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Circadian regulation of metabolism and healthspan in Drosophila

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Abstract

Circadian clocks generate daily rhythms in gene expression, cellular functions, physiological processes and behavior. The core clock mechanism consists of transcriptional-translational negative feedback loops that turn over with an endogenous circa 24 h period. Classical genetic experiments in the fly *Drosophila melanogaster* played an essential role in identification of clock genes that turned out to be largely conserved between flies and mammals. Like in mammals, circadian clocks in flies generate transcriptional rhythms in a variety of metabolic pathways related to feeding and detoxification. Given that rhythms pervade metabolism and the loss of metabolic homeostasis is involved in aging and disease, there is increasing interest in understanding how the clocks and the rhythms they control change during aging. The importance of circadian clocks for healthy aging is supported by studies reporting that genetic or environmental clock disruptions are associated with reduced healthspan and lifespan. For example, arrhythmia caused by mutations in core clock genes lead to symptoms of accelerated aging in both flies and mammals, including neurodegenerative phenotypes. Despite the wealth of descriptive data, the mechanisms by which functional clocks confer healthspan and lifespan benefits are poorly understood. Studies in *Drosophila* discussed here are beginning to unravel causative relationships between the circadian system and aging. In particular, recent data suggest that clocks may be involved in inducing rhythmic expression of specific genes late in life in response to age-related increase in oxidative stress. This review will summarize insights into links between circadian system and aging in *Drosophila*, which were obtained using powerful genetics tools available for this model organism and taking advantage of the short adult lifespan in flies that is measured in days rather than years.

Graphical abstract

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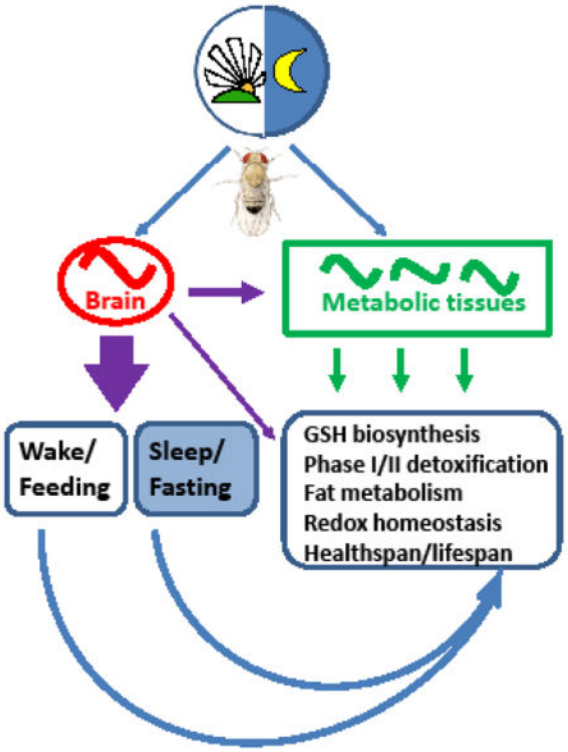

Keywords

longevity; metabolic rhythms; circadian clocks

1. Clock mechanism and the organization of the circadian systems in *Drosophila*

Circadian clocks are cell-autonomous molecular feedback loops that impose daily rhythms on gene transcription, protein activity, metabolic functions, physiological processes and behavior. Their functions have been mostly studied in young organisms, but there is increasing interest in the analysis of clock mechanism across lifespan [1]. Early experiments determined that daily behavioral rhythms are not simply a response to day/night cycles but can persist in constant darkness with a “circa” 24 hour period, suggesting their endogenous nature [2]. The genetic basis of circadian rhythms was convincingly demonstrated in *Drosophila melanogaster* by the discovery of the gene named *period* (*per*) [3]. A null allele of this “clock” gene resulted in loss of rhythmic behaviors, while missense alleles resulted in flies with short (~19h) or long (~29h) free-running periods of two behavioral rhythms [3]. Subsequent studies in *Drosophila* resulted in the cloning of the *per* gene and the discovery of several other conserved core clock genes that form transcriptional-translational negative feedback loops that turn over every ~24hr [4]. Molecular principles of clock organization are well conserved between flies and mammals including humans [5] and, remarkably, the three scientists instrumental in deciphering fly clock mechanism (Drs. Jeff Hall, Michael Rosbash,

and Michael Young) were awarded the 2017 Nobel Prize in Physiology or Medicine. This award consolidates the value of *Drosophila* as an excellent model in biomedical studies.

