Cell. Mol. Life Sci. (2010) 67:1397–1406
DOI 10.1007/s00018-009-0232-y

Cellular and Molecular Life Sciences

REVIEW

A comparative view of insect circadian clock systems

Kenji Tomioka · Akira Matsumoto

Received: 31 August 2009 / Revised: 8 December 2009 / Accepted: 9 December 2009 / Published online: 25 December 2009

Abstract Recent studies revealed that the neuronal network controlling overt rhythms shows striking similarity in various insect orders. The *pigment-dispersing factor* seems commonly involved in regulating locomotor activity. However, there are considerable variations in the molecular oscillatory mechanism, and input and output pathways among insects. In *Drosophila,* autoregulatory negative feedback loops that consist of clock genes, such as *period* and *timeless* are believed to create 24-h rhythmicity. Although similar clock genes have been found in some insects, the behavior of their product proteins shows considerable differences from that of *Drosophila*. In other insects, mammalian-type *cryptochrome* (*cry2*) seems to work as a transcriptional repressor in the feedback loop. For photic entrainment, *Drosophila* type *cryptochrome (cry1)* plays the major role in *Drosophila* while the compound eyes are the major photoreceptor in others. Further comparative study will be necessary to understand how this variety of clock mechanisms derived from an ancestral one.

Keywords Insect · Circadian rhythm · Clock genes · Entrainment · Molecular mechanism · Neural network

K. Tomioka (✉)
Graduate School of Natural Science and Technology,
Okayama University, Okayama 700-8530, Japan
e-mail: tomioka@cc.okayama-u.ac.jp

A. Matsumoto
Department of Biology, Juntendo University
School of Medicine, 1-1 Hiraga Gakuendai, Inba-gun,
Chiba 270-1695, Japan

Introduction

Circadian rhythms of about 24-h periodicity are commonly observed in a variety of physiological functions of organisms from bacteria to humans. The rhythm is governed by a mechanism called the circadian system, generally including a circadian clock that generates a 24-h oscillation, a photoreceptor that is necessary for the clock to synchronize to the light–dark cycles (LD), and an output system that relays the clock information to various tissues to regulate their overt rhythmicity [1, 2].

The rhythm has some common, basic characteristics: it persists for long periods in constant conditions; its period is temperature-compensated and maintains a similar value over a wide range of temperatures; and it synchronizes to daily environmental cycles, using light and temperature as major synchronizing agents or zeitgebers. Insects provide good models for dissecting the circadian system both at the physiological and molecular levels. The circadian clock that regulates the overt behavioral rhythms has been localized in the brain. For example, the optic lobe is the clock locus for crickets, cockroaches, and beetles [3–5], while the central brain is known for moths, flies, and mosquitoes [6–8]. The compound eyes are known as the principal circadian photoreceptor for photic entrainment in most insects, but ocelli and the extraretinal photoreceptors also play a role in others (see [2]).

Recent studies have promoted our understanding of the circadian system at molecular and cellular levels. The circadian oscillatory mechanism has now been uncovered at the molecular level, and the circadian network for generating rhythmicity has been elucidated in detail in fruit flies [9–12]. Molecular and cellular dissection has also been started in other non-drosophilid insects, promoting the comparative and general formulation of the underlying mