Journal of Applied Biology & Biotechnology Vol. 7(06), pp. 88-95, Nov-Dec, 2019
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2019.70614

Feedback loops in circadian clocks of *Drosophila* and mammals

Gunja Snehi*
Department of Zoology, Jai Narain Vyas University, Jodhpur, India

ARTICLE INFO

Article history:
Received on: July 31, 2019
Accepted on: September 10, 2019
Available online: November 12, 2019

Key words:
Circadian rhythm, clock genes, SCN, feedback loops, period genes

ABSTRACT

Circadian rhythm is 24-hour cycle rhythmicity in organisms, which is endogenous, entrained by environmental cues, and temperature-compensated. Circadian rhythm is driven by circadian clock, which is present in all the cells and tissues of the body. Small ventral lateral neurons located in the lateral brain in *Drosophila* and suprachiasmatic nucleus in mammals are the central oscillators which regulate all the peripheral clocks present throughout the body. The circadian rhythm is maintained by a conserved transcriptional–translational autoregulatory loop, which generates oscillations in the expression of clock genes. Here, this review focuses on the interconnected feedback loops present in *Drosophila* and mammals.

1. INTRODUCTION

Circadian rhythm is 24-hour cycle rhythmicity in organisms, which is endogenous and can be seen externally as changes in behaviour or may be internal in form of gene expression. These rhythms are synchronized or entrained to environmental signals called zeitgebers [1]. There are three important features of circadian rhythm—a 24-hour endogenous free running period, entrainment (adjustment of the period to the surrounding environmental signals, such as light and temperature, in time-dependent manner), and temperature compensation (the periodicity of the circadian rhythm is maintained even when there is a variation in the temperature within a physiological range) [1–4].

Circadian clock, that drives circadian rhythm [5], is present in all the cells and tissues of the body [6]. The circadian clock is mainly of two types—primary or central and peripheral. The central clock in *Drosophila* is a group of 5–6 bilaterally symmetric small ventral lateral neurons located in the lateral brain [5]. The circadian clock in mammals was first discovered in central nervous system [3]. The central clock is located in the suprachiasmatic nucleus (SCN) of ventral anterior hypothalamus [5]. The other oscillators, besides the central clock, present in the body are the peripheral oscillators. In *Drosophila*, peripheral clocks can be directly synchronized by the environmental signals [1]. In mammals, the peripheral clock is entrained by SCN through some hormonal signals, such as glucocorticoids [7]. It is slow in response to light because it takes a feeding time of 4–12 hours to receive the signal from the SCN [1] and its rhythmicity is lost just after 4–5 days [8].

Three components are involved in the maintenance of circadian timing system—entrainment/input pathway which connects the pacemaker to the environment through retinohypothalamic tract (RHT); pacemaker, which generates the circadian signal [2] and output pathway which activates behavioural and physiological processes [9] and involves both neuronal and hormonal signals [2].

The circadian system constitutes non-visual photoreceptor cells of retina, SCN, pineal gland, and many peripheral oscillators [10]. Retina contains non-visual photoreceptor cells, called intrinsically photosensitive retinal ganglion cells (ipRGCs) [6]. These cells express a photopigment called melanopsin which makes ipRGCs photosensitive. SCN, the central clock of mammals, acts as a co-ordinator between the external environmental cues and the body of an organism by receiving the environmental cues like light and by sending the signals to the circadian time-keeping system. The entrainment of the central clock is rapid in response to light [1] and the rhythm in response to entrainment is maintained for more than 15 days [8]. The SCN receives information from the ipRGCs through RHT which is helpful in entrainment [1,6]. Pineal gland is an endocrine gland which synthesizes a hormone, called melatonin (N-acetyl-5-methoxytryptamine). This hormone is secreted in a

*Corresponding Author
Gunja Snehi, Department of Zoology, Jai Narain Vyas University, Jodhpur, India. E-mail: gunjasnehi01@gmail.com

circadian manner. It plays a role as a hormone of darkness because it is secreted maximally at night in the absence of light [11].

2. CLOCK GENES

Studies in many organisms, such as photosynthetic bacteria, *Arabidopsis thaliana*, *Drosophila*, *Chlamydomonas*, *Neurospora*, hamster, and mice have shown that oscillations in transcription of some clock genes generate and maintain circadian rhythm [12]. Clock genes identified in *Drosophila* are period (*PER*), timeless (*TIM*), clock (*CLK*), cycle (*CYC*), and doubletime (*DBT*) [13]. In mammals, some of the genes are circadian locomoter output cycles kaput protein (*CLOCK* or *CLK*) gene and its paralogs, such as neuronal PAS domain protein2 (*NPAS*2), period1 (*PER*1), period2 (*PER*2), period3 (*PER*3), brain and muscle arnt-like1 (*BMAL*1 or *ARNTL*, or *MOP*3), cryptochrome1 (*CRY*1), and cryptochrome2 (*CRY*2) [1,2,6].

3. PERIOD GENES

In *Drosophila*, *PER* gene is located on the X chromosome. It encodes PER protein which is essential for the circadian rhythms in eclosion (emergence of the adult fly from the pupa) and locomoter activity [4].

In mammals, three period genes were discovered—*PER*1 [14], *PER*2 [14,15], and *PER*3 [15,16]. A fourth human period gene, *PER*4 [17] has been identified as a pseudogene, which is descended by retrotransposition of *PER*3 gene [18]. The four *PER* paralogues have been evolved via two genome duplications from a single ancestral gene. The duplicated gene, which is of functional importance are retained, such as *PER*3 and the others, are lost from the genome such as *PER*4 [19].

