observed even at the molecular level in the form of increased transcriptional noise in aged tissue (Bahar et al. 2006).

Calorie restriction (CR), a treatment that increases lifespan in many species, entrains the central clock in the SCN (Challet et al. 2003; Challet et al. 1998; Mendoza et al. 2005). It also upregulates clock genes in several tissues, among the top three biological processes thus affected (Swindell 2008). Thus, CR affects the clock and metabolism both centrally and peripherally. The Sirt1 protein deacetylase forms a complex with the Clock acetylase. This complex regulates the activity

of clock-controlled genes through chromatin remodeling (Nakahata et al. 2008) and by acetylating/deacetylating Bmal1 and Per2 (Asher et al. 2008), which are other components of the core clock (Zheng and Sehgal 2012). There is evidence that homologs of the yeast longevity gene *SIR2* play a role in the lifespan extension afforded by CR, and the activity of the mammalian Sir2 homolog, Sirt1, promotes metabolic responses similar to those found in CR (Canto and Auwerx 2009). By most criteria, the circadian system constitutes the essence of the gene–environment interface that plays such an important role in aging.

Metabolism, and especially energy metabolism, changes markedly over the lifespan (Kim and Jazwinski 2015). Calorie restriction, which extends lifespan and promotes health span, reverses many of these changes (Westbrook et al. 2014). Signaling via insulin/IGF-1(van Heemst 2010) and PI3K/Akt/Foxo (Hay 2011) plays a key role in lifespan extension, while regulating key components of metabolism. Similarly, AMPK has dual effects on metabolism and lifespan (Onken and Driscoll 2010). TOR, the cell sensor of nutrient status, plays a crucial role in coupling protein synthesis to nutrient availability, among its several functions, and it is the most widely encountered lifespan regulating device across phylogeny (Johnson et al. 2015); and, the TOR inhibitor, rapamycin, is one of only a few drugs that actually extend lifespan and health span reproducibly (Harrison et al. 2009). Finally, Sirt1 responds to NAD availability to regulate lipid metabolism and mitochondrial biogenesis (Canto and Auwerx 2009). Activation of this protein improves various features of energy metabolism, and recently it has been shown to increase mouse longevity (Mercken et al. 2014). Sirt1 is also an accessory protein to the core clock machinery (Asher et al. 2008; Zheng and Sehgal 2012).

Mitochondria, which play a central role in energy metabolism, become dysfunctional with age (Lai et al. 2002). Removal of such compromised organelles is essential to preserve youthful function, but this quality-control, autophagic process likewise becomes deficient during aging (Chistiakov et al. 2014). This deficiency is exacerbated in certain neurodegenerative disorders associated with aging (Nixon and Yang 2012). Interestingly, TOR regulates autophagy, in addition to its direct metabolic effects (Johnson et al. 2015). Thus, we must consider mitochondrial respiratory ability, ROS production, biogenesis, and turnover, in evaluating the impact of circadian regulation of metabolism on aging. The importance of ROS as a cause of aging is somewhat controversial at present, but it is clear that ROS can cause significant damage to cell components, including DNA. Melatonin, often called the hormone of darkness, is secreted by the pineal gland on stimulation by the SCN, and it is a critical output of the circadian, central pacemaker that helps to entrain both the central and peripheral circadian oscillators throughout the body (Reiter 1991, 1994; Claustrat and Leston 2015). It is also synthesized in numerous other tissues, and it is a potent antioxidant that combats ROS (Hardeland 2015).

This brief introduction highlights some of the important ways in which the circadian system impacts the aging process. It stresses the central role the circadian system plays in the integration of physiologic functions across the organism. The chapters in this book explore these topics in great detail, and they introduce the nuances associated with these and other aspects of these relationships.

Chapter 1 (Circadian Dysregulation and Melatonin Rhythm Suppression in the Context of Aging) by Reiter et al. introduces the circadian system, its operation, and the central role of the SCN and melatonin signaling. It also outlines the broad impact of this system on physiology and pathology, especially as it relates to aging.

Chapters 2–4 amplify on the themes introduced in the first chapter. Chapter 2 (Pulmonary Diseases, a Matter of Time) by Sanchez discusses the circadian system's impact on the pathophysiology of the aging lung. This chapter presents some of the recent research in this area at the cell and molecular levels. Chapter 3 (Circadian Regulation of Bone) by Maria and Witt-Enderby focuses on the impact of the clock genes on the activity of osteoblasts and osteoclasts and how this translates to bone metabolism, so important especially during aging. Chapter 4 (Aging and the Circadian Control of the Gastrointestinal System: from the Brain to the Gut Microbiome (and back)) by Cassone et al. talks about the effects of aging on the circadian clocks of the gastrointestinal system. These authors discuss novel mechanisms of entrainment and the complicity of the gut microbiome in circadian control.

Chapters 5–12 dig more deeply into cellular and molecular mechanisms associated with circadian system aging. Chapter 5 (Circadian System and Aging in Rodent Models) by Panchenko et al. presents the changes that occur in the circadian system during rodent aging and their impact on physiology and disease. This chapter describes the use of both genetic and environmental manipulations to unravel the mechanisms involved. Chapter 6 (The Circadian System and Aging of *Drosophila*) by Giebultowicz introduces the powerful *Drosophila* genetic model organism. This chapter, like the previous one, engages both genetic and environmental interventions to tease out circadian-based mechanisms of aging. It also describes novel findings related to rhythmic gene expression during aging. Chapter 7 (Circadian Control of Mitochondrial Dynamics) by Jacobi et al. expands on the metabolic threads in the previous two chapters, with an emphasis on mitochondria. These authors discuss the involvement of the core clock gene BMAL1 in mitochondrial dynamics and in mitophagy in mice and worms and the impact of these processes on both aging and lifespan. Chapter 8 (Circadian Rhythms and Proteostasis in Aging) by Desvergne and Friguet establishes a link between the circadian system, redox homeostasis, and two other fundamental cellular processes, autophagy and proteostasis. These processes undergo age-related deterioration, as demonstrated in yet another aging model, cell senescence. Gupta and Kondratov discuss the connections between the circadian clocks and mTOR signaling in Chap. 9 (Circadian Clocks and mTOR Signaling). The clocks and mTOR together play an essential role in the sensing of nutritional status and in regulation of metabolism. They impact aging, and their disruption is associated with various pathologies in animal models. In Chap. 10 (Aging and the Biological Clock), Judge et al. draw our attention to the *Neurspora* genetic model, which has played such an important role in circadian research. These authors take a systems biology approach based on the complete knowledge of the organism's transcriptional network. They demonstrate that several different metabolic pathways link aging and the clock in a reciprocal interaction. Nohara et al. in Chap. 11 (Developing Circadian Therapeutics Against Age-related Metabolic Decline) review evidence for the intimate relationship between energy homeostasis,

aging, and the circadian clock. They build on this to describe current efforts to identify small-molecule therapeutic agents that enhance circadian and metabolic functions. Such chronotherapeutics may promote healthy aging by delaying metabolic decline. Chapter 12 (The Possible Role of Epigenetics in the Memory Impairment Elicited by Circadian Rhythm Disruption) by Deibel and McDonald closes this set of chapters by presenting epigenetics as a mechanism for generation and synchronization of circadian rhythms. They discuss how memory is affected by circadian rhythms and the potential role of epigenetics in this relationship.

