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Aging signaling pathways and circadian clock-dependent metabolic derangements

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Abstract

The circadian clock machinery orchestrates organism metabolism in order to ensure that development, survival and reproduction are attuned to diurnal environmental variations. For unknown reasons, there is a decline in circadian rhythms with age, concomitant with declines in the overall metabolic tissues homeostasis and changes in the feeding behavior of aged organisms. This disruption of the relationship between the clock and the nutrient sensing networks might underlie age-related diseases; overall, greater knowledge of the molecular mediators of and variations in clock networks during lifespan may shed light on the aging process and how it may be delayed. In this review we address the complex links between the circadian clock, metabolic (dys)functions and aging in different model organisms.

Keywords

aging; circadian clock; glucose metabolism; insulin; flies; mice; nutrients

Aging signaling pathways and control of nutrient metabolism

Whether aging might be regarded as a disease or as a consequence of development, in molecular terms it can be understood as a decline of the homeostatic mechanisms that ensure function of cells, tissues, organs and organ systems¹⁻⁴. During evolution, a set of interconnected signaling pathways were selected to sense environmental cues and modulate

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these fundamental homeostatic and physiological functions: proliferation/growth, repair/survival and reproduction. Thus, it should be no surprise that environmental conditions that influence longevity often exert their effects through such pathways ¹⁻⁴.

Nutrient sensing pathways in model organisms

In yeast, nutrient signaling allows colonies to synchronize cell division and adapt their metabolism to the surrounding environment. In scarce nutrient conditions, 90-99% of the colony undergoes cell death in an altruistic fashion ⁵, allowing the remaining cells to survive on the restricted nutrients. This contributes to the main goal of a clonal population: to maximize the future replication of its shared genome, in this case by assuring that a small number of individuals will survive the harsh environmental conditions.

In multicellular organisms a set of tissues became specialized in nutrient sensing. For example, in *C. elegans*, nutrient signaling takes place primarily in neurons, the gonad and the intestine. As in higher animals, primary nutrient signaling pathways include insulin/insulin-like growth factor (IIS) and target of rapamycin (TOR), both of which are well-known to modulate lifespan ^{6, 7}. Insulin-like peptides synthesized in response to nutrient sensation are bound by the insulin receptor in neurons. Loss of DAF-2, the only insulin/IGF-1 like receptor in the worm, enhances lifespan through signaling to the intestine and gonad ⁸. Germline loss, which mobilizes intestinal fat stores and increases autophagy, also promotes longevity ^{9, 10}. Finally, reductions in TOR activity enhance lifespan and mimic the effects of caloric restriction ¹¹. Altogether, these data show that modulation of nutrient signaling in development and reproduction also affects aging and lifespan in *C. elegans*.

Growth and reproduction are matched to the environment through long-range signals, primarily insulin, to provide whole-body coordination of energy consumption and storage. As with *C. elegans*, in *Drosophila* and mammal IIS signaling is integrated through AKT/PI3K to the TOR pathway to promote growth and proliferation ^{3, 4, 12, 13} (Figure 1). In *Drosophila*, reduction in the activity of TOR or IIS pathways leads to developmental delays and small-sized flies with reduced fecundity, but increased longevity ^{11, 13}. Similar interventions in adult flies also enhance lifespan and slow the appearance of age related characteristics ^{11, 13}. Furthermore, mice lacking ribosomal S6 protein kinase 1 (S6K1), a downstream component of the TOR pathway, have longer lifespan and exhibit resistance to age-related pathologies ¹⁴⁻¹⁷. Feeding worms, flies or mice with rapamycin, which reduces TOR signaling, also extends lifespan ¹⁶. Reduction of the anabolic hormones growth hormone (GH), IGF-1, or insulin, leads to long-lived mice and resistance to age related pathologies ^{18, 19}. Mutant mice with reduced plasma IGF-1 are smaller in size and show lifespan extension ²⁰. Similarly, mice mutant for insulin receptor substrate 1 (IRS1: a component of the IGF-1 pathway) show decreased insulin sensitivity but are long-lived ²¹ (Figure 1). In humans, increased insulin sensitivity, evidenced by reduction in plasma insulin, IGF-1 and plasma glucose levels, is a hallmark of increased longevity ^{4, 22}. Indeed, individuals with Laron syndrome (see Glossary), who are deficient in GH receptor, show remarkable reduction in pro-aging signaling, cancer, and diabetes ²³.