3.1. Period1

*PER*1 gene is located on chromosome 17 (17p13.1) in humans (GenBank accession number AF022992) and contains 24 exons (Gene ID 5187). *PER*1 is rhythmically expressed in mammalian SCN and peripheral tissues [9]. This gene is also called morning-phase clock [20]. PER1 protein is involved in learning and memory [21]. It protects from hepatic inflammatory damage induced by endotoxin [22]. PER1 also acts as a link between stress and peripheral circadian clock as it is induced by stress [23].

3.2. Period2

*PER*2 is located on chromosome 2 (2q37.3) (GenBank accession number AF035830) and contains 23 exons in humans [24]. *PER*2 is expressed in most of the SCN cells [20] and in a smaller proportion of neurons in the brain outside SCN [25]. One study suggests *PER*2 as an afternoon-phase clock gene [20]. *PER*2 regulates *PER*1 and is the most important oscillator for circadian rhythm generation in central and peripheral organs [26]. Compared with other clock genes, *PER*2 expression in the brain regions other than SCN is more sensitive to physiological changes such as feeding behaviour [27].

3.3. Period3

*PER*3 is present on human chromosome 1 (1p36.23) (GenBank accession number AF050182) and has 21 exons of which exon 18 shows polymorphism which encodes an 18-amino-acid domain which is repeated four or five times [28]. Its mRNA level fluctuates in SCN and eyes. *PER*3 is expressed in peripheral organs as well. Unlike *PER*1 and *PER*2 genes, the levels of *PER*3 mRNA do not fluctuate by light pulses during night [16]. PER3 has very much similarity in amino acid sequences with mammalian PER1 and PER2. It also has a PAS (Period–Arnt–Single-minded) domain which has highest similarity with those of PER1 and PER2. PER3 makes organisms more sensitive to light [29].

4. TRANSCRIPTION/TRANSLATION FEEDBACK LOOPS (TTFLS)

Approximately, 43% of all transcriptomes of the mammalian body shows circadian rhythmicity in organ-specific manner. Organ-specificity means the clock genes are active in the whole body but the output is different in each organ. The rhythmic genes are clustered together in the genome and are longer than non-rhythmic genes. Liver has the largest number of circadian genes and the brain regions, such as hypothalamus, have the minimum number [30].

A nearly 24-hour period oscillation in levels of *PER* mRNA and PER proteins occurs which are expressed rhythmically [31]. There is a rapid transcription of *PER* gene just after sunset/lights off (zeitgeber time, ZT12), accumulation of PER protein in the cytoplasm 2–6 hours after sunset (ZT10-17) and at ZT18, PER reaches its maximum level. These proteins are translocated to the nucleus 5–8 hours after lights off, where they repress their own transcription. PER protein levels become the lowest during the sunrise/lights on (ZT0/24) [32] (Fig. 1). The delay between PER expression and its repressor activity in the nucleus acts as a checkpoint for a stable circadian oscillation generation [33]. Moreover, a recent study suggests that transcription delay of *PER2* and *CRY1* and degradation rates of *CRY1* and *REV-ERBα* are the major factors which influence phases of the genes involved in circadian timing network [34].

There are two feedback loops—positive and negative, which interact with each other to drive the circadian rhythm correctly. These loops are discussed in the following section for *Drosophila* and mammals.

4.1. TTFLs in *Drosophila*

4.1.1 Transcriptional activators

In *Drosophila*, the bHLH-PAS (basic helix–loop–helix, Period–Arnt–Single-minded) transcription factors, CLOCK (d*CLK*) and CYCLE (*CYC*) are the activators of positive feedback loop. d*CLK* is rhythmically expressed which means that its RNA and protein levels oscillate over 24-hour period but *CYC* is constitutively expressed [5,31,35].

4.1.2. Transcriptional inhibitors

PER and TIM are the transcriptional inhibitors of this feedback loop. The levels of RNAs and proteins of both *PER* and *TIM* oscillate rhythmically with same period and phase [31,36]. Though PER is required for the expression of TIM in the nucleus [36], it is primary repressor of the transcription of the positive elements of

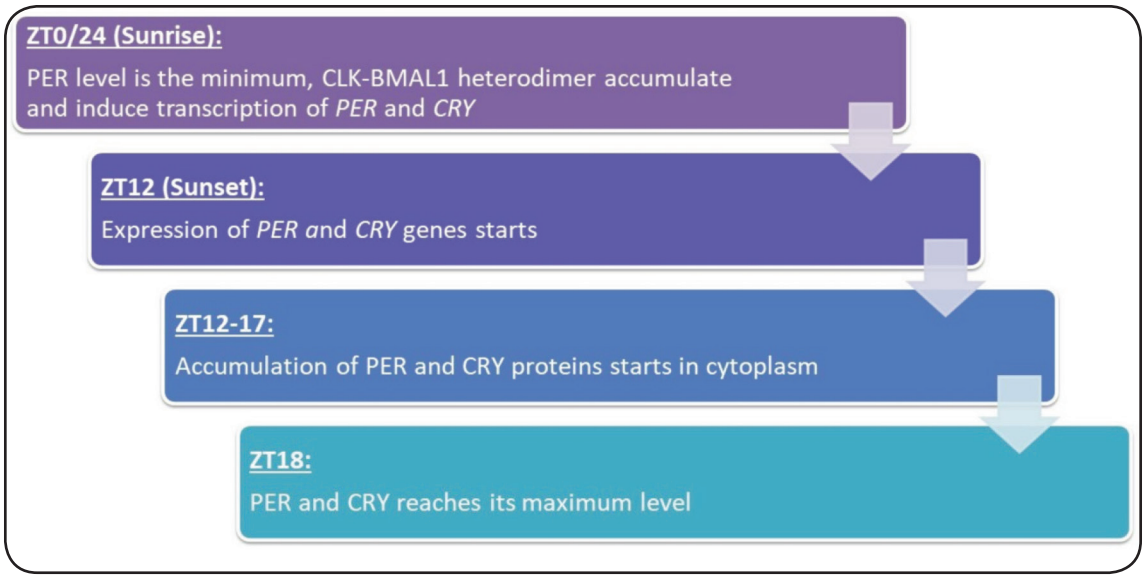

Figure 1: Circadian rhythm pattern.

the loop [31]. Degradation of TIM is rapid through a proteasome-mediated pathway and is due to the formation of heterodimer with dCRY in response to light [5,36]. In *Drosophila*, dCRY plays a role in circadian photoreception [37].