The simplified version of the core feedback loop in *Drosophila* is shown in Fig. 1. Two transcription factors encoded by the genes *Clock* (*Clk*) and *cycle* (*cyc*) act as the positive limb of the clock whereby CLK-CYC form heterodimers and bind to the E-box sequences in the promoters of *period* (*per*) and *timeless* (*tim*) genes, stimulating their transcription in the early night. PER and TIM proteins act as a negative limb of the clock when they accumulate in the cell nuclei late at night and repress CLK-CYC activity. This results in the suppression of *per* and *tim* transcription until the repressive PER and TIM are degraded and the positive clock limb can restart [4]. Clock oscillations are additionally enhanced via posttranslational modifications of clock proteins, especially via sequential phosphorylation [4]. While clock oscillations persist in experimental conditions of constant darkness (DD), they are normally entrained to daily light/dark (LD) cycles in flies via light-sensitive CRY protein encoded by the *cryptochrome* (*cry*) gene. Upon activation by light, CRY binds to TIM leading to its degradation. Because TIM stabilizes PER, the latter is also degraded within few hours of lights-on. However, TIM (and PER) are also degraded in DD by a different mechanism [6]. While most core clock genes are highly conserved between *Drosophila* and mammals [5], flies have a single copy of each clock gene, whereas mammals often have two or more paralogs that are partially redundant, so that multiple genes have to be knocked down to make an animal arrhythmic [7].

Based on early observations of behavioral rhythms in sleep/activity, feeding and cognitive functions, it was assumed that the clock would reside in specialized neurons. Indeed, the circadian clocks with anatomical and functional similarities have been identified in the brains of mammals and insects using perturbation of locomotor activity rhythms as a readout of clock function [5]. These master clocks are composed of multiple neurons, which are organized in populations with different morphology sub-serving different functions [8]. The mammalian central clock consist of thousands of neurons located in the suprachiasmatic nucleus (SCN) while the fly central clock consists of the network of less than 200 pacemaker neurons controlling different aspects of sleep-activity rhythms [9]. It is now well established that animals possess multi-oscillatory circadian systems with master clocks residing in the central nervous system and peripheral clocks in cells forming most other tissues. The existence of peripheral clocks was first demonstrated in insects [10, 11] and later in mammals [12]. In flies, *bona fide* clock mechanisms reside in a multitude of peripheral cells such as retinal photoreceptors, olfactory and gustatory neurons, glial cells, fat bodies, as well as gut and excretory epithelia [13–19]. These clocks are called peripheral because they do not contribute directly to the behavioral sleep/activity rhythms, which persist in flies displaying clock function exclusively in central pacemaker neurons [20, 21].

In contrast to mammals [22], peripheral clocks in flies are directly entrained by LD cycles whereby the light-activated CRY protein interacts with TIM protein leading to its degradation [23]. There is ample evidence that peripheral clocks can function independently of the central clock in flies [24, 25]; however, emerging data (discussed below) suggest that, similar to mammals, the outputs of the fly central clock can regulate rhythmic transcription of specific genes in peripheral tissues.