Dietary input and lifespan

The incidence of insulin resistance, diabetes, metabolic syndrome and cardiovascular diseases increases with age in humans ^{3, 4}. Aging-related modifications in body composition that contribute to insulin resistance and metabolic syndrome are represented by abdominal obesity and sarcopenia ²⁴. Abdominal obesity is observed in the elderly, despite a normal body mass index and visceral fat is associated with changes in proinflammatory cytokines, and interferes with insulin action. Sarcopenia contributes to the loss of skeletal muscle and to insulin resistance through reduction in energy expenditure ²⁴.

Caloric restriction (CR) is the only intervention known to slow down aging across taxa^{25, 26}. While the positive effects of CR on longevity are clear in nematodes, flies, and inbred mouse strains²⁷, results on wild-caught mice²⁸, primates²⁹⁻³¹, and human populations^{25, 26, 32, 33}, suggest that the genetic background, the age of onset of CR, and the composition of the diet may affect the longevity outcomes in mammals. More studies are required to understand the effects of CR in lifespan and healthspan in higher vertebrates, as well as species-specific nutrient requirements throughout lifespan. This will also help to better understand which type of dietary restriction (DR) improves healthspan in each species²⁶. The mechanisms underlying CR effects are poorly understood. Evidence for involvement of insulin signaling was obtained in *dilp* (*Drosophila insulin like peptides*) mutant flies, which mimic the effects of CR³⁴. Flies fed a high fat diet (HFD) show alterations in insulin and glucose homeostasis, as well as other age related pathologies, similar to those observed in mammals. Down-regulation of the insulin-target of rapamycin (TOR) pathway ameliorates these effects³⁵.

Similarly, CR improves insulin sensitivity and age related diseases in mice³⁶ and in rhesus monkeys^{25, 29}. As in flies, mice subjected to a HFD show TOR hyperactivation, which contributes to insulin resistance and obesity, both exacerbated during aging¹⁷. Instead, mice null in ribosomal protein S6 kinase beta-1 (*S6K1*) do not show insulin resistance associated with HFD¹⁷ (Figure 1). Further, preserved glucose tolerance and insulin sensitivity are common features of centenarians when compared to other age groups of elderly subjects^{25, 32}. Evidence in humans suggests that short term fasting protects against various cancers, possibly by downregulating the IIS/nutrient signaling pathway³⁷. Altogether, the evidence suggests that IIS/TOR pathways and dietary inputs cross-talk during the lifespan of an organism. Is this cross-talk, then, related to another major nexus between metabolism and diet, the circadian rhythm?

Circadian clock machinery and nutrient metabolism

Daily rotation of the Earth on its axis and yearly revolution around the Sun impose periodicity on most organisms. Molecular circadian clocks might have evolved to synchronize internal metabolic rhythms to predictable environmental cycles, in order to most advantageously time functions such as feeding, mating, and general patterns of activity³⁸. Moreover, a growing body of evidence suggests reciprocal links between nutrient sensing pathway and circadian clocks³⁹.

Circadian signaling in worms

C. elegans appear to use a different mechanism to generate daily rhythms than flies and mammals⁴⁰⁻⁴². Though most core circadian genes are conserved in *C. elegans*^{40, 43}, these genes generally function in timing the periodic moults in nematode larval development^{41, 44}, and are not expressed with any known periodicity in adulthood⁴². Nevertheless, these animals do have detectable circadian rhythms that can be entrained by light:dark or warm:cool cycles. This rhythmicity is measurable in both larvae and adults, in parameters such as motility^{45, 46}, stress-resistance^{47, 48}, pathogen-resistance⁴⁹, food and oxygen consumption⁵⁰, and gene expression⁴². Further, conserved signaling molecules are involved in the *C. elegans* circadian process. First, pigment dispersing factor (PDF) and its receptor, which in insects play a role in transducing circadian oscillations to downstream processes (a function analogous to that of VIP: Vasoactive Intestinal Peptide in mammals)^{51, 52}, have been identified in *C. elegans*, and shown to be involved in circadian rhythmicity in locomotion⁵³. Second, circadian cycling of the levels of melatonin and its synthase has recently been identified⁵⁴, though not yet functionally characterized. Further downstream, profiling of genes expressed in rhythmic fashion during and after 12-hour light:dark or warm:cool cycles identified GO terms relating to a broad diversity of biological