TIM lacks a PAS domain, so it associates with PER by a heterotypic protein interaction [31]. The interaction between TIM and PER occurs at two sites as follows:

1) Cytoplasmic localization domains (CLDs) of both proteins; which not only helps in formation of TIM-PER heterodimer but also allows the nuclear entry of the complex

2) between PAS of PER protein with nuclear localization signal (NLS) of TIM [33].

Nuclear entry of each protein is prevented by the CLD either by binding the monomeric PER and TIM to the cytoplasmic anchor or by inhibiting the NLSs of both proteins [31]. Thus, TIM is essential for PER stabilization and its transport to the nucleus [32,33] possibly due to the physical association of both PER and TIM that may suppresses the activity of CLD [33].

4.1.3. Positive feedback loop

The positive feedback loop involves *dCLK* transcription regulation. Transcripts of *dCLK* and those of *PER* and *TIM* oscillate out of phase. Thus, when the level of *dCLK* mRNA peaks (in the late night and in early morning), the levels of *PER* and *TIM* transcripts are the lowest [5,31]. dCLK-CYC heterodimer enhances the transcription of negative elements, *PER* and *TIM* genes. The complex constitutively binds specifically to CACGTG nucleotide sequence of E-box enhancer elements of the target gene promoters [31,38]. The RNA levels of *PER* and *TIM* increase, and thus PER and TIM proteins accumulate in the cytoplasm as heterodimer [31].

4.1.4. Negative feedback loop

The negative feedback loop involves repression of the positive element dCLK-CYC. This repression is achieved when PER and TIM form a heterodimer due to increase in the amount of PER and TIM proteins. This heterodimer moves into the nucleus and causes a conformational change in dCLK-CYC or decreases its DNA-binding activity without affecting the association between dCLK and CYC which causes fall in the levels of PER and TIM proteins [38]. According to Bae *et al*. [35], CYC is present in abundant amount, approximately 200 times more than the amount of dCLK. PER and TIM bind with dCLK or dCLK-CYC complex rather than with the free CYC. Thus, dCLK acts as a limiting agent for the *PER-TIM* and *dCLK* feedback loops [35]. Figure 2 illustrates that dCLK-CYC heterodimer enhances the transcription of negative elements, *PER* and *TIM* genes. The RNA levels of *PER* and *TIM* increase, and thus PER and TIM proteins accumulate in the cytoplasm as heterodimer. This heterodimer moves into the nucleus and inhibits dCLK-CYC heterodimer which causes fall in the levels of PER and TIM proteins. DBT is a kinase which degrades monomeric PER but does not show its activity on PER-TIM heterodimer. Shaggy, another protein kinase, is constitutively expressed. It phosphorylates TIM protein and also helps in nuclear translocation of PER/TIM.

4.2. TTFLs in mammals

Mammalian core clock acts via enhancer elements in their promoters, such as E-boxes, D-boxes, and ROR-elements [39] and includes mainly five regulators, such as activators *BMAL*1 and *DBP* (D-box regulator) and the inhibitors *PER*2, *CRY*1, and *REV-ERBα*. There are three feedback loops, which are essential for circadian rhythm generation. These are autoinhibitions of *PER* and *CRY*, *BMAL*1/*REV-ERBα* loops [40] and repressilator motifs which contains *PER*2, CRY1, and *REV-ERBα* genes [41]. These loops are tissue-specific. The primary feedback loop *PER-CRY*

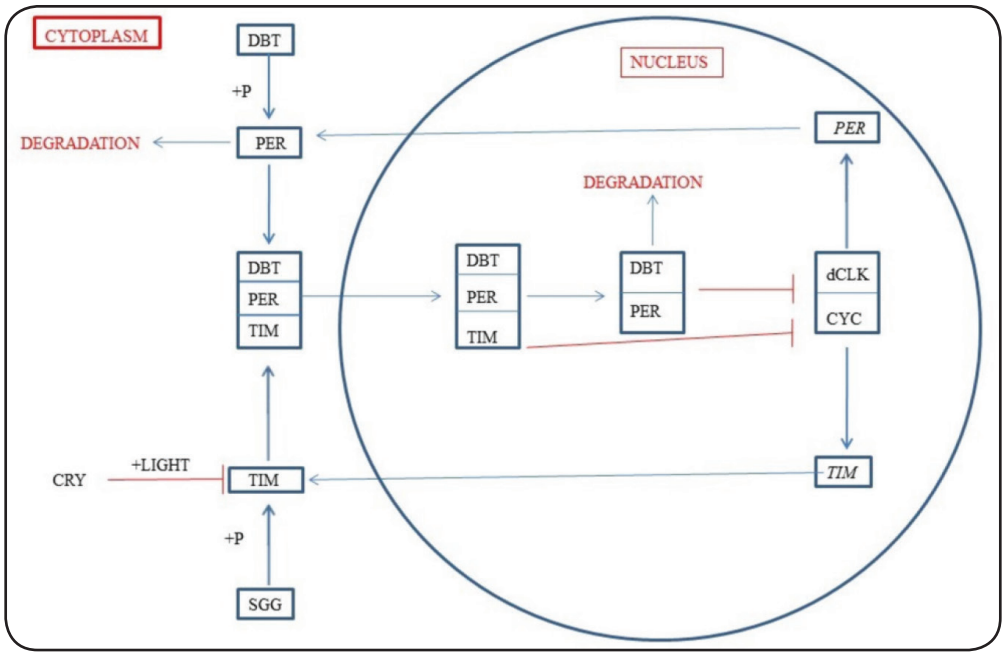

Figure 2: Feedback loop in *Drosophila*.