Stone and Tranah discuss the impact of disruption of 24-hr activity patterns in older adults on health and mortality risk in Chap. 13 (Circadian Sleep-wake Activity Patterns During Aging). These effects include increased risk of mild cognitive impairment and dementia. The authors point to the need for studies to test interventions that regulate circadian activity rhythms on health outcomes in the elderly. One of these interventions is physical activity. This book ends with Chap. 14 (Effects of Physical Activity on Circadian Rhythms in the Elderly), in which Bessot discusses the effects of physical activity on circadian rhythms. He also presents several potential mechanisms that may be involved, pointing to new avenues of investigation.

We know a good deal about circadian rhythms and how they change during aging. There is ample evidence that disruption of these rhythms accelerates aging and shortens lifespan. Often, this disruption leads to outright disease and degeneration. The impact can be readily observed at the level of fundamental cellular processes, as well as at the physiologic level. Core clock genes through their impact on the transcriptome are clearly part of the underlying mechanism. Tantalizingly, there appear to be reciprocal interactions between the circadian system and its outputs. The circadian system is the ultimate interface of the organism with the environment. It is beginning to emerge that epigenetics resides at this interface. The impact of the loss of circadian activities during human aging is manifold, and it includes cognitive function. There is promise that relatively simple interventions, such as physical activity, can reverse this decline by reinforcing circadian activity. These simple interventions may be joined by chronotherapeutics which are already in early stages of testing.

This recitation of the accomplishments of current research in the field of chronobiology of aging points to how much more we need to learn to be able to manipulate circadian activity to enhance health in an aging population. These efforts will require a systems biology approach because the circadian system impacts so many processes. Furthermore, the core clock is not only fundamentally a feedback system, but it also is subject to feedback from its distal outputs. These challenges make future research all the more exciting. It is also reassuring that interventions, such as physical activity, that have systemic effects already show considerable promise. In sum, we expect the next few years will bring us much activity in the field of the chronobiology of aging.

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Chapter 1
Circadian Dysregulation and Melatonin Rhythm Suppression in the Context of Aging

Russel J. Reiter, Sergio A. Rosales-Corral, Dun Xian Tan, Moises Alatorre-Jimenez and Carlos Lopez

Abstract Fifty years ago, little was known of the role of the prevailing light:dark environment in terms of its impact on the circadian pathophysiology of organisms. In the intervening years the field of photoperiodic regulation of the master circadian oscillator, i.e., the suprachiasmatic nucleus (SCN), has advanced at a rapid pace. The importance of the regulatory actions of the light:dark cycle, and particularly of perturbed light:dark cycles, not only on the SCN but also on the circadian production of pineal melatonin as well as the cyclic metabolism of cells throughout the body are by no means trivial. When the regular cyclic information generated and dispensed by the SCN is dysregulated, the negative consequences in terms of cellular and organismal physiology can be dire to the extent that the rate of aging and the onset and progression of a variety of age-related diseases have now been at least provisionally linked to circadian disruption and/or melatonin suppression. While the findings are not definitive, there is certainly credible data to warrant the conclusion that regular circadian rhythms at multiple levels, including a stable day: night melatonin cycle, enhance life quality and potentially delay senescence and forestall diseases normally associated with advanced age. As a result, the prolonged health span may also predispose to a longer life span. In view of the critical role of an abnormal or unusual light environment in terms of perturbing essential circadian physiological events, serious consideration should be given to rational thought about the misuse of artificial light and the consequences thereof.

Keywords Senescence · Age-related diseases · Health span · Life span Suprachiasmatic nucleus · Slave oscillators

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1.1 Introduction

Properly-timed circadian rhythms are a *sine qua non* for optimal cellular and organismal health and for the prolongation of health span, which may also translate into an enhanced life span (Agorastos and Lindhorst 2016). In the absence of well-regulated circadian biology, the molecular physiology of cells is in a state of relative chaos, the degree of which presumably is determined in part by the severity of the dysregulation (chronodisruption) and the frequency and duration of the circadian insults. There is compelling evidence that subcellular turmoil due to any influence is associated with altered metabolic pathways that jeopardize the health of cells (Hardeland et al. 2012; Reiter et al. 2012a, b). Unhealthy cells promote less vigorous organs which surely compromise the welfare and survival of organisms. The "bottom line" is that regular circadian rhythms improve cellular physiology at the molecular level.

An estimated 15% of the genes possessed by cells relate to circadian rhythms (Scott 2015). The rhythms resulting from the expression of these genes at the cellular level have been referred to as slave oscillations given that many of them are under the influence of a more superior time-giver (Lamia et al. 2008; Golombek and Rosenstein 2010; Hardeland et al. 2012; Coelho et al. 2015; Buijs et al. 2016). While there are several factors that influence the expression of rhythms within cells, a major one is the cyclic information received from the master circadian oscillator or pacemaker, the suprachiasmatic nuclei (SCN) (Pauls et al. 2016). In mammals including the human, the activity of the SCN in turn is governed in large part by the light:dark environment as perceived by the eyes (Hughes et al. 2016). The unique and intricate molecular mechanisms whereby specific, especially blue, wavelengths of light impact distinctive intrinsically photosensitive retinal ganglion cells (*ip*RGC) in the outer layer of the mammalian retinas along with the retinohypothalamic tract which transfers this information to the SCN are well described elsewhere and are not reviewed here (Lucas et al. 2014).

Once the photoperiodic information is received by the SCN, it must convey the associated message to the slave oscillators at the cellular level, whether or not this information is accurate relative to regularly-changing daily and seasonal changes in the light:dark environment as determined by the rotation of the Earth on its axis and its journey around the sun. The SCN has two known routes by which to inform the peripheral elements about the photoperiodic state. Thus, the central oscillator apprises the more distant cells via either a neural or humoral pathway (Fig. 1.1).