processes, chief among them metabolic processes, enriched in the genes that can be driven and/or entrained by cycling temperature in particular ⁴². Of note, this study found that light versus temperature cycling entrained distinct sets of genes (unlike the case in *Drosophila*, for example), suggesting that there could be multiple independent clock mechanisms or differential damping of genes entrained by different processes.

Circadian signaling in flies

Unlike in *C. elegans*, circadian clock genes and mechanisms seem to be largely conserved between *Drosophila* and humans. In flies and mammals, circadian clocks consist of transcriptional and translational feedback loops working in a cell autonomous manner (Figure 2). At the core of the *Drosophila* circadian clock there are four clock genes *Clock* (*Clk*), *cycle* (*cyc*, an ortholog of BMAL1 in mammals), *timeless* (*tim*), and *period* (*per*) ⁵⁵. They interact in a negative feedback loop, whereby expression levels of *per* and *tim* are regulated by transcriptional activators encoded by *Clk* and *cyc*. This leads to periodic increase in the levels of PER and TIM proteins, which accumulate in cell nuclei, and repress CLK/ CYC activators, causing suppression of *per* and *tim* transcription ⁵⁵. In addition to *per* and *tim*, CLK/ CYC heterodimers activate genes that participate in secondary clock feedback loops and a substantial number of clock output genes ^{55, 56}.

The central clock in flies consists of ~150 central pacemaker neurons (CPNs) controlling rest/activity rhythms ⁵⁷, similar to the suprachiasmatic nucleus (SCN) in mammals. In addition, peripheral clocks are located in most fly cells, such as sensory neurons, glia, fat, and excretory system; these oscillators are CPN-independent and serve tissue-specific functions ⁵⁸. Some clock output genes are rhythmic in both central and peripheral clock cells, such as the enzymes involved in glutathione biosynthesis ⁵⁹.

Circadian signaling in mammals

As in flies, the mammalian circadian clock is constituted by a coordinator center, the SCN, which integrates cues derived from peripheral clocks located in peripheral tissues (liver, adipose tissue, reproductive organs among others) with external light:dark cycles ³⁸. At a molecular level, the “clock genes” constitute a similar network as that found in *Drosophila*. CLOCK and BMAL1 are transcriptional activators that bind the enhancer sequences of *Per* and *Cry* (*Cryptochrome*) ³⁸. In turn, PER and CRY proteins accumulate in the cytoplasm and translocate to the nucleus to inhibit CLOCK:BMAL1 ³⁸. CLOCK/BMAL1 also activates transcription of the nuclear receptors (NRs) reverse transcript of erythroblastosis gene (REV-ERB) α/β and retinoic acid related (RAR) orphan receptor (ROR) $\alpha/\beta/\delta$ and γ . REV-ERB α and REV-ERB β act as negative regulators of ROR. ROR and REV-ERB proteins compete for ROR regulatory element (RRE) binding sites in the promoter region of *Bmal1* and regulate its transcription ^{38, 60}. SIRT1, a NAD-dependent deacetylase, interacts directly with CLOCK and deacetylates BMAL1 and PER ⁶¹. In addition, the nutrient sensor AMPK directly phosphorylates and destabilizes CRY1 ⁶², and acts upstream of PER. AMPK can in turn regulate SIRT1 activity ⁶³, participating with the core circadian clock machinery in an intricate nutrient sensing signaling loop that is now starting to be unraveled at the whole organism level (Figure 2).