autoinhibition is particularly found in SCN, whereas *BMAL*1/*REV-ERBα* loops are found in the heart. *BMAL*1/*REV-ERBα* loop and repressilators form the largest group of oscillators in liver. The co-existence of these feedback loops provides redundancy and enhances robustness and flexibility of the circadian core clock [40].

4.2.1. Transcriptional activators

CLK (or NPAS2) and BMAL1 are the bHLH-PAS transcription factors constituting the positive elements of the loop. Unlike d*CLK*, mammalian *CLK* is expressed constitutively [5] in SCN but its oscillations are cyclic in peripheral tissues [42], whereas *BMAL*1 RNA and protein levels have a cycle of over 24-hour period [5,31].

4.2.2. Transcriptional inhibitors

mCRY and mPER are the transcriptional inhibitors of the loop. mCRY, a member of the blue light-sensitive family of photoreceptor proteins [43] and the primary repressor in mammals [44] has two types—CRY1 and CRY2 which are rhythmically expressed [45]. Although CRY1 is a stronger transcriptional repressor than CRY2 and can maintain the rhythm alone [46], CRY1 and CRY2 bind to CLOCK and BMAL1 with the same affinity when PER2 is co-expressed [47].

PER1 and PER2 respond differentially toward light [14]. This is because the regulatory region of PER1 mainly responds to light but that of PER2 is affected by hormonal and other signals [14]. Though m*PER*s (mPER1 and mPER2) are expressed rhythmically [48], there is a delay of 4 hours between their expressions. *PER*1 transcripts are formed prior to *PER*2 transcripts may be due to the fact that a minimum amount of PER1 is required to initiate *PER*2 expression [14].

CRY repressor activity depends upon its synthesis, post-translational modifications, nuclear shuttling, and its degradation. These activities are regulated by the interaction of CRY to different proteins, such as PER1/2 [49], E3 ligases (FBXL3 and FBXL21), and CLOCK. Conserved core structure called photolyase homology region of CRY binds with all these proteins. PER competes for the binding of CRY to CLOCK/BMAL1 complex [50]. Binding site of PER to CRY overlaps with the binding site of FBXL3 and CLOCK/BMAL1 which regulates the degradation and repression activities of CRY. Another site, Ser71 of CRY1 is phosphorylated by nutrient-responsive AMP-activated kinase (AMPK) which enhances the binding of FBXL3 and reduces the stability of CRY1. CRY1 also entrain peripheral clocks metabolically by phosphorylation of AMPK through nutrient signals [51]. Cys412 of CRY1 forms an intramolecular disulfide bond with its Cys363 [52], which weakens mCRY1-mPER2 interactions and an intermolecular disulfide bond with FBXL3. Moreover, zinc enhances the formation of the reduced state of mCRY1 and stabilizes the mCRY1/mPER2 complex [53].

Constitutively nuclear mammalian homolog of *Drosophila* TIM shows no change in its RNA and protein levels and is not degraded by light exposure [48]. mTIM does not affect PER1 nuclear translocation but when PER1 enters the nucleus, mTIM interferes in CLK-BMAL1-mediated transcription [48].

4.2.3. Positive feedback loop

It involves the *BMAL*1 transcription regulation. There is an interval of 12 hours between the peak RNA levels of *BMAL*1 and *PER* & *CRY*. CLK (or NPAS2) and BMAL1 heterodimer remains bound specifically to CACGTG nucleotide sequence of E-box enhancer elements of the target gene promoters constitutively. During the day, when co-activators such as p300, which occupy the binding site of CRY1 [54], are bound to this heterodimer, it enhances the

transcription of negative elements, *PER* and *CRY* genes and also activates transcription of retinoic acid-related orphan nuclear receptor gene—*REV-ERBα* through E-box enhancers. REV-ERBα protein then represses the transcription of *BMAL*1 gene. Thus, the RNA levels of *BMAL*1 fall and those of *PER* and *CRY* increase [55]. RORα, another orphan nuclear receptor protein, competes with REV-ERBα to bind retinoic acid-related orphan receptor response elements present in the *BMAL*1 promoter. It enhances the transcription of *BMAL*1 gene [56]. An E3 ubiquitin ligase, TNF receptor-associated factor 2, decreases the stability of BMAL1, thus decreases *PER*1 mRNA expression [57]. CLK-BMAL1 heterodimer also regulates the expression of clock-controlled genes (CCG) such as genes related to cell growth, apoptosis and DNA repair [58].