Since the SCN are located near the base of the telencephalic/diencephalic interface, they are in close proximity to the "head ganglion" of the autonomic nervous system, i.e., the hypothalamus. The central pacemaker has solicited the autonomic sympathetic/parasympathetic pathways as the neural route for contacting peripheral cells. One limitation of using the autonomic nervous system is that its postganglionic fibers are restricted in terms of their peripheral distribution. To solve this problem, the SCN also selected a humoral route for communication. To achieve this, the SCN utilizing the central and peripheral sympathetic nervous system,

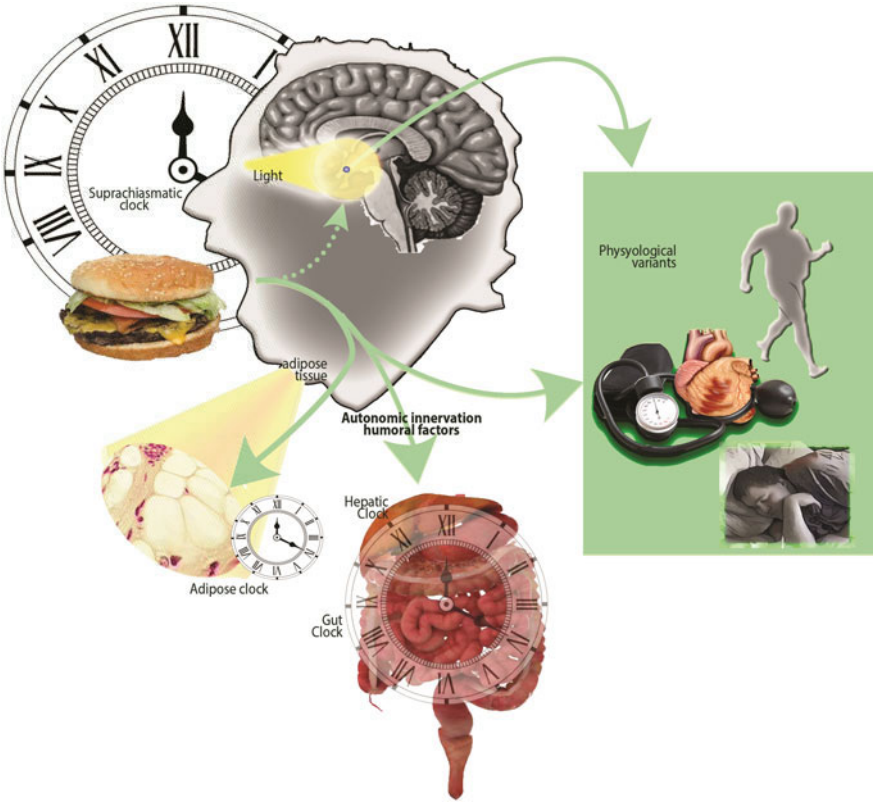

Fig. 1.1 Regulation of the master circadian oscillator (the suprachiasmatic nuclei or SCN) and its impact on peripheral cellular rhythms. The major regulatory input to the SCN comes from the light:dark cycle as perceived by melanopsin in the intrinsically photoreceptic ganglion cells of the eyes. Other factors that impact the circadian system, but to a lesser degree than the light:dark environment, include physical activity and eating. The SCN communicates its circadian information to peripheral cellular clocks via two routes, the autonomic nervous system and by means of a humoral signal, most conspicuously the melatonin cycle. Desynchronization of circadian rhythms leads to pathological disorders, represented here by the "physiological variants." Chronic disruption of the circadian system likely contributes to aging and age-related diseases

communicates with the pineal gland and dictates its metabolism (Stehle et al. 2011). In the absence of the light, the message is stimulatory at the level of the pinealocytes such that they are induced to synthesize and secrete, both into the blood (Vaughan et al. 1976) and into the cerebrospinal fluid (CSF) (Skinner and Malpaux 1999), a humoral mediator, i.e., melatonin, which contacts every cell in the organism. Any cell capable of "reading" this message, therefore, knows the status of the external light:dark environment. By utilizing both neural and humoral routes, information from the SCN is imposed on the genome of every cell in vertebrates.

With the advent of the introduction of artificial light in 1879 when the light bulb was invented and with the development of rapid transmeridian travel via airplanes, the photoperiodic information received by the SCN from the *ip*RGC is often not representative of the true daily/seasonal changes in the light:dark environment. Since the SCN is not allowed a choice in passing the altered information forward, every cell sometimes receives misinformation which disrupts their cycles and leads to circadian disturbances, i.e., chronodisruption (Erren and Reiter 2009). This contributes to malfunction of intrinsic molecular processes which create the chaos mentioned above; chaos always translates into accelerated cellular deterioration, i.e., aging. There are factors in addition to the light:dark cycle, specifically the rest/activity cycle and the feeding regimen, that also influence the function of the SCN (Fig. 1.1). This review, however, is primarily concerned with the instructions the SCN receives from the retinas and how perturbations of this information impacts cellular and organism senescence.

A major point of this report is that the aging-related consequences of chronodisruption cannot be easily distinguished from those caused by a perturbed melatonin cycle. Altered SCN rhythms are always accompanied by either a changed melatonin rhythm or a total suppression of melatonin synthesis and secretion. Given that this humoral message reaches every cell, circadian disorder becomes widespread. The problem is confounded by the fact that the plasma and CSF melatonin cycles are also designed to strengthen the central circadian clock message (Cassone 1990; Reiter et al. 2014b). In the absence of this regularly-repeating humoral message, the function of the SCN is weakened causing additional disorder which, via feedback and feedforward processes, may become a vicious cycle of gradually deteriorating molecular physiology. This leads to an acceleration of age-related processes.

The most common example of chronodisruption is that caused by light at inappropriate times, i.e., light at night. Throughout the world, well-developed societies, due to electrification, are experiencing something that organisms have never experienced during a very long period of evolution; they are experiencing what is referred to as the "end of night." It was the dependence of organisms on the regularly-repeating periods of light and darkness during evolution that was surely consequential in the evolution and the development of the SCN; after all, it was the most reliable environmental variable on which to evolve a "clock."

Light pollution is unquestionably a major factor that negatively impacts the function of the central circadian oscillator and, by necessity, the slave oscillators as well. Many studies have documented that light-at-night (LAN) changes the circadian output of the SCN, typically measured as perturbations in the blood melatonin cycle (Lewy et al. 1980) or in the urinary melatonin metabolite (Dumont et al. 2012) rhythms in humans and animals. These disturbances in the melatonin cycle have been linked to a variety of diseases/disorders that contribute to aging per se and indirectly to age-related diseases (Reiter 1995, 1997; Hardeland 2013; Hardeland et al. 2015; Opie and Lecour 2016), i.e., the promotion of pathologies. Of special note is the accelerated cancer growth, changes in glucose metabolism, skin deterioration, etc. (Kleszczynski and Fischer 2012; Haus and Smolensky 2013;

Cipolla-Neto et al. 2014) Again, the reader is reminded that any alteration in the melatonin rhythm does not occur without a corresponding change in the neural message conveyed to the periphery by the SCN. Hence, it is never possible to distinguish whether an age-related change or pathology is a specific consequence of a malfunctional melatonin rhythm or abnormal circadian neural information; most likely, they are due to a combination of these factors (Reiter et al. 2012a; Hardeland 2013, 2015).