Lessons from modulating circadian signaling pathways

Genetic manipulations in flies leading to increased dTOR signaling, specifically in a subset of the central pacemaker neurons, significantly lengthen the circadian period of locomotor activity, whereas mutations reducing AKT activity shorten the circadian period ⁶⁴. In the fly brain, the modulation of circadian period is mediated by the S6K and activity of glycogen synthase kinase-3 (GSK3), which also affects core clock proteins ⁶⁴. In the mouse SCN, TOR is also regulated by circadian function ⁶⁵. Thus, conserved nutrient sensing pathways

are integrated into circadian clock network, likely to ensure organism-wide temporal homeostasis and survival.

Another process integrated by the circadian clock network in mammals, and probably also in flies ⁶⁶, is glucose homeostasis ³⁸. In mammals, the hormones cortisol and melatonin also convey the rhythmic output of the SCN to peripheral oscillators and modulate glucose homeostasis ⁶⁷⁻⁶⁹. Circadian rhythmicity of cortisol secretion is implicated in nycthemeral changes of insulin sensitivity ⁶⁷. Melatonin modulates glucose-stimulated insulin secretion ^{70, 71}.

The circadian control of glucose homeostasis is demonstrated in mice with depletion of clock genes in the whole organism or in peripheral metabolic organs. *Clock* hypomorphs, *Bmal1*^{-/-} and *Per2*^{-/-} mice show impaired glucose metabolism ⁷²⁻⁷⁴. Furthermore, mouse models of diabetes and obesity, where glucose homeostatic mechanisms are altered, exhibit altered circadian behavior ^{73, 74}. This bidirectional relationship between circadian disruption and metabolic pathologies also appears in humans. Diabetic patients exhibit dampened amplitude of rhythms of glucose tolerance and insulin secretion ⁷⁵; conversely, a higher incidence of metabolic diseases and cardiovascular events is observed in shift workers ^{76, 77}. Forced circadian misalignment (simulating shift work) has been shown to impact on neuroendocrine control of glucose metabolism ⁷⁸. Moreover, genomic variations in clock genes have been linked to obesity and metabolic syndrome in humans ^{79, 80}.

Interestingly, eating habits (timing and amount of food) may contribute to not only body weight gain and insulin sensitivity, but also to the circadian pattern of clock genes expression in the liver as well as the activity of nutrient sensing pathways like TOR, AMPK and SIRT1 ⁸¹⁻⁸³. A HFD with a time restricted feeding schedule, leads to reduced cholesterol levels and delayed onset of insulin resistance and glucose intolerance ^{81, 83}. Time restricted feeding can restore the shifts induced in the oscillations of clock genes as well as the levels of expression of nutrient sensing molecules induced by a HFD ^{81, 83, 84}. Such studies are relevant for human health since re-alignment of the clock through feeding could constitute a non-pharmacological treatment against metabolic diseases.

Aging molecular networks and the circadian clock

For reasons yet unknown, during aging there is a concomitant decline in circadian rhythms and the overall homeostasis of the organism. Because circadian clocks integrate a wide range of physiological functions, better knowledge of the molecular mediators and variations in clock networks during lifespan might lead to a better understanding of the aging process and how it may be delayed.

Circadian rhythms and aging in worms

Genome wide expression analysis in *C. elegans* identified a set of light and temperature driven oscillating transcripts, suggesting that these transcripts might be under circadian control ⁴². Moreover, the GO term “multicellular organismal aging” is enriched in the light-driven and light-entrained transcript sets. Further, the aging-relevant transcription factors pha-4, skn-1, sod-3 and daf-16 oscillate in warm:cool cycles (but not in light:dark cycles); however none can be entrained to continue free-running oscillation in the absence of periodic stimulus. The physiological relevance of these observations remains unclear. However, extended-longevity phenotypes are well known to correlate with stress-resistant in *C. elegans* ⁸⁵; as such, the observation that resistance to osmotic, oxidative, and pathological challenges varies in circadian fashion ⁴⁷ suggests a circadian modulation of pathways relevant for lifespan determination.