4.2.4. Negative feedback loop

It involves the repression of the positive element CLK-BMAL1 by the negative regulators. As the amount of PER and CRY proteins increases, these proteins stabilize by forming heterodimer involving PAS domain. The heterodimer may be between any one of the three PER proteins with one of the two cryptochrome proteins (CRY1 and CRY2). PER2 has a stabilization sequence that stabilizes the complex. As PER-CRY heterodimer moves into the nucleus, CRY proteins repress the transcription of *PER* and *CRY* and also that of *REV-ERBα* by inhibiting the positive regulators of the loop. As a result, the levels of PER and CRY fall and those of BMAL1 rise during night [55]. Recently, it is shown in mouse liver cell extracts that the three PER proteins form a mature cytoplasmic multi-globular complex with the two CRYs and casein kinase 1 delta which migrates to the nucleus and inhibits CLOCK/BMAL1 [59]. Figure 3 illustrates feedback loops in mammals. ipRGCs of retina receive light signal and convey the signal to the SCN core through RHT which releases glutamate and pituitary adenylate cyclase activating polypeptide (PACAP) onto cells in the SCN core. It results in phosphorylation of cAMP-response-element-binding protein which binds to the cAMP-response element present in the promoter of *PER*1 and *PER*2, inducing their transcription. Different neurotransmitters, such as VIP, GRP and GABA, help in communication between the SCN core and SCN shell. CLOCK-BMAL1 heterodimer binds to E-boxes in the promoter region of *PER*, *CRY*, *REV-ERBα*, *RORα*, and other CCG and induces their transcription. High levels of PER and CRY proteins in the cytoplasm cause them to dimerize which then binds with casein kinase 1 and translocate to the nucleus where they inhibit the activity of CLOCK–BMAL, thus inhibiting their own transcription. At the same time, REV-ERBα inhibits the transcription of BMAL1.

The interaction between these positive and negative loops is thus essential for the proper functioning of circadian rhythm [9,55]. The entire cycle takes approximately 24 hours to complete. The yield of PER and CRY proteins is regulated by E3 ubiquitin ligase complexes [6]. The nuclear entry and repressor activity of PER-CRY complex occur through the interaction of PER with KPNB1, an importin-β component, without the involvement of importin-α [60]. CLOCK/BMAL1 recruits Ddb1 (DNA damage binding protein 1)–Cullin-4 (Cul4) E3 ubiquitin ligase to E-boxes of the target gene which enhances mono-ubiquitination of H2B histone protein at Lys-120. This enzymatic action helps in the stable interaction between the PER complex and CLOCK/BMAL1 complex which causes the modification in the chromatin and thus repression of transcription [61]. CRY binds to BMAL1's C-terminal transactivation domain with its C-terminal α-helix tail and competes with other coactivators for binding to BMAL1 [62]. The secondary pocket of CRY binds with the PAS-B domain of CLOCK protein [63].

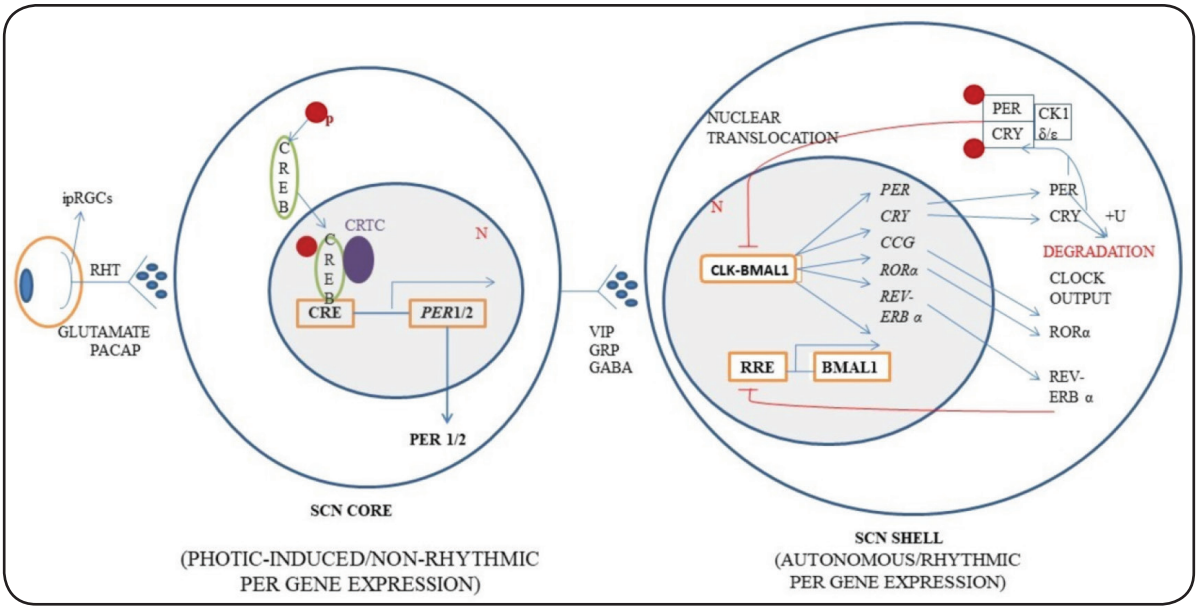

Figure 3: Feedback loop in mammals [Modified from figure 1 (Antle & Silver, 2005)].

Since *PER* has a rate-limiting role in the formation of a negative-feedback complex with CRY, its rhythmic expression is critical in the circadian oscillations. PER2 has a great binding affinity with CLOCK and BMAL1 which connects the negative complex (PER:CRY) to the positive complex (CLOCK:BMAL1), a key step to regulate the circadian rhythm [64]. PER2 LCCLL motif between the two PAS domains triggers the interaction of PER2 with CLOCK or BMAL1. This motif may not be flexible enough or may not be accessible to interact with CLOCK/ BMAL1 in PER1. PER2 also binds to the nuclear receptors, peroxisome proliferator-activated receptor-α (PPARα, present in the liver) and REV-ERBα which regulates the transcription of *BMAL*1 gene [65].