1.2 Hallmarks of Aging: Effects of Melatonin

Aging, the rate of which varies widely among animal and plant species, is usually characterized by a time-related functional deterioration of living creatures (Jones et al. 2014). More formally, aging is defined "as a progressive loss of physiological integrity leading to impaired function and increased vulnerability to death." Historically, aging was judged on the basis of longevity in addition to the propensity of an individual to develop overt diseases. In the current era, however, aging is being investigated at the molecular level with the goal of identifying the processes that actually contribute to the aging phenotype. When these molecular processes are defined, it is the assumption that they will be modifiable and an increased healthy life span can be realized.

In a recent review, Lopez-Otin et al. (2013) suggested nine markers of aging which they feel characterize the degenerative processes at the subcellular level. While these markers are listed as being distinctly different entities, in fact, they have significant functional overlap consistent with the undoubtedly great complexity of the aging process. This entanglement will likely make identifying the specific mechanisms that underlie aging very difficult and equally problematic to treat.

A common denominator for many of the processes that are characteristics of aging is the oxidative microenvironment within cells (Forman 2016). Cells utilize oxygen as a basis of metabolism in the production of energy. Because of the relative inefficiency of the mitochondrial electron transport chain, the generation of oxygen free radicals and related non-radical oxygen-based derivatives is unavoidable (Brand 2016). This occurs when electrons are fumbled during their transfer between respiratory complexes and chemically reduce molecular oxygen to the superoxide anion radical ($O_2^{\bullet -}$). While there is a formidable antioxidant defense system, some toxic species escape detoxification and harm neighboring molecules. As a consequence, over the course of a life time, oxidatively-damaged molecules gradually accumulate which impedes efficient molecular functions. These less than proficient systems further contribute to physiological disability and the greater deterioration typical of older individuals (Sohal and Allen 1990).

Oxidative stress is certainly not the only factor that accounts for the aging phenotype; but it surely subsidizes the amount of damage molecules sustain. If these injured molecules are not repaired or removed, they become a burden to cellular physiology. Accumulated oxidative stress is mentioned as a feature of many

theories that have attempted to explain aging (Ames 1989). Melatonin, the endogenous levels of which diminish with age in most individuals, normally functions as a potent antioxidant to reduce the accumulation of oxidized molecules which accelerate senescence and aging.

Cells typically have a finite number of cell divisions they can undergo before they functionally collapse. This phenomenon was described by Hayflick several decades ago (Hayflick 1979). It is our prediction that properly-timed melatonin exposure would extend the Hayflick limit, i.e., defer cellular aging. This assumption is based on melatonin's ability to both organize the circadian biology of cells and to its actions as a multifaceted direct free radical scavenger and its indirect actions in the promotion of enzymatic antioxidative defense processes. Moreover, melatonin's high concentration in mitochondria (Venegas et al. 2012), a site where free radical formation is abundant, is consistent with the option. In this regard, melatonin has recently been designated as a mitochondria-targeted antioxidant (Reiter et al. 2016) which was proven equivalent to or more effective than synthetic antioxidants, Mito E and Mito Q, in resisting oxidative damage and inflammation. Mito E and Mito Q are industry-produced antioxidants that, because of their increased lipid solubility, concentrate in the mitochondria up to 100-500-fold (Oyewole and Birch-Machin 2015). Despite this, they are no more effective than endogenous-produced melatonin in preventing free radical-mediated molecular damage resulting from the simultaneous exposure of animals to two highly toxic bacterial molecules, lipopolysaccharide and peptidoglycan (Galley 2010; Lowes et al. 2013). Ramis et al. (2015) have recently reviewed the literature related to the relative effectiveness of synthetic antioxidants and melatonin in determining the degree of oxidative stress. Given that melatonin is so effective in curtailing the actions to toxic free radicals and the fact that its levels in both animals and many humans wane with advancing age (Scholtens et al. 2016), it seems safe to assume that the loss of melatonin including the suppression of its rhythm which contributes to circadian disruption in late life contributes to the aging phenotype which is a consequence of accumulated oxidatively-damaged molecules that occur throughout life.

More than 15 years ago, we reported that early-life pinealectomy in rats, which deprived the animals of a circadian melatonin message and dropped their circulating melatonin concentrations to barely-measurable values, caused augmented oxidative damage to all tissues in which it was measured when the rats reached 24 months of age; rats of this age are generally considered old (Reiter et al. 1999). These results likely related to the high efficiency of melatonin as a multifaceted antioxidant which, due to its low levels, allowed many mitochondrial-generated free radicals to go uncontested. This would certainly contribute to accelerated aging. These animals, however, likely had weakened circadian rhythms at the peripheral cellular level which also presumably supported the generation of an increased numbers of partially-reduced toxic oxygen derivatives. This illustrates the difficulty in distinguishing which factor, melatonin deprivation or circadian disorder, contributes most significantly to the more abundant oxidative stress in the old animals.

Some resolution to this conundrum could perhaps be gained by comparing the degree of oxidative damage in different tissues of old animals (that have been

pinealectomized at an early age). In the study of Reiter et al. (1999), for example, all tissues in which lipid peroxidation and protein carbonyls were measured (lung, liver, skin, pancreas, kidney, etc.), normally rely on the blood melatonin cycle for the majority of their circadian direction. Other tissues (e.g., salivary glands) which also received a significant amount of their circadian information via the autonomic nervous system, may exhibit less oxidative damage than cells which are not directly innervated by the sympathetic/parasympathetic fibers. These glands, since the activity of the SCN is still synchronized by the prevailing light: dark environment as perceived by the *ip*RGC, may receive regular circadian information which may better synchronize their inherent cellular rhythms thereby reducing free radical generation.

The melatonin rhythm, as a reflection of information provided by the SCN, as suggested above, likely has a significant role in directing the circadian biology of peripheral cells. The melatonin cycle, unlike the SCN-mediated circadian information conveyed via the autonomic nervous system, contacts every cell in the organism. That melatonin is in fact capable of synchronizing cellular rhythms has been documented (Jung-Hynes et al. 2010). This group showed that the intrinsic circadian rhythms of cultured cells were brought into synchrony when melatonin was added to the culture medium. This action would likely aid in slowing molecular degeneration that contributes to aging. This circadian regulatory action of melatonin may also be an explanation for the recent findings which document that melatonin sensitizes previously chemotherapeutic-resistant cancer cells to these treatments (Martin et al. 2010; Xiang et al. 2015). Given the findings of Jung-Hynes et al. (2010) relative to melatonin's ability to properly direct circadian gene expression at the peripheral level, the necessity of maintaining a regular alternating light:dark environment which propels the melatonin rhythm seems essential if aging is to be slowed. These findings are also consistent with the observations that excessive light pollution or frequent long-haul transmeridian travel, both of which cause chronodisruption and melatonin rhythm alterations/suppression, are associated with an increased risk of developing cancer and other age-related diseases. Melatonin has been commonly used by frequent long-haul travelers to combat the sleep deprivation and fatigue related to travel across multiple time zones (Cardinali et al. 2002). Besides helping to correct these disturbances, melatonin's use for this reason may also reduce the likelihood of developing age-related cancer predictably linked to frequent long-haul travel. Whether these beneficial effects of melatonin derive from is intrinsic oncostatic activity or as a result of its rhythm-synchronizing activity is difficult to determine.