Circadian rhythms and aging in flies and mammals

In flies and mammals, including human, robust high-amplitude circadian rhythms are observed in young individuals but lose their strength with age⁸⁶⁻⁸⁸. Daily rhythms in hormone levels, body temperature, sleep/wake cycles, and other physiological and behavioral variables are diminished during aging^{88, 89}. In mammals, disruption of circadian rhythms with age might be associated with a reduced sensitivity of SCN to entrainment signals^{90, 91}. For example, aged mice are less sensitive to light entrainment than young ones and have reduced *Per2* expression^{92, 93}, reduced responsiveness to melatonin⁹⁴ and serotonin in the SCN⁹⁵.

Studies performed in aging mammals reported an either reduced or normal expression of different clock genes, depending on the organs and species examined⁹⁶. More recent evidence, both in mammals and flies, suggest aging is associated with reduced expression of specific clock genes in peripheral clocks but not in the Central Pacemaker Neurons (CPNs) or SCN^{86, 96, 97}. Moreover, aging affects differentially the re-entrainment responses of central and peripheral circadian clocks^{90, 91}. Altogether, these data indicate that synchrony between all the clocks in the body and the CPN/SCN as well as within the central clock might be crucial to prevent aging.

Possible mediators of the aging circadian network

In mammals, disruption of circadian rhythms with age might be associated with a reduced sensitivity of SCN to entrainment signals^{90, 91}. For example, aged mice are less sensitive to light entrainment than young ones and have reduced *Per2* expression^{92, 93}, reduced responsiveness to melatonin⁹⁴ and serotonin in the SCN⁹⁵. *Clock*^{-/-} and *Bmal1*^{-/-} mice, on top of impaired glucose homeostasis, exhibit premature age-associated disorders and reduced lifespan^{72, 87, 98, 99}, as well as increased sensitivity to genotoxic stress¹⁰⁰. Although, downstream effectors remain largely unknown, a likely candidate is the redox system, which oscillates daily and affects BMAL1 activity^{93, 101}. Indeed, *Bmal1*^{-/-} mice suffer from chronic redox stress¹⁰⁰, which could explain the sensitivity to genotoxic stress and short lifespan. A recent study showed that reactive oxygen species (ROS) oscillate in a circadian manner in wild-type mouse brains, though exhibit a stochastic behavior in *Bmal1*^{-/-} brains¹⁰¹. Likewise, in *Drosophila*, ROS levels oscillate in wild-type heads and accumulate to higher levels in aging *per*^{-/-} flies^{102, 103}. Therefore, ROS species are plausible candidates to mediate the effects of decaying clocks on the impairments of brain function and locomotion that are observed in mutant and aged mice^{87, 104}. Melatonin, which has antioxidant neuroprotective effects¹⁰⁵, oscillates daily^{67, 106, 107}, and decreases with age^{69, 95, 107, 108}, is another putative link between the decline of brain function and circadian clock. Nevertheless, there is no conclusive data regarding locomotor impairment, memory loss, mood and/or sleep disorders in mice mutant for clock genes⁸⁷. Furthermore, given the role of clock genes in growth/proliferation and cancer¹⁰⁰, other possible mediators of clock genes are the cell cycle checkpoint genes, which could explain high cancer susceptibility, and hence short lifespan in mice mutant for certain clock genes'.

Age related SCN derangement

In humans and rhesus monkeys, the circadian timing system shows progressive age-related derangements⁸⁸. Overt degeneration in the human SCN occurs later than the functional changes in circadian organization⁸⁷: reduction in SCN volume and neuron number begin at 80 years of age, whereas behavioral and sleep pattern changes are reported in 50 to 60 year-old subjects¹⁰⁹. Considering that SCN oscillators drive metabolic pathways through autonomic nervous system innervation to the pancreas and liver¹¹⁰, the progressive deterioration of neuronal networks in the circadian system may lead to increasing

destabilization of the array of rhythms that underlies the maintenance of organism metabolic homeostasis (Figure 3).