2. Metabolic rhythms related to feeding

One of the major functions of mammalian circadian systems is to coordinate periods of food intake with digestion and nutrient processing to maintain metabolic homeostasis [26]. Clock-dependent feeding rhythms have been also reported in *Drosophila* [16, 27]. It was then established that clocks in the fly fat body cells (which serve as both fat tissue and liver in insects) drive rhythmic expression of many genes involved in metabolism; however, some of these genes remain rhythmic when the fat body clock is genetically disrupted [28]. More recent experiments revealed that the rhythmic transcription of those genes depends upon clocks in neurons expressing neuropeptide F (NPF) [29]. Interestingly, the mammalian ortholog of NPF called neuropeptide Y (NPY), functions similarly to regulate cycling of specific metabolic genes in the mouse liver [29]. Another study demonstrated rhythmic control of metabolism via insulin-producing cells in the fly brain that are functionally connected to the central clock neurons; this endocrine axis appears necessary for rhythmic expression of a lipase transcript in the fat body [30]. Taken together, these studies uncover circuit level coordination and systemic signaling between central and peripheral clocks that may synchronize food intake and nutrient utilization. In fact, the coordination between functional circadian clocks in the brain and peripheral tissues was shown to optimize metabolic homeostasis as measured by reproductive fitness [28]. Moreover, the metabolic state of the fly can influence the central clock to affect locomotor and foraging rhythms [31]. In summary, these studies show that systemic signals from the brain are required for transcriptional rhythms not only in the mammalian liver but also in the *Drosophila* fat body. Thus, another facet of circadian regulation is evolutionary conserved and genetic manipulations in flies may help to understand reciprocal relationships between the circadian and metabolic system.

3. Metabolic rhythms related to redox maintenance and detoxification

Several lines of evidence suggest that circadian clocks regulate processes that protect organisms from oxidative stress. First, genome-wide analyses of the circadian transcriptome in fly heads by microarray [32–35] or RNA-seq [36, 37] uncovered that the expression of many genes involved in defense from reactive oxygen species (ROS) occur in a circadian manner. Remarkably, genes belonging to the GO term “Glutathione metabolism” were significantly enriched among rhythmic transcripts identified by RNA-seq in fly heads [37]. These transcriptional oscillations are consistent with previous functional studies showing that the levels of ROS and oxidatively damaged (carbonylated) proteins fluctuate in a daily rhythm in heads of wild type flies, whereas these parameters are non-rhythmic and significantly higher in flies with a null mutation in the *per* clock gene [38]. Accordingly, mortality after a constant dose of hydrogen peroxide varied with time of its application in wild type flies but was constitutively higher in flies with disrupted clock [38]. Apparently, the circadian system affects redox pathways and regulate fly survival in concert with the presence or absence of detoxifying enzymes.

Consistent with the fluctuations in oxidative stress response, the circadian clock was shown to modulate pathways involved in the synthesis of glutathione (GSH), which plays a central role in antioxidant defenses that minimize the accumulation of oxidative damage [39, 40].

We demonstrated that circadian clocks in the fly heads regulate concentrations of GSH as well as the level of its precursor, gamma-glutamylcysteine [41]. In addition, significant rhythms were observed in mRNA levels of genes encoding the glutamate-cysteine ligase (GCL) holoenzyme. This is a rate limiting enzyme in GSH synthesis comprising the catalytic (*Gclc*) and modulatory (*Gclm*) subunits, both showing rhythmic transcription. These rhythms were abolished in flies with mutations in core clock genes, thus linking glutathione production and utilization to the circadian system [41]. Subsequent studies showed that rhythmic expression of *Gclc* mRNA occurs independently of the central pacemaker neurons, because it persisted in heads of behaviorally arrhythmic flies with a disabled central clock but intact peripheral clocks [21]. Among cells harboring peripheral clocks, the glial cells appear to generate rhythms in *Gclc* mRNA levels as genetic disruption of clocks only in glia abolished *Gclc* mRNA oscillations [21]. Taken together, these studies suggest the involvement of circadian clocks in redox regulation in flies, adding to the ample evidence from mammalian studies that these systems are tightly interlinked [42]. Additional support for clock-redox links can be gleaned from a study that reported rhythmic peroxiredoxin oxidation patterns in heads of wild-type flies, which persisted in clock mutants albeit with altered circadian phase relative to wild type flies [43]. Whether these rhythms are involved in regulating cellular ROS has not been determined in flies.