5. CONCLUSION

The feedback loops generate the molecular mechanism of circadian clock. The feedback loops are tissue-specific. *PER-CRY* autoinhibition are characteristic for SCN clocks, while *BMAL*1/ *REV-ERBα* loops are found in the heart and repressilators motifs are found in liver. Tissue-specific use of a network of co-existing synergistic feedback loops could account for functional differences between organs. These co-existing feedback loops enhances robustness and flexibility of the circadian core clock [40]. Further work on the details of molecular clocks is needed to know their roles in peripheral tissues and to know how these are associated with the behavioural and physiological systems of the body. Some clock-related disorders can also be cured by knowing these associations.

ACKNOWLEDGMENT

The author would like to express his special thanks of gratitude to UGC, New Delhi, for providing fellowship throughout his work period.

ABBREVIATIONS

SCN	suprachiasmatic nucleus
RHT	retinohypothalamic tract
ipRGC	intrinsically photosensitive retinal ganglion cell
PER	period
TIM	timeless
CLK	clock
CYC	cycle
DBT	doubletime
*NPAS*2	neuronal PAS domain protein2
*BMAL*1	brain and muscle arnt-like1
CRY	cryptochrome
PAS	Period–Arnt–Single-minded
bHLH	basic helix–loop–helix
CLD	cytoplasmic localization domain
NLS	nuclear localization signal
AMPK	AMP-activated kinase
CCG	clock-controlled genes
Ddb1	DNA damage binding protein 1
Cul4	cullin-4
PPARα	peroxisome proliferator-activated receptor-α

REFERENCES

1. Bell-Pedersen D, Cassone V, Earnest D, Golden SS, Hardin PE, Thomas TL, et al. Circadian rhythms from multiple oscillators: Lessons from diverse organisms. Nat Rev Genet 2005;6(7):544–56; doi:10.1038/nrg1633
2. Lowrey P, Takahashi J. Genetics of circadian rhythms in mammalian model organisms. Adv Genet 2011;74:175–230.
3. Vaze K, Sharma V. Circadian Rhythms. Resonance 2013;(November): 1032–50.
4. Tataroglu O, Emery P. Studying circadian rhythms in *Drosophila melanogaster*.Methods2014;68(1):140–50;doi:10.1016/j.ymeth.2014.01.001
5. Glossop N, Hardin P. Central and peripheral circadian oscillator mechanisms in flies and mammals. J Cell Sci 2002;115(Pt 17):3369–77; doi:10.1523/JNEUROSCI.3559-12.2012
6. Mohawk J, Green C, Takahashi J. Central and peripheral circadian clocks in mammals. Ann Rev Neurosci 2012;35:445–62; doi:10.1146/annurev-neuro-060909-153128
7. Balsalobre A, Brown SA, Marcacci L, Tronche F, Kellendonk C, Reichardt HM, et al. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. Science 2000;289(5488):2344–7. Available via http://www.ncbi.nlm.nih.gov/pubmed/11009419
8. Yamazaki S. Resetting central and peripheral circadian oscillators in transgenic rats. Science 2000;288(5466):682–5; doi:10.1126/science.288.5466.682
9. Kiss Z, Ghosh P. Circadian rhythmicity and the influence of "clock" genes on prostate cancer. Endocr Relat Cancer 2016;23(11):T123–34; doi:10.1530/ERC-16-0366
10. Guido M, Garbarino-Pico E, Contin M, *et al*. Inner retinal circadian clocks and non-visual photoreceptors: novel players in the circadian system. Prog Neurobiol 2010;92(4):484–504; doi:10.1016/j.pneurobio.2010.08.005
11. Filadelfi A, Castrucci Am. Comparative aspects of the pineal/melatonin system of poikilothermic vertebrates. J Pineal Res 1996;20(4):175–86; doi:10.1109/JSSC.2006.886523
12. Vitaterna MH, King DP, Chang AA, Kornhauser JM, Lowrey PL, McDonald JD, et al. Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. Science 1994;264(5159):719–25; doi:10.1126/science.8171325
13. Young M. The molecular control of circadian behavioral rhythms and their entrainment in *Drosophila*. Ann Rev Biochem 1998;67:135–52; doi:10.1146/annurev.biochem.67.1.135
14. Albrecht U, Sun Z, Eichele G, Lee C. A differential response of two putative mammalian circadian regulators, mper1 and mper2, to light. Cell 1997;91(7):1055–64; doi:10.1016/S0092-8674(00)80495-X.
15. Takumi T, Taguchi K, Miyake S, Sakakida Y, Takashima N, Matsubara C, et al. A light-independent oscillatory gene mPer3 in mouse SCN and OVLT. EMBO J 1998;17(16):4753–9; doi:10.1093/emboj/17.16.4753
16. Zylka M, Shearman L, Weaver D, Reppert S. Three period homologs in mammals: differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain. Neuron 1998;20(6):1103–10; doi:10.1016/S0896-6273(00)80492-4
17. Clayton J, Kyriacou C, Reppert S. Keeping time with the human genome. Nature 2001;409(6822):829–31; doi:10.1038/35057006
18. Gotter A, Reppert S. Analysis of human Per4. Brain Res Mol Brain Res 2001;92(1–2):19–26. Available via http://www.ncbi.nlm.nih.gov/pubmed/11483238
19. Archer S, Schmidt C, Vandewalle G, Dijk D. Phenotyping of PER3 variants reveals widespread effects on circadian preference, sleep regulation, and health. Sleep Med Rev 2018;40:109–26; doi:10.1016/j.smrv.2017.10.008
20. Takumi T, Matsubara C, Shigeyoshi Y, Taguchi K, Yagita K, Maebayashi Y, et al. A new mammalian period gene predominantly expressed in the suprachiasmatic nucleus. Genes Cells 1998;3(3):167–76; doi:10.1046/j.1365-2443.1998.00178.x