1.3 Consequences of Disruption of Circadian Rhythms

During aging, there is a clear diminution in the strength of circadian rhythms which compromises organismal homeostasis in a negative way; the resulting changes contribute to morphological and physiological aging. The molecular aging

phenotype is manifested at many levels. In old individuals of any species, molecular damage comes in a variety of forms and has been studied for many years. This damage includes elevated levels of lipid peroxidation products (Grinna 1977; Zs-Nagy 1978), increased damaged protein concentrations (Steinberg and Witztum 1990; Clarke et al. 1991) and enhanced amounts of oxidized DNA (Alexander 1967; Imlag and Linn 1988). These changes also are a result of a number of factors including disease processes and perturbations of circadian rhythms, among others.

A more recently defined disorder that contributes to aging and especially to age-related diseases is the gradually-developing instability of the genome. This instability becomes greater as organisms age and is best known for its association with conditions such as progeroid-like syndromes where aging is highly accelerated and in cancer (Campisi 2005; Vijg and Suh 2013). A relationship of the deteriorating stability of the genome and cancer incidence is not unexpected since specific mutations are a requirement for a cancer to develop. While humans possess genes, e.g., SIRT1, that foster longevity (Jazwinski et al. 2010; Kim et al. 2012), the longest lived individuals often do not develop cancer; this possibly aids in their long survival (Kovacic and Somanthan 2014). SIRT1 also influences *PER2* in the master circadian clock which aids in stabilizing circadian rhythms which helps to delay/prevent disease of aging (Asher et al. 2008).

The stability of the genome declines during aging due to a variety of factors; many of these relate to an unhealthy life style (cigarette smoking, poor diet, etc.) and those that predispose to cancer (exposure to volatile carcinogens, ingestion of heavy metals, ionizing and ultraviolet radiation). A more recently defined, previously thought to be innocuous factor that aggravates genomic stability is light-at-night (LAN), i.e., circadian rhythm disturbance and/or melatonin suppression (Belancio et al. 2015). These occur especially during night shift work, late night recreational activities (so called, social jet lag), light exposure at night due to light pollution and intentional light after darkness onset when awakening. Each of these disturbs the function of the master biological clock and suppresses the melatonin synthesis/secretion cycle thereby contributing to circadian dysregulation and genomic instability (Fig. 1.2). Equally as disruptive to the circadian system are rapid changes in time zones, especially when moving in an eastwardly direction; the resulting jet lag has been linked to an increased likelihood of developing cancer (Ptacek et al. 2007), probably due to suppression of melatonin (Reiter et al. 2007; Hill et al. 2015). Genomic instability due to any reason leads to accelerated DNA damage and the consequences thereof; this includes accelerated aging.

Recently, Belancio et al. (2010a) have summarized the data that defines the role of transposable elements in genomic instability. These elements are capable of rearranging the genetic material and support for their involvement in cancer has accumulated over the last decade (Gasior et al. 2006; Belancio et al. 2010b; Lee et al. 2012; Scott et al. 2016). Genomic instability contributes to many different lesions in DNA including single base-pair substitutions or deletions and large genomic rearrangements including deletions, insertions, inversions and translocations (Belancio et al. 2010a). The large genomic changes likely contribute to the aging phenotype (Vijg and Dolle 2002; Hsieh et al. 2013).

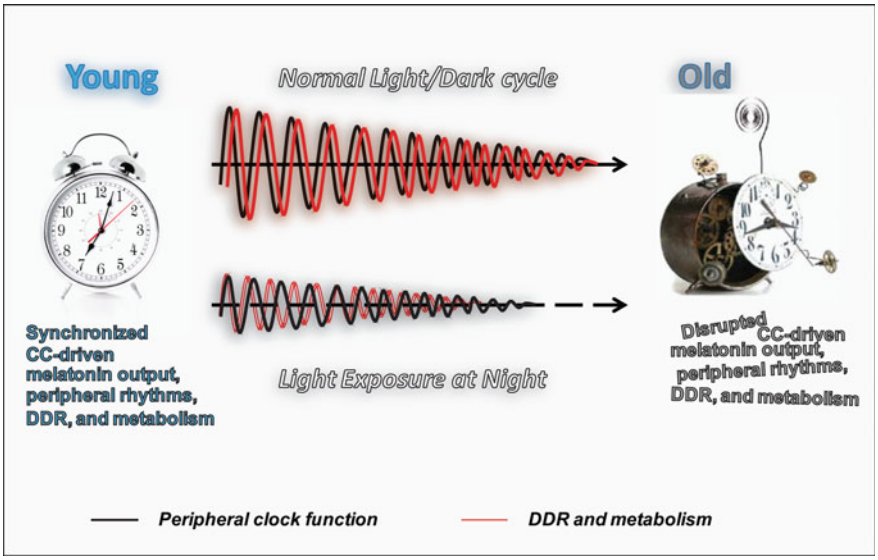

Fig. 1.2 Light exposure at night disrupts the function of the master circadian oscillator, the central clock (CC; also known as the suprachiasmatic nuclei), which results in an associated deterioration in the normally synchronized oscillations in peripheral cells. Under regularly alternating light:dark cycle, these DNA damage response (DDR) are well synchronized. The perturbed rhythms due to light-at-night negatively impacts the melatonin cycle, DDR and metabolism all of which are believed to contribute to aging and/or age-related diseases. From Belancio et al. (2015) with permission

Research has shown there are clear connections between genomic stability transposable elements (retrotransposons), circadian organization, metabolism and aging (Belancio et al. 2010a). Retro elements are mobile genetic entities that consist of two related groups, i.e., long terminal repeat (LTR) and non-LTR retrotransposons; these are represented by long (LINE) and short interspersed elements (SINE) (Belancio et al. 2008). There is now credible evidence showing that L1 activity and the circadian system are inextricably linked (deHaro et al. 2014). Hence, in an in vivo cancer model, the interaction of melatonin with its MT1 membrane receptor suppressed L1 expression; melatonin receptor had a similar effect in cultured cells. Given that melatonin is an important component of the endogenous circadian networks, it is plausible that L1 expression is under the influence of the circadian system.