GSH is an integral part of organismal detoxification system, which is controlled by circadian clock at many levels. In phase I of detoxification pathway, enzymes such as cytochrome P450 oxidases introduce reactive or polar groups into xenobiotics. These modified compounds are then conjugated to polar compounds in phase II reactions performed by enzymes such as glutathione S-transferases that catalyze the conjugation of the reduced GSH to xenobiotic substrates. Genome-wide circadian transcription studies determined that many genes encoding xenobiotic metabolizing enzymes are rhythmically expressed in *Drosophila* [44]. Similarly in mammals, both basal and inducible xenobiotic detoxification in the liver is regulated in a circadian fashion via set of clock-controlled transcription factors [45]. Insects including *Drosophila* encounter a variety of xenobiotics such as potentially toxic food components produced by plants or introduced by humans as pesticides. Since feeding in flies is rhythmic (see previous section) it may be expected that susceptibility to toxins is clock-controlled. Indeed, flies display daily variation in mortality after exposure to the same dose of specific toxic compound at different time of day [46]. Daily rhythms in xenobiotic response were associated with diurnal fluctuations in the activity of several phase-I xenobiotic metabolizing enzymes [46]. Further genetic experiments established that the positive clock transcription factors CLK and CYC are necessary for rhythmic expression of several Cytochrome P450 enzymes, and that survival of flies with a null mutation in the *cyc* gene is decreased after toxin exposure compared to wild-type flies [47]. Thus, the functional circadian clock contributes to survival of flies challenged with chemical compounds by helping the organism to be most resistant at the time that toxins are most likely to be encountered as a consequence of the feeding rhythm [28]. Due to conservation of the clock mechanism and detoxification system, flies could serve as an effective model for chronotoxicological and chronopharmacological studies, a very active field due to well-known circadian responses to many therapeutic compounds including anti-cancer drugs [48–50].

4. Links between circadian rhythms and aging

4.1 Functional clocks support healthy aging in flies

As discussed above, many metabolic pathways are controlled by circadian clocks; therefore young flies with disrupted clock mechanisms show higher susceptibility to oxidative stress and toxic compounds [38, 46]. These results suggest that the disruption of the clock function may affect aging organisms even more adversely. Indeed, several studies demonstrated that longevity is compromised in flies with loss of functions mutations in core clock genes (for review see [51]). Disruption of circadian clock also affect healthspan, which can be defined broadly as the time of robust organismal function before the onset of age-associated decline. For assessment of healthspan in clock-deficient flies, middle-aged per-null (per^{01}) mutants or control flies were exposed to 24-h of mild oxidative stress (100% oxygen) and their survival was monitored. While none of the flies died during the 24-h of hyperoxia, subsequent mortality rates increased significantly in per^{01} mutants compared to age-matched wild-type flies exposed to this stress [52]. This suggests that flies with disrupted clocks have a reduced ability to survive homeostatic challenge during aging. Other experiments confirmed that per^{01} mutants are physiologically older when tested at the same chronological age as control flies with functional clocks. For example, the aging per^{01} mutants show significantly higher accumulation of oxidatively damaged proteins and lipids along with accelerated loss of vertical climbing ability [52]. Poor climbing ability in per^{01} flies was associated with increased neurodegeneration [52], suggesting that clocks may have neuroprotective functions during aging.

Neuroprotective roles of clocks were further supported by testing effects of disrupted clocks on two neurodegeneration-prone mutants. One of the genes, *sniffer* (*sni*) encodes carbonyl reductase involved in protection against oxidative stress-induced neurodegeneration and apoptosis [53] The other gene, *swiss cheese* (*sws*) encodes a transmembrane protein that hydrolyzes phosphatidylcholine and supports health of neurons and glia [54, 55]. Aging parameters were tested in flies carrying either *sni* or *sws* mutation in *per*-positive and per^{01} backgrounds. Importantly, double mutants combining both *sni* and *sws* mutation with per^{01} exhibited shortened lifespan and more severe neurodegeneration at a younger age compared to either *sni* or *sws* single mutants with normal clock function [56]. Together, these results provide substantial evidence for detrimental pro-aging effects of circadian disruption and suggest that a functional circadian system plays neuroprotective roles during aging, presumably by coordinating temporal homeostasis in the aging brain. Similarly to flies, accumulating evidence in mammals suggests that circadian disruption contributes to accelerated aging [57, 58] and neurodegenerative pathologies [59–61], but understanding of the mechanisms involved will require further studies to identify critical clock-controlled genes involved in maintaining neuronal homeostasis.