Effects of aging on the circadian clock-dependent changes in peripheral nutrient metabolism

Energy requirements are among the physiological variables the most robustly change both during the day and throughout the life of an organism. An example of crosstalk between the nutrient sensing pathways, clock genes and the environment is observed in feeding behavior and the fluctuations of metabolic hormones, which are altered during physiological aging^{87, 107}, and in metabolic diseases^{67, 68, 110}.

Declining clock genes expression in aging flies may have multiple metabolic consequences

In *Drosophila*, clock proteins are expressed in the fat body, an organ combining the fat and liver functions of mammals. The fat body clock has been linked to circadian rhythm in feeding and flies lacking clocks in these tissues display increased food consumption and higher sensitivity to starvation^{111, 112}. Overall, the input of neuronal clocks in the brain and that of peripheral clocks in metabolic tissues appear coordinated to provide effective energy homeostasis^{111, 112}. More specifically, basic metabolite levels are coordinated in a clock-dependent manner and that clock function is required to maintain lipid homeostasis⁶⁶. However, the important question of how declining expression of clock genes may affect metabolism in aging flies remains to be addressed. This role may be substantial, though: the clock in the *Drosophila* fat body drives rhythmic expression of genes involved in metabolism, detoxification, and hormone regulation¹¹³. Restricted feeding (RF) drives rhythmic gene expression in the fat body but not in the brain clocks. Limiting food to a time of day when consumption is low leads to desynchronization of internal rhythms and to reduced egg production, showing that the circadian clock interacts with metabolic physiology to influence reproductive fitness. Further, clocks are also important for rhythmic expression of xenobiotic-metabolizing enzymes in flies¹¹⁴ and mammals¹¹⁵.

Additionally, in mammals, there are daily circadian oscillations in hormones such as leptin, insulin and glucagon, which affect blood glycemia^{67, 68, 110}. However, if and how these oscillations change throughout lifespan, and which molecular signaling pathways are involved, remains to be addressed. It is possible that decaying clocks affect the rhythmic oscillations of metabolic enzymes, through the action of melatonin and cortisol, leading to age-associated insulin resistance, diabetes, or glucose intolerance.

Diet effects on circadian rhythms

Like the fat bodies in flies, the role of mammalian pancreatic and liver clocks in aging might be substantial. However, these effects may be indirect: the environment (i.e. diet, stress insults) can affect aging through feeding, and the feeding regime can affect circadian rhythms^{116, 117}. In fact, before they become obese, mice fed on a HFD start feeding during the day⁷³. A HFD modifies circadian synchronization to light¹¹⁷. Recent data on a diurnal rodent, *Arvicanthis ansorgei*, indicates that a hypocaloric diet can also affect the circadian system, in particular *Per2* light induced expression in the SCN¹¹⁸.

As in *Drosophila*, mice under RF have a deranged peripheral clock without affecting the SCN pacemaker^{81, 84, 116}. Nevertheless, RF improves the overall health, in particular, metabolic homeostasis^{81, 83, 84}. Depending on the age of administration, intermittent RF increased both mean and maximum lifespan in inbred mice¹¹⁹. It is yet to be determined if this is the case for all types of diets and what the influence of the genetic background may

be. Mice placed under CR live longer and show resistance to age associated diseases ²⁵⁻²⁷. These mice also consume most of their food within a few hours, however their peripheral clocks are well synchronized with the SCN ¹¹⁶. Considering that mice under RF have a deranged peripheral clock and lifespan extension requires further research, it has been speculated that this synchrony observed in mice under CR could be the source of lifespan extension ¹¹⁶. Though synchrony appears important for lifespan extension, the integrity of the SCN, which governs the feeding behavior, and the time of feeding appear important for metabolic homeostasis and healthspan.

Further, α MUPA mice may be a very useful model for elucidating the links between aging, caloric intake and circadian rhythms because they show spontaneous reduction in appetite, live as long as CR mice, and show higher amplitude in circadian rhythms ^{116, 120}.