21. Rawashdeh O, Parsons R, Maronde E. Clocking in time to gate memory processes: the circadian clock is part of the ins and outs of memory. Neural Plast 2018;2018:6238989; doi:10.1155/2018/6238989
22. Wang T, Wang Z, Yang P, *et al*. PER1 prevents excessive innate immune response during endotoxin-induced liver injury through regulation of macrophage recruitment in mice. Cell Death Dis 2016;**7**:e2176; doi:10.1038/cddis.2016.9
23. Al-Safadi S, Al-Safadi A, Branchaud M, Rutherford S, Dayanandan A, Robinson B, et al. Stress-induced changes in the expression of the clock protein PERIOD1 in the rat limbic forebrain and hypothalamus: role of stress type, time of day, and predictability. PLoS One 2014;9(10):e111166; doi:10.1371/journal.pone.0111166
24. Toh K, Jones C, He Y, Eide EJ, Hinz WA, Virshup DM, et al. An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. Science 2001;291(5506):1040–3; doi:10.1126/science.1057499
25. Frederick A, Goldsmith J, de Zavalia N, Amir S. Mapping the co-localization of the circadian proteins PER2 and BMAL1 with enkephalin and substance P throughout the rodent forebrain. PLoS One 2017;12(4):e0176279; doi:10.1371/journal.pone.0176279
26. Kim M, de la Peña J, Cheong J, Kim H. Neurobiological functions of the period circadian clock 2 gene, Per2. Biomol Ther 2018;26(4):358–67; doi:10.4062/biomolther.2017.131
27. Blancas-Velazquez A, Unmehopa U, Eggels L, Koekkoek L, Kalsbeek A, Mendoza J, et al. A free-choice high-fat high-sugar diet alters day-night Per2 gene expression in reward-related brain areas in rats. Front Endocrinol 2018;9(9):154; doi:10.3389/fendo.2018.00154
28. Jenkins A, Archer S, von Schantz M. Expansion during primate radiation of a variable number tandem repeat in the coding region of the circadian clock gene period3. J Biol Rhythms 2005;20(5):470–2; doi:10.1177/0748730405278442
29. Pereira D, van der Veen D, Gonçalves B, Tufik S, von Schantz M, Archer SN, et al. The effect of different photoperiods in circadian rhythms of per3 knockout mice. BioMed Res Int 2014;2014:170795; doi:10.1155/2014/170795
30. Zhang R, Lahens N, Ballance H, Hughes M, Hogenesch J. A circadian gene expression atlas in mammals: implications for biology and medicine. Proc Natl Acad Sci USA 2014;111(45):16219–24; doi:10.1073/pnas.1408886111
31. Allada R, Emery P, Takahashi J, Rosbash M. Stopping time: the genetics of fly and mouse circadian clocks. Ann Rev Neurosci 2001;24:1091–119; doi:10.1146/annurev.neuro.24.1.1091
32. Curtin K, Huang Z, Rosbash M. Temporally regulated nuclear entry of the *Drosophila* period protein contributes to the circadian clock. Neuron 1995;14(2):365–72; doi:10.1016/0896-6273(95)90292-9
33. Saez L, Young M. Regulation of nuclear entry of the *Drosophila* clock proteins period and timeless. Neuron 1996;17(5):911–20; doi:10.1016/S0896-6273(00)80222-6
34. Mavroudis P, DuBois D, Almon R, Jusko W. Modeling circadian variability of core-clock and clock-controlled genes in four tissues of the rat. PLoS One 2018;13(6):e0197534; doi:10.1371/journal.pone.0197534
35. Bae K, Lee C, Hardin PE, Edery I. dCLOCK is present in limiting amounts and likely mediates daily interactions between the dCLOCK-CYC transcription factor and the PER-TIM complex. J Neurosci 2000;20(5):1746–53. Available via http://www.ncbi.nlm.nih.gov/pubmed/10684876
36. Hunter-Ensor M, Ousley A, Sehgal A. Regulation of the *Drosophila* protein timeless suggests a mechanism for resetting the circadian clock by light. Cell 1996;84(5):677–85; doi:10.1016/S0092-8674(00)81046-6
37. Emery P, Stanewsky R, Helfrich-Förster C, Emery-Le M, Hall J, Rosbash M. *Drosophila* CRY is a deep brain circadian photoreceptor. Neuron 2000;26(2):493–504; doi:10.1016/S0896-6273(00)81181-2
38. Ishida N, Kaneko M, Allada R. Biological clocks. Proc Natl Acad Sci USA 1999;96(16):8819–20; doi:10.1073/pnas.96.16.8819
39. Ukai H, Ueda H. Systems biology of mammalian circadian clocks. Ann Rev Physiol 2010;72(March):579–603; doi:10.1146/annurev-physiol-073109-130051
40. Pett J, Kondoff M, Bordyugov G, Kramer A, Herzel H. Co-existing feedback loops generate tissue-specific circadian rhythms. Life Sci Alliance 2018;1(3):e201800078; doi:10.26508/lsa.201800078
41. Pett J, Korenčič A, Wesener F, Kramer A, Herzel H. Feedback loops of the mammalian circadian clock constitute repressilator. PLoS Comput Biol 2016;12(12):e1005266; doi:10.1371/journal.pcbi.1005266
42. Wang Q, Tikhonenko M, Bozack SN, Lydic TA, Yan L, Panchy NL, et al. Changes in the daily rhythm of lipid metabolism in the diabetic retina. PLoS One 2014;9(4); doi:10.1371/journal.pone.0095028
43. Cashmore A, Jarillo J, Wu Y, Liu D. Cryptochromes: blue light receptors for plants and animals. Science 1999;284(5415):760–5.
44. Ye R, Selby C, Chiou Y, Ozkan-Dagliyan I, Gaddameedhi S, Sancar A. Dual modes of CLOCK:BMAL1 inhibition mediated by cryptochrome and period proteins in the mammalian circadian clock. Genes Dev 2014;28(18):1989–98; doi:10.1101/gad.249417.114
45. Kume K, Zylka M, Sriram S, Shearman LP, Weaver DR, Jin X, et al. mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 1999;98(2):193–205; doi:10.1016/S0092-8674(00)81014-4
46. Khan S, Xu H, Ukai-Tadenuma M, Burton B, Wang Y, Ueda HR, et al. Identification of a novel cryptochrome differentiating domain required for feedback repression in circadian clock function. J Biol Chem 2012;287(31):25917–26; doi:10.1074/jbc.M112.368001
47. Rosensweig C, Reynolds KA, Gao P, Laothamatas I, Shan Y, Ranganathan R, et al. An evolutionary hotspot defines functional differences between CRYPTOCHROMES. Nat Commun 2018;9(1):1138; doi:10.1038/s41467-018-03503-6
48. Hastings MH, Field MD, Maywood ES, Weaver DR, Reppert SM. Differential regulation of mPER1 and mTIM proteins in the mouse suprachiasmatic nuclei: new insights into a core clock mechanism. J Neurosci 1999;19(12):RC11.
49. Yagita K, Tamanini F, Yasuda M, Hoeijmakers J, van der Horst G, Okamura H. Nucleocytoplasmic shuttling and mCRY-dependent inhibition of ubiquitylation of the mPER2 clock protein. EMBO J 2002;21(6):1301–14; doi:10.1093/emboj/21.6.1301
50. Ye R, Selby C, Ozturk N, Annayev Y, Sancar A. Biochemical analysis of the canonical model for the mammalian circadian clock. J Biol Chem 2011;286(29):25891–902; doi:10.1074/jbc.M111.254680
51. Lamia K, Sachdeva U, DiTacchio L, Williams EC, Alvarez JG, Egan DF, et al. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science 2009;326(5951):437–40; doi:10.1126/science.1172156
52. Czarna A, Berndt A, Singh H, *et al*. Structures of *Drosophila* cryptochrome and mouse cryptochrome1 provide insight into circadian function. Cell 2013;153(6):1394–405; doi:10.1016/j.cell.2013.05.011
53. Schmalen I, Reischl S, Wallach T, Klemz R, Grudziecki A, Prabu JR, et al. Interaction of circadian clock proteins CRY1 and PER2 is modulated by zinc binding and disulfide bond formation. Cell 2014;157(5):1203–15; doi:10.1016/j.cell.2014.03.057
54. Koike N, Yoo SH, Huang HC, Kumar V, Lee C, Kim TK, et al. Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. Science 2012;338(6105):349–54; doi:10.1126/science.1226339
55. Reppert S, Weaver D. Coordination of circadian timing in mammals. Nature 2002;418(6901):935–41; doi:10.1038/nature00965
56. Ko C, Takahashi J. Molecular components of the mammalian circadian clock. Hum Mol Genet 2006;15(2):R271–7; doi:10.1093/hmg/ddl207
57. Chen S, Yang J, Yang L, Zhang Y, Zhou L, Liu Q, et al. Ubiquitin ligase TRAF2 attenuates the transcriptional activity of the core clock protein BMAL1 and affects the maximal Per1 mRNA level of the circadian clock in cells. FEBS J 2018;285(16):2987–3001; doi:10.1111/febs.14595