4. 2. The circadian system is involved in anti-aging interventions

As discussed above, disruptions of the circadian system is associated with accelerated aging; however, molecular bases of these links are poorly understood. Emerging evidence suggest that functional clocks may be involved in known life-extending pathways, such as dietary restriction (DR). A recent study addressing this question showed that functional clocks are

necessary for the lifespan responses to low protein DR in *Drosophila* since knockout of the core clock genes *tim* or *per* abolished lifespan extension by DR [62]. The lifespan extension in flies appears to be mediated through enhanced fat turnover, whereby the clock gene *tim* is necessary for cycling of medium chain triglycerides under DR [62]. Importantly, DR increased the amplitude of cycling in most circadian clock genes in heads and bodies of wild type flies [62]. Similar results were obtained in mice where the amplitudes of expression of several clock genes were significantly induced by calorie restriction in the liver [63] and also in the SCN [64]. Taken together, these studies indicate that the clock mechanism becomes more robust under low nutrients, and DR regulates the expression of core clock gene in different organisms and in different tissues. This opens an important question whether peripheral clocks are affected by the metabolic changes associated with low nutrients directly or via strengthening of central clock. Secondly, identification of putative metabolites that are targeted by enhanced circadian system could be of high clinical importance.

Another approach to DR, namely time-restricted feeding (TRF) was also shown to improve the amplitude of oscillation of circadian clock components and clock controlled genes in mouse liver and to provide overall health benefits [65]. Remarkably, TRF also had anti-aging effects on neural, peripheral, and cardiovascular physiology in *Drosophila*. In this study, flies (which are diurnal) had access to food only during 12 h of light in the 12:12 LD cycle or had constant ad libitum (AL) access to food [66]. Improved sleep patterns, prevention of body weight gain, and deceleration of cardiac aging were observed under TRF relative to age-matched AL flies, despite that total caloric intake and activity levels were similar in both groups of flies. Importantly, these effects were dependent on a functional clock, as the imposition of TRF was insufficient for protecting against cardiac aging in flies with disrupted clock function [66]. Altogether, these studies suggest that a cross-talk between DR or TRF and functional circadian clocks may delay aging; however, understanding of the nature of reciprocal links between dietary interventions and clock mechanisms will require further studies.

Involvement of the clock in healthy aging in *Drosophila* is also supported by two studies showing that the overexpression of specific clock genes could slow down deterioration of rest/activity rhythms that occurs in old flies [67]. The first study focused on the pigment dispersing factor (PDF) peptide, which is necessary for maintaining robust central clock network [68]. It was shown that overexpression of PDF specifically in the PDF-positive neurons partially rescued behavioral rhythms in old flies and shortened their free-running periods, causing apparent rejuvenation of these rhythms [69]. The second study tested effects of the overexpression of the *cry* gene encoding a blue light sensitive protein CRY, which is also required in peripheral clocks for their free-running oscillations in constant darkness [70, 71]. Overexpression of CRY in all clock cells strengthened rest/activity rhythms in constant darkness late in life [72]. Importantly, flies with elevated CRY levels also showed better climbing ability and decreased oxidative damage pointing to their extended healthspan [72]. Interestingly, overexpression of cry in PDF-positive central clock neurons alone was not sufficient to restore rest/activity rhythms suggesting that peripheral clocks also play an active role in delaying behavioral and physiological aging [72]. Consistent with these findings, in the DR study discussed above, overexpression of *tim* in peripheral tissues, especially fat body extended lifespan under ad libitum feeding [62].

5. Changes in the expression of clock genes across lifespan

As mentioned above, significant weakening of sleep/activity rhythms was reported in aging *Drosophila* with more frequent and shorter sleep bouts and declining strength of overall rest/activity rhythms [67]. These observations opened the question of whether the dampening of rest/activity rhythms is caused by age-related changes in the expression of core clock genes or rhythm impairments downstream of the clock. To address this, two studies compared diurnal rhythms of clock gene expression in heads of young and old flies [73, 74]. Measuring mRNA levels by quantitative RT-PCR, it was determined that all clock genes retained rhythmic expression, albeit several showed reduced oscillatory amplitude in heads of old flies compared to young [73, 74]. Cell-type specific analysis of the PER protein revealed that PER maintained relatively strong rhythms in central pacemaker neurons of old flies [69, 73], while its levels were significantly reduced in retinal photoreceptor of the compound eyes in old flies compared to young [73, 74]. Photoreceptors form the bulk of clock cells in the head and significant age-dependent decreases in the levels of PER protein were also detected by Western blotting [73, 74].