As noted above, LAN is a major factor capable of disturbing the function of the master circadian oscillator, the SCN (Ikeno and Yan 2016). Since the prevailing light:dark environment drives the circadian network including the intrinsic rhythms of the slave oscillators in perhaps all mammalian cells, when darkness is interrupted by light genomic stability is also impacted as suggested by the findings of deHaro et al. (2014). The manifestations of the genomic damage that likely occurs as a result of interruption of darkness probably accounts, at least in part, for the reported

elevated cancer risk in night shift workers (Lewy et al. 1980; Reiter et al. 2007; Erren and Reiter 2008). Beyond this, LAN also accelerates the growth of already established cancer because of the suppression of melatonin (Blask et al. 2005; Hill et al. 2015). Finally, there are other metabolic consequences resulting from circadian disruption due to LAN; some of these include obesity (Cipolla-Neto et al. 2014; Coomans et al. 2015; Scott 2015) and diabetes (Ingenwerth et al. 2016) and at the cellular level depressed SIRT1 activity (Asher et al. 2008; Jung-Hynes et al. 2010) and clock gene expression (Granados-Fuentes et al. 2015). These perturbations contribute to the functional decline normally known as aging and certainly in part relate to chronodisruption- and melatonin suppression-mediated genomic instability.

1.4 Cellular Aging: The Case of Stem Cell Senescence

The ability of melatonin to forestall senescence of stem cells grown in vitro and subjected to multiple passages was recently documented. Judging from the number of approved clinical trials, it seems likely that MSC-based therapies will continue to be a common treatment paradigm in the field of regenerative medicine (Caplan and Correa 2011). The recovery of stem cells for therapy is often a limiting factor because of their numbers; this is particularly the case with certain stem cells (Jones and Wagers 2008). For example, bone marrow donation remains rather infrequent and even when marrow samples are collected there is a very low ratio of stem cells to the total number of blood forming cells. Hence, in vitro expansion of the relatively small number of stem cells that are recovered is critical to obtain the necessary number for implantation therapy. Repeated in vitro passaging of stem cells, however, introduces the likelihood of a number of disorders which compromise their usefulness, i.e., they lose their stemness and exhibit signs of senescence (Baker et al. 2015).

In an attempt to preserve bone marrow MSC (BMMSC) in a more youthful state during multiple passaging, Shuai et al. (2016) compared four low molecular weight molecules (rapamycin, resveratrol, quercetin and melatonin) in terms of preserving rat and human BMMSC in a more original state. These molecules were selected as candidates because of their previously-reported anti-aging and antioxidant actions and for their ability to enhance stem cell protection.

Shuai et al. (2016) initially showed that BMMSC lost their self-renewal potential and their osteogenic differentiation capacity during growth through 15 passages. When the four molecules (each at 10 nM) were compared for their efficacy in maintaining stemness, melatonin proved to be far superior to the other three molecules (rapamycin, resveratrol and quercetin). For example, melatonin proved highly effective in maintaining the ectopic osteogenic activity of BMMSC during long-term passaging and when the cells were transplanted into nude mice (for 8 weeks). In other models in which melatonin was tested (calvarial defect repair,

bone loss after ovariectomy, reduction of immune competence), it also had a very positive effect on the outcome measured and clearly delayed senescence.

To establish the mechanism by which melatonin functioned in their study, Shuai et al. (2016) explored the possibility that the potent antioxidant functions of the indoleamine (Manchester et al. 2015; Reiter et al. 2016) accounted for its protective actions given that cellular senescence during in vitro passaging of stem cells is known to be related to ROS generation (Busuttil et al. 2003; Parrinello et al. 2003). The incubation of BMMSC with melatonin reduced ROS formation by more than 50%; this drop was associated with a 2-fold elevation in the expression of superoxide dismutase 2 (SOD2). Likewise, the p53 pathway is stimulated by both ROS and as a result of telomere shortening limits stem cell proliferation and renewal (Bonizzi et al. 2002). When Shuai et al. (2016) examined p53 and the downstream regulator, p16, melatonin was found to significantly prevent the rise in p53 which normally would have suppressed stem cell renewal. Finally, clusters of master genes, one of which is designated NONAG, control the multipotency of stem cells by preserving them in an undifferentiated state (Tsai et al. 2012). NONAG expression is normally lost during senescence of BMMSC. Melatonin treatment preserved NONAG expression at a level equivalent to that in just-isolated stem cells. Thus, the collective results show that melatonin preserves stemness of BMMSC by reducing oxidative stress, inhibiting the p53 pathway and maintaining NONAG expression. These beneficial actions seem not to involve MT1/MT2 melatonin membrane receptors (Luchetti et al. 2014) but rather melatonin's direct scavenging actions on ROS (Tan et al. 1993; Reiter et al. 2014a) and its ability to promote antioxidant enzymes (Rodriguez et al. 2004). In the study reported by Shuai et al. (2016), treating BMMSC with luzindole (an MT1/MT2 receptor antagonist) did not interfere with melatonin's ability to safeguard these cells; thus, these receptors are not involved in anti-senescent action of melatonin.

These findings are directly applicable to aging and age-related diseases. Elevated free radical generation is commonly associated with advanced age as metabolic pathways become less efficient (Hocman 1979; Allen 1990). Simultaneously, blood melatonin levels wane as does the antioxidant capacity of this fluid (Benot et al. 1999) (Fig. 1.3). Also, early-life pinealectomy, which deprives animals of their daily melatonin rhythm and contributes to circadian dysregulation exaggerates the amount of oxidatively-damaged molecules these animals accumulate when they are 24 months of age (Reiter et al. 1999). Interestingly, the premier means of delaying aging, i.e., caloric-restriction (Meites 1990; Rae 2004), preserves molecular and cellular function and, likewise, prevents the normal reduction in pineal melatonin synthesis associated with aging (Stokkan et al. 1991). Mechanistically, the preserved melatonin production is accompanied by the retention of β-adrenergic receptors on the pinealocyte membranes; these receptors mediate the sympathetic stimulation of melatonin synthesis (Henden et al. 1992). Whether the conserved melatonin rhythm contributed to the preserved physiology of the caloric-restricted old animals has yet to be proven.