The cross-talk between circadian clock circuitry and metabolic pathways could represent a fundamental hinge in the pathophysiological mechanisms of the aging process. The negative effects on human health deriving from alterations of the correct timing of the endogenous circadian clock (chronodisruption) are an important cause of diseases (metabolic, degenerative, cancer) leading to premature aging ¹²¹ (Figure 3). Recent studies have aimed to determine individual “body time” by measuring the oscillation of metabolites in individual blood samples ¹²², potentially paving the way to individualized health interventions. Overall, such metabolic oscillators may link circadian rhythmicity with nutrient sensing pathways, and impairment of these links may underlie or aggravate many of the pathologies associated with aging.

Concluding remarks

Model organism data and epidemiological evidence discussed here indicate that disruption of circadian rhythms has detrimental effects on health and can lead to metabolic diseases such diabetes and obesity. At the same time, circadian clocks are tightly coupled to cellular metabolism and respond to light and feeding cycles. A regular lifestyle in terms of exposure to daylight, physical exercise, sleep/wake and feeding patterns has beneficial impact on our health probably because it helps to maintain such synchrony. Thus, it will be important to determine why circadian rhythms diminish during aging, and whether such decline could be reversed to boost temporal tissue homeostasis. Ongoing ¹²³ and future studies that include understanding the impact of alterations in the biological clock system on chromatin remodeling, gene expression, translation, signaling, and function of individual cell types and peripheral tissues will advance our knowledge towards personalized therapeutic approaches. In the meantime progress in circadian research appears to provide scientific validation of the maxim “Early to bed, early to rise, makes a man healthy, wealthy and wise”.

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Glossary

αMUPA mice	these animals carry a transgene encoding urokinase-type plasminogen activator, a member of the plasminogen/plasmin system that functions in fibrinolysis and extracellular proteolysis. These mice spontaneously consume less food when fed <i>ad libitum</i>
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	and live longer compared with wild-type control mice. α MUPA mice are obesity resistant and share many similarities with calorically restricted animals ^{116, 120}
Caloric restriction (CR)	the reduction of caloric intake (typically by 20–40% of ad libitum consumption) while maintaining adequate nutrient intake ²⁶
Dietary restriction (DR)	restriction of one or more components of intake (typically macronutrients) with no reduction in total caloric intake ²⁶
Chronotherapy	use of circadian or other rhythmic cycles in the application of therapy
Glucose intolerance	a pre-diabetic state of hyperglycemia that is associated with insulin resistance and increased risk of cardiovascular pathology
Insulin sensitivity	a physiological condition in which cells respond to the normal actions of the hormone insulin. Insulin-sensitive cells are able to take in glucose, amino acids and fatty acids
Melatonin	a hormone secreted by the pineal gland in the brain that promotes sleep, and helps to regulate circadian rhythms and temporal homeostasis
Metabolic syndrome	a combination of medical disorders that, together, increase the risk of developing cardiovascular disease and diabetes. These include obesity, raised triglycerides, reduced HDL cholesterol, raised blood pressure and raised fasting plasma glucose
Laron syndrome	an autosomal recessive disorder, also known as Growth Hormone Receptor Deficiency, characterized by an insensitivity to growth hormone (GH), caused by a splice site mutation in exon 6 of the growth hormone receptor gene. Phenotypically, this syndrome causes short stature with normal body proportions in childhood but child-like proportions in adults. Individuals have reduced TOR expression and low plasma insulin, which leads to increased insulin sensitivity. These could be the causes of the observed resistance to diabetes and cancer in this individuals ²³
Restricted feeding (RF)	animals are fed <i>ad libitum</i> but food is provided for only a short time (e.g. 2 h) during each circadian cycle. RF can change phase of clock oscillations in liver but not in the SCN leading to desynchronization
Sarcopenia	age related degenerative loss of muscle mass and strength
Serotonin	a monoamine neurotransmitter that is biochemically derived from tryptophan

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Outstanding Questions BOX

- What are the main factors that determine individual body time? Can genetic and environmental factors be quantified? Can restricted feeding improve temporal homeostasis?
- Is there a differential role for TOR in the SCN or in peripheral clocks?
- How is the clock system altered in mutants for IIS signaling? How does the IIS pathway influence metabolite oscillations and body time?
- How does declining expression of clock genes affect metabolism in aging organism?
- What is the role of chromatin remodeling factors in determining body time?