Recent comparison of clock gene expression in heads of young and old flies around the clock by direct RNA-sequencing (RNA-seq) technology rather than qPCR did not detect age-related reduction in the amplitude of most clock genes; in fact, the oscillations of *per* mRNA were enhanced with aging [75]. On the other hand, this study confirmed a significant decrease in the PER protein levels in whole heads of old flies [75]. Since PER repression of CLK-CYC activity reduces *per* transcription via negative feedback loop (Fig. 1), diminished repression due to PER deficiency could result in the age-related increase of *per* mRNA. In summary, the core circadian mechanism known in young flies is altered during aging mainly by weakening of the repressive arm of the clock feedback loop. Nevertheless, the clocks remain functional throughout fly lifespan and, interestingly, studies in mammals lead to similar conclusions, at least in the entrained conditions [76].

While patterns of clock gene expression do not change dramatically in flies entrained by LD cycles across lifespan, aging appears to weaken cross-talk between cellular oscillators in constant conditions. Significant dampening of clock gene oscillations was observed in constant darkness (DD) both in flies [73] and mice [76]. The latter study found that the amplitude of PER2::LUC rhythms in single cells differed only slightly between SCN explants from young and aged animals under LD conditions, while under DD conditions, the PER2::LUC rhythms of aged animals showed markedly lower amplitudes because the rhythms of individual cells became desynchronized very rapidly. Aging in flies is associated with reduced expression of the PDF peptide, which is necessary for maintaining robust central clock network [69]. As discussed above, overexpression of PDF specifically in the PDF-positive neurons improved behavioral rhythms in old flies [69]. Altogether, these data suggest that aging degrades circadian network properties resulting in decreased pacemaker robustness that could contribute to age-related sleep and circadian disturbances.

6. Age related changes in the expression of clock-controlled genes

An important mechanism for producing circadian output that is used by all known molecular clock circuits is to generate rhythms in the expression of clock-controlled genes (CCGs). Oscillating clock components impose rhythmic expression on a diverse set of target CCGs in young flies [36, 37, 77]. The CCGs encode transcription factors, metabolic enzymes, and regulators of neuronal processes and cellular redox [28, 44, 46]. How does aging affect expression of CCGs? To address this question for a single gene, we conducted a functional study of GSH biosynthesis in old flies, which revealed that aging is associated with abolished daily oscillations and increased constitutive expression of *Gclc* mRNA, leading to the significant increase in the catalytic activity of the GCL holoenzyme [78]. This may be due to the "overriding" of the clock by oxidative stress-induced pathways, which are known to stimulate *Gclc* transcription in a clock-independent fashion in mammals [79].

To elucidate changes in CCGs at the genome-wide level, we recently measured diurnal gene expression profiles in heads of young and old female flies by RNA-seq [75]. Data analysis revealed that several hundred CCGs maintained robust rhythmicity from young to old flies while other genes changed expression in a variety of ways (Fig 2). For example, a very strong rhythm in NAD-dependent oxidoreductase (photoreceptor dehydrogenase, *Pdh*) was detected in heads of young and old flies with the peak occurring at the same phase (Fig. 2A). In contrast, a group of few genes showed significant rhythm in young and old flies but with large shift in the expression phase; among them was Phosphoenolpyruvate carboxykinase (*Pepck*) (Fig. 2B), an enzyme essential for glucose and glycerol homeostasis [80]. However, many other CCGs showed dampened or abolished oscillations in old flies. Some genes become constitutively low, including those encoding important signaling molecules, for example Insulin-like peptide 2 (*Ilp2*, Fig. 2C). Other genes become constitutively high, for example *Gclc* (Fig 2D) in agreement with our published data [78].