The ability of melatonin to forestall the functional capacity of stem cells is not exclusive to long-term passaged senescing BMMSC. Similarly, aging of the

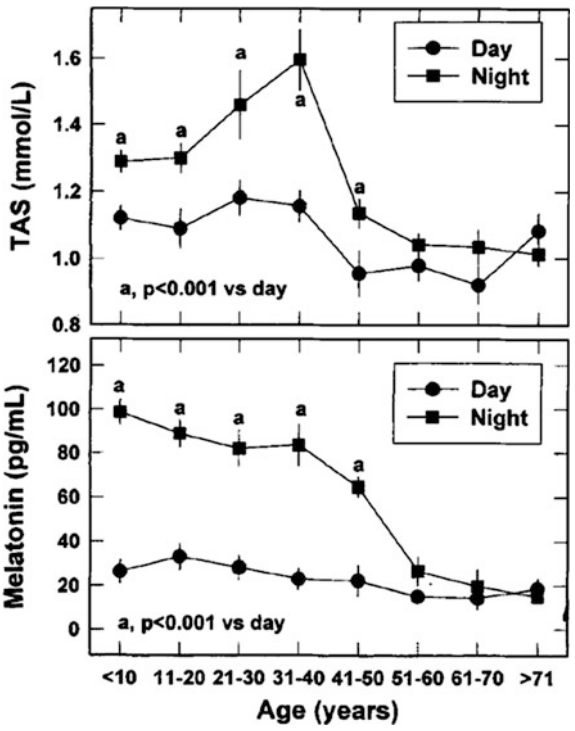

Fig. 1.3 The nocturnal levels of circulating melatonin usually drop as humans age (*bottom panel*). Associated with the nighttime reduction in melatonin is a loss of the total antioxidant status (TAS) (*upper panel*) of the blood. Thus, as humans age the reduction in the level of the antioxidant, melatonin, likely contributes to the accumulation of free radical damage to cells which hastens aging. From Benot et al. (1999) with permission

umbilical cord-derived (Lee et al. 2014) and adipose-derived stem cells (Yip et al. 2013) is also reduced when they are grown in a melatonin-containing medium. By retarding the aging of these cells, melatonin treatment also preserves their growth and physiology when they are used for transplantation therapy (Chen et al. 2014a, b; Yip et al. 2013). Finally, Zhou et al. (2015) reported that melatonin prevented MSC premature senescence when the cells were treated with the oxidizing agent, H_2O_2. This reversal was associated with an upregulation of the SIRT1 pathway, an event known to be associated with anti-aging processes (Favero et al. 2015; Ghosh and Zhou 2015). In addition to prolonging the functional half life of MSC, we had earlier predicted that culturing more differentiated cells in a melatonin-containing medium would allow them to undergo more mitoses than prescribed by the Hayflick limit (Hayflick 1979; Reiter et al. 2016).

Beyond upregulating the expression of the longevity-promoting gene, SIRT1, in MSC by melatonin, little is known about other molecular events that were changed as a consequence of melatonin treatment. Thus, while melatonin's antioxidant functions are usually used as an explanation for the enhanced functional state and prolonged survival of MSC, the circadian biology of these cells under the influence of melatonin is yet to be examined. Under in vivo situations, melatonin presumably provides stem cells with timing information as it does for terminally-differentiated elements.

Collectively, the ability of melatonin to maintain a more optimal physiological state of stem cells and reduce the rate at which they become non-functional and undergo apoptosis and aging, has applications to a number of human conditions beyond aging per se. The more rapid stabilization of dental implants, wound healing and bone fracture repair are examples where melatonin's capability to improve the functions of stem cells may come into play (Cutando et al. 2011; Clafshenkel et al. 2012; Lee et al. 2014). Even more important may be the use of melatonin to prolong the survival of stem cells when they are injected in vivo to restore tissues such as the cardiomyocytes and neurons which have no capability of regeneration. Typically, the vast majority of stem cells die when injected before they have any restorative benefit. Since the pathological conditions that require stem cell treatment are most common in the elderly, the improved function due to the use of melatonin may enhance the quality of life in older individuals. Again, in these conditions the circadian-organizing actions of melatonin should not be overlooked. Perhaps older individuals with stem cell implants should be given melatonin at a specific time of the day (night) to aid in the synchronization of the circadian rhythms of not only stem cells but other non-diseased cells as well. This may be of importance since the endogenous melatonin signal is often severely weakened in older patients because of the diminished melatonin cycle.

In vivo, stem cells are maintained in their optimal functional state by a complex signaling network (Hawkins et al. 2014). Under culture conditions due to a change in their microenvironment, these essential signals are lost; as a result researchers have sought means to defer senescence of these cells when undergoing multiple passages (Coutu et al. 2011; Eom et al. 2014). These procedures, however, have disadvantages (Guitart et al. 2010). Here, we propose that melatonin is one signaling molecule that should be tested for its ability to prolong the undifferentiated state of the cells. Other signaling molecules have already been used for this purpose (Danet et al. 2003; Lin et al. 2012) and have been shown to have only slight efficacy in this regard. Based on the comparative investigation of Shaui et al. (2016), however, melatonin should be seriously considered as an agent to delay aging of stem cells in vitro as well as after their injection. Finally, melatonin should be considered for use to prolong the survival of differentiated cells in vivo which are limited in the number of mitoses they can experience (Hayflick 1979).

1.5 Circadian Disruption and Age-Related Diseases

Epidemiological data have long suggested that circadian dysregulation due to light exposure at night (LAN) (or rapid transmeridian travel) is associated with an increased cancer risk (Hansen 2001; Erren et al. 2010; Rao et al. 2015). These findings also have strong support from experimental laboratory studies. Blask et al. (2005) and Hill et al. (2015) have repeatedly shown that LAN stimulates xenografted human tumors transplanted into immune-compromised rodents grow more aggressively; this exaggerated growth is likewise inhibited by properly-timed

melatonin administration. As noted in this survey, LAN always simultaneously disrupts the circadian melatonin cycle which also perturbs circadian rhythms more generally.

That disruption of the master clock promotes cancer growth was also recently documented in a circadian-based study reported by Papagiannakopoulos et al. (2016). Using a genetically-engineered mouse model that can be induced to develop lung adenocarcinoma, this group reported that circadian disruption due to imposed jet lag or genetic manipulation of central clock components promoted lung cancer growth and reduced the survival of the animals. Loss of the central clock components, *Per2* and *Bmal* were associated with elevated c-Myc expression, enhanced tumor cell proliferation and metabolic disruption. Melatonin levels were not measured in these animals nor was it given as a supplement to determine whether it would reverse the observed effects on exaggerated tumor growth.

The studies summarized above are only a few of the many that document that circadian disintegration and melatonin suppression may be consequential in the increased cancer risk experienced by aging individuals (Erren et al. 2016). The majority of cancers are age-related and both accurate circadian regulation and a well-preserved melatonin rhythm decrease with age. The implication is that disturbances in these two related systems contribute to cancer development and, more generally, to the degenerative changes of aging (Reiter et al. 2007).

In addition to cancer, a number of degenerative nervous system disorders are presumed to be mediated by the gradual loss of a robust melatonin cycle in the elderly; moreover, during aging the intrinsic central circadian clock is weakened and the 24-hour cycles are progressively disrupted. As an example of this, the sleep/wake cycle is often significantly advanced and sleep itself is fragmented in the elderly. The loss of adequate and restful sleep may itself be a contributing factor to aging.