58. Yang S, Ren Q, Wen L, Hu J, Wang H. Research progress on circadian clock genes in common abdominal malignant tumors. Oncol Lett 2017;14(5):5091–8; doi:10.3892/ol.2017.6856
59. Aryal RP, Kwak PB, Tamayo AG, Gebert M, Chiu PL, Walz T, et al. Macromolecular assemblies of the mammalian circadian clock. Mol Cell 2017;67(5):770–82.e6; doi:10.1016/j.molcel.2017.07.017
60. Lee Y, Jang A, Francey L, Sehgal A, Hogenesch J. KPNB1 mediates PER/CRY nuclear translocation and circadian clock function. eLife 2015;4:1–16; doi:10.7554/eLife.08647
61. Tamayo A, Duong H, Robles M, Mann M, Weitz C. Histone monoubiquitination by Clock-Bmal1 complex marks Per1 and Per2 genes for circadian feedback. Nat Struct Mol Biol 2015;22(10):759–66; doi:10.1038/nsmb.3076.Histone
62. Xu H, Gustafson C, Sammons P, Khan SK, Parsley NC, Ramanathan C, et al. Cryptochrome 1 regulates the circadian clock through dynamic interactions with the BMAL1 C terminus. Nat Struct Mol Biol 2015;22(6):476–84; doi:10.1038/nsmb.3018
63. Michael AK, Fribourgh JL, Chelliah Y, Sandate CR, Hura GL, Schneidman-Duhovny D, et al. Formation of a repressive complex in the mammalian circadian clock is mediated by the secondary pocket of CRY1. Proc Natl Acad Sci USA 2017;114(7):1560–5; doi:10.1073/pnas.1615310114
64. Chen R, Schirmer A, Lee Y, Lee H, Kumar V, Yoo SH, et al. Rhythmic PER abundance defines a critical nodal point for negative feedback within the circadian clock mechanism. Mol Cell 2009;36(3):417–30; doi:10.1016/j.molcel.2009.10.012.Rhythmic
65. Schmutz I, Ripperger J, Baeriswyl-Aebischer S, Albrecht U. The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors. Genes Dev 2010;24(4):345–57; doi:10.1101/gad.564110

How to cite this article:
Snehi G. Feedback loops in circadian clocks of *Drosophila* and mammals. J Appl Biol Biotech2019;7(06):88–95. DOI: 10.7324/JABB.2019.70614