The most surprising outcome of the genome wide comparative analysis of CCGs was the identification of the gene subset that showed significantly larger expression amplitudes or even de novo rhythmicity in old flies [75]. Stress-response genes were enriched among transcripts with age-induced rhythmicity, and subsequent experiments demonstrated that exogenous oxidative stress can induce rhythmic expression of these genes in young flies [75]. These data suggest that late life activation of stress responsive genes may be an organismal response to endogenous oxidative stress that tends to accumulate during aging [52]. In summary, this study revealed dynamic reprogramming of the subset of circadian transcriptome including unexpected outcomes, such as age-related gain of rhythmicity in the expression of several genes. Remarkably, recent comparison of young and old mice revealed extensive reprogramming of the circadian transcriptome in a tissue-specific manner with genes gaining or losing rhythmicity in age-related fashion [81, 82]. Together, these new findings demonstrate that the degradation of the physiological and behavioral rhythms in aging flies and mammals is not caused by the general dampening of the clock controlled genes or global arrhythmia. While some genes become less rhythmic, physiological aging is also associated with de novo or increased rhythmicity in other genes. Further studies are needed to determine whether late life transcriptional rhythmicity of these genes is regulated by the circadian clock and what is the functional significance of these oscillations. The fact

that circadian genomic signatures of aging in the liver are reverted by caloric restriction [81] suggests caution in interpreting age-related transcriptional changes as generally beneficial.

7. Conclusions and future directions

Studies on the reciprocal links between circadian clocks and aging are in an exciting exploratory phase with many questions awaiting further research. It is becoming clear that the central clock mechanism is functional during aging, yet the rest/activity rhythms become impaired by normal or pathological aging despite strong oscillations of clock genes in the master clock neurons [73, 83, 84]. It is possible that peripheral clocks in the fly head, while dispensable for strong rest/activity rhythms in young flies may be important in old flies via their role in maintaining temporal homeostasis in the nervous system and metabolic functions. To address these questions, future research needs to focus on age-related changes in clock-controlled genes and processes in specific tissues, as well as their functional significance in the regulation of healthy aging.

This review highlights *Drosophila* as a model for studying metabolic rhythms and age-associated changes in the clock mechanism and clock-controlled processes. Features that make flies a great model for such studies include their short lifespan (in the range of 50–80 days), the vast array of genetic tools, and evolutionary conservation of molecular pathways involved in both the circadian system and aging processes between flies and mammals. It is our hope that studies on flies can provide a better understanding of the important outstanding issues in the field, for example: What are the consequences of age-related reprogramming of the circadian transcriptome for heathy aging? How this reprogramming is regulated? Non-clock transcription factors are likely to be involved and it would be important to identify them. Recent studies suggest that circadian clocks are “called upon” to mediate various anti-aging interventions, such as DR. Furter studies in flies should help to define circadian conditions and interventions that would be applicable to promoting healthy aging in humans.

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Highlights

- Circadian systems are evolutionary conserved between Drosophila and mammals.
- Circadian clocks regulate feeding, metabolic processes and detoxification.
- Age-related increase in oxidative stress induce rhythmic expression of stress response genes.
- Circadian transcriptome changes in variety of ways during aging.

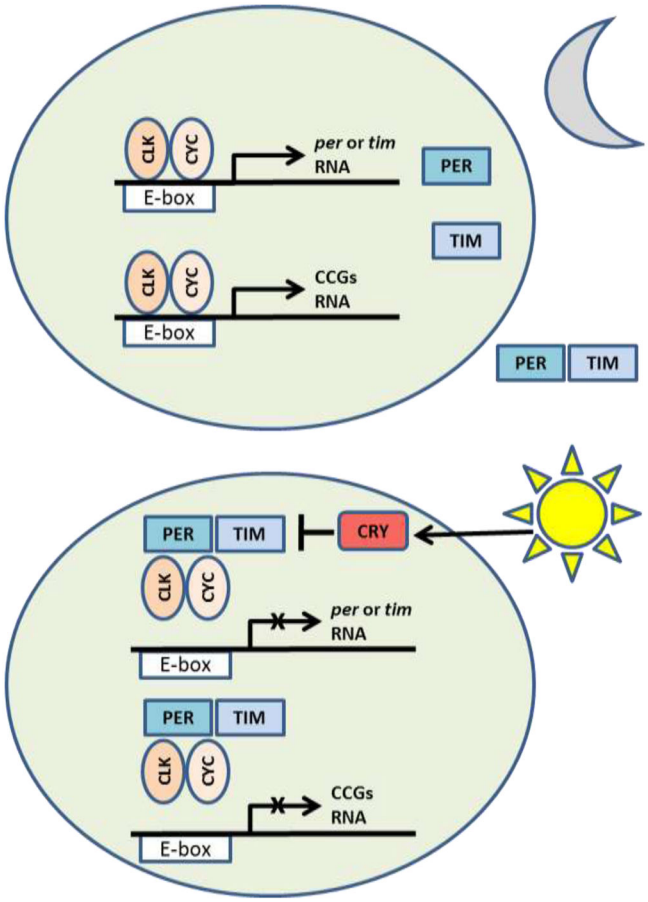

Fig. 1.