nutrients in the environment / dietary input

Figure 1. Nutrient sensing pathways crosstalk during lifespan

Several distinct pathways sense dietary input from nutrients in the environment. Amino acids are sensed by the complex TSC1/TSC2 of the TOR pathway. This signal is relayed by a series of mediators, including TOR kinase to the downstream effectors S6K1 and 4E-BP. S6K and 4E-BP enhance translation, and hence, anabolism. Glucose is transported into the cell and converted from ADP and AMP to ATP, which ratios are sensed by the AMPK pathway. AMPK interacts with TSC1/TSC2 to ensure cell homeostasis. Circulating insulin and IGF (IIS) are sensed by the Insulin Receptor (IR) and transmitted to the Insulin Receptor Substrate (IRS, *chico* in flies). The activated IRS relays the signal to PI3K, which activates AKT, which in turn leads to transcriptional activation of target genes. AKT links the IIS pathway to the glucose sensors and TOR signaling and promotes anabolic processes. Reciprocally, signals from the TOR pathway are relayed via S6K to the IRS in the IIS pathway. As an organism's energy requirements vary throughout lifespan, these signaling

pathways operate to maintain energy homeostasis during periods of high demand, such as development and reproduction. Decay in the relays of signals and/or crosstalk leads to aging and age related diseases like insulin resistance, glucose intolerance, diabetes, obesity, metabolic syndrome and cancer.

Figure 2. The circadian clock network is conserved in flies and mammals

Nematodes appear to use non-conserved mechanism to generate circadian rhythms. Light inputs generate oscillations on gene expression and locomotion, among other variables, and PDF and melatonin might be mediators of environmental cues. In higher species, the circadian clock is constituted by the CPN in flies and the SCN in mammals. In *Drosophila* there are four clock genes, which interact in a negative feedback loop: *Clock* (*Clk*), *cycle* (*cyc*, an ortholog of BMAL1 in mammals), *timeless* (*tim*), and *period* (*per*). CLK and CYC activate and regulate expression levels of *per* and *tim*. This leads to periodic increase in the levels of PER and TIM proteins, which accumulate in cell nuclei, and repress CLK/ CYC activators, causing suppression of *per* and *tim* transcription. PDF is expressed in a subset of CPNs and affects circadian period. Increased nutrient sensing via dTOR signaling lengthens the circadian period through S6K and GSK3, which also affects TIM. In mammals, CLOCK and BMAL1 are transcriptional activators that bind the enhancer sequences of *mPer* (*Per 1*, *Per 2* and *Per 3*) and *mCry* (*Cryptochrome*; *Cry1* and *Cry2*). PER and CRY proteins accumulate in the cytoplasm and translocate to the nucleus to inhibit CLOCK:BMAL1. The CLOCK/BMAL1 complex also activates transcription of the nuclear receptors REV-ERB α/β and ROR $\alpha/\beta/\delta$ and γ . REV-ERB acts as negative regulators of ROR. ROR and REV-ERB proteins compete for binding sites in the promoter region of *Bmal1* and regulate its transcription. SIRT1 interacts with CLOCK and deacetylates BMAL1 and PER. AMPK modulated levels of CRY1 and PER and regulates SIRT1. mTOR is also regulated in a circadian manner in the mouse SCN. VIP is expressed in a subset of neurons of SCN and

affects the circadian period. These interactions ensure temporal homeostasis during development and reproduction.

Figure 3. The circadian clock network and nutrient sensing pathways cross-talk during lifespan
 In mammals, the circadian coordinator center, the suprachiasmatic cell nuclei (SCN), integrates light/dark cycles and nutrient cues from the environment. The SCN then relays signals to peripheral clocks. Nutrient sensing pathways in the SCN cross-talk with peripheral clocks, in a yet undefined manner. The crosstalk between nutrient sensing pathways and clock network leads to the oscillation of metabolites, ROS and hormones. These oscillations constitute individual “body time” and lead to the overall physiological homeostasis of the organism during development and reproduction. Desynchronization, disruption or decay of the clock network is associated with aging and age-associated diseases.