Neural conditions that may be aggravated by the deteriorating melatonin cycle and a more general disturbance of the biological clock include Alzheimer disease (AD) (Pappolla et al. 1997; Hardeland 2012; Rosales-Corral et al. 2012; Ali and Kim 2015; Miller et al. 2015; Zhang et al. 2016), Parkinson disease (PD) (Mayo et al. 2005; Santos, 2012), Huntington disease (HD) (Escribano et al. 2014; van Wamelen et al. 2015), amyotrophic lateral sclerosis (ALS) (Anderson and Rodriguez 2011; Farez et al. 2016), and multiple sclerosis (MS) (Kashani et al. 2014; Wen et al. 2016). These conditions often are associated with altered circadian rhythms; whether these disturbances are a cause or an effect of these states has not been unequivocally determined. The ability of melatonin to defer the progressive development of these conditions in limited clinical trials, however, has been documented (Weishaupt et al. 2006; Medeiros et al. 2007; Cardinali et al. 2012; Lopez-Gonzalez et al. 2015). Moreover, preliminary studies with the use of melatonin to treat AD patients also suggest that this treatment may retard the progression of this devastating condition in humans (Hardeland 2016). While it is usually surmised that the antioxidant actions of melatonin account for its ability to slow the causes of AD, as noted this indole also has the capability of synchronizing the circadian system generally as well as promoting more successful sleep (Erren

et al. 2016) which may aid in forestalling CNS degeneration. Finally, melatonin may also reduce aging in the central nervous system by stimulating hippocampal progenitor cell proliferation which leads to the restoration of lost neurons in the dentate gyrus (Ethuwapranee et al. 2015).

That the rhythmic machinery underlying the sleep/wake cycle is altered becomes apparent in the older population where the timing of sleep onset shifts to an earlier time. Additionally, sleep architecture is changed since there is an increased frequency of awakenings during the nighttime sleep period along with a reduction in the total amount of non-rapid eye movement (REM) sleep (Lo et al. 2016; Mattis and Sehgal 2016). Concurrent with these changes there is often a marked attenuation of the nocturnal peak of circulating melatonin. On the other hand, there are reports of some elderly individuals who do not experience a substantial drop in maximal night melatonin values. To the knowledge of the current authors, there are no reports which examined whether the retained robust melatonin rhythm was associated with sleep architecture of younger individuals with fewer nocturnal awakenings, etc. Also, whether the well preserved melatonin cycle in a small percentage of the aged are beneficial in maintaining sleep quality seems not to have been investigated.

The differential rate of deterioration of the melatonin cycle and sleep efficiency is of special interest in light of data published by Dauchy et al. (2013) using animals. This group reported that the quality of daytime light has a major impact on the amplitude of the nighttime melatonin peak; when rats were exposed to blue wavelength-enriched light during the day, the maximal nighttime melatonin levels were increased up to ten-fold. This remarkably augmented nighttime melatonin peak enhanced the ability of the animals to resist the growth of transplanted cancer cells. Given the other actions of melatonin, the animals would be expected to have a much higher total antioxidant capacity and perhaps greater longevity. The findings also have potential implications for older individuals who may be able to improve nighttime sleep by changing their daytime light exposure to one rich in blue wavelengths.

Cardiovascular physiology also exhibits changes that have been classified as negative during aging. It is common that mean arterial blood pressure (MAP) gradually increases as individuals advance in age (Shen et al. 2015). This hypertensive response may be related to the concurrent drop in the nocturnal circulating melatonin peak and the perturbed circadian system in the elderly. In young individuals, the nighttime rise in melatonin is functionally correlated with a reduction in nocturnal systolic blood pressure (Pechanova et al. 2014). The loss of this drop in systolic pressure at night contributes to a rise in the MAP throughout life. This is consistent with what occurs in the aged. As the amplitude of the melatonin rhythm declines, the MAP rises. Hypertension is an independent risk factor for cardiovascular death and, therefore, it reduces longevity. Melatonin's benefits, besides mediating the nocturnal dip in systolic blood pressure, via its antioxidant actions likely contribute to its protection of the myocardium, as seen during cardiac ischemic/reperfusion injury (Dominguez-Rodriguez et al. 2012; Liu et al. 2015). Additionally, melatonin has been shown to exert cardiovascular

protection due to its capacity to improve the bioavailability of nitric oxide which leads to vasodilation and a drop in blood pressure (Simko et al. 2016). Thus, the loss of a stout nocturnal melatonin rise in the aged may well contribute to the elevation of MAP during late life. Elevated blood pressure invariably compromises longevity if it goes untreated.

In addition to melatonin making nitric oxide more available to provide relief from elevated MAP, its loss during aging also impacts the autonomic nervous system which may contribute to mortality. Melatonin influences sympathetic drive by enhancing GABA-ergic signaling involved in inhibiting the paraventricular nucleus by the SCN (Wang et al. 2003). Additionally, neurons in the area postrema, which are central to blood pressure regulation, are epigenetically influenced by melatonin (Irmak and Sizlan 2006). Finally, intermittent ventricular dyssynchrony has been shown to be treatable with melatonin (Dominguez-Rodriguez et al. 2016). Clearly, the melatonin rhythm has direct effects on blood pressure as well as indirect actions via its ability to modulate circadian rhythms of the autonomic nervous system (Simko et al. 2016).

1.6 Concluding Remarks and Perspectives

Even though no one has ever avoided aging and death and they are unquestionably inevitable, there is still unbounding interest in the possibility of deferring these conditions when means of delaying aging are discussed. The major consideration usually relates to prolonging life. Increasing longevity without maintaining optimal health, i.e., without extending health span, may, however, be counterproductive. On the other hand, prolonging good health into advanced age may concurrently contribute to enhanced longevity. In experimental rodents, the most reproducible means of extending life and limiting pathologies is to rather severely limit caloric intake; but the success of this procedure when applied in primates has been equivocal (Roth et al. 1999; McKiernan et al. 2011; Mattison et al. 2012). Thus, the potential benefits of reducing food intake for the purpose of stretching the life span of human remains obscure. This, however, has not dissuaded scientists from trying to identify molecules that can be consumed and that mimic the cellular molecular actions observed in underfed rodents (Ingram and Roth 2015). The amount of resources dedicated to identifying elixirs that may slow down time is currently massive and even well-established, renowned scientists are self-testing molecules that may defer age-related diseases and enhance longevity.

In the current report, we examined the data that suggests a relationship between circadian biology and the processes of aging. Circadian rhythm disturbances cannot be considered in terms of their effects without taking into account the melatonin cycle. This is a requirement since the central Zeitgeber, i.e., the SCN, and the melatonin rhythm are mutually interactive. Any disturbance of the message sent out from the SCN is always accompanied by a perturbation and often a suppression of the pineal melatonin production and release. Moreover, any alteration in melatonin