Schematic depiction of the negative feedback loop that forms the core mechanism of the *Drosophila clock*. At night (upper panel) the CLK/CYC heterodimers bind to E-box sequences in *per* and *tim* promoters and activate transcription of these genes. Resulting PER and TIM proteins form heterodimers, enter the nucleus and bind to CLK/CYC repressing further transcription of *per* and *tim*. Morning light activates CRY protein (lower panel) which binds to TIM causing its degradation. PER, which is stabilized by TIM, also degrades ending repressive phase of the clock and allowing positive arm of the clock to restart. Note that degradation of TIM and PER proteasome is also accomplished in constant darkness. Many clock-controlled genes (CCGs) also contain E-box in their promoters and their transcription is directly stimulated by CLK/CYC. Some of these CCGs encode transcription factors, which indirecly generate rhythmic transcription of additional CCGs. Figure based on reference [1].

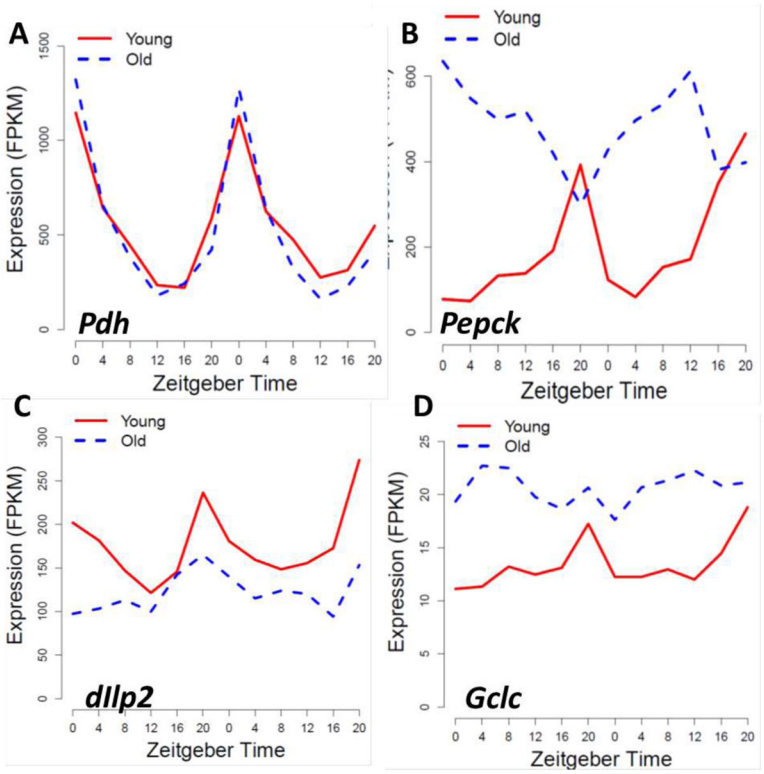

Fig. 2.

Comparative analysis of diurnal transcriptome in heads of young and old flies revealed several age-dependent trends in gene expression. Several hundred genes that were rhythmic in young flies remained rhythmic in old, such as gene encoding photoreceptor dehydrogenase, *Pdh* (A). A few genes remain rhythmic in heads of old flies but adopt a new phase, with a prominent example of Phosphoenolpyruvate carboxykinase, *Pepck* (B). Many genes showed significantly reduced oscillatory amplitude or loss of rhythmicity with age, for example, Insulin-like peptide2, *Ilp2* (C) or glutamate-cysteine ligase catalytic subunit, *Gclc* (D). Gene expression profiles shown are based on data obtained by RNA-seq and published recently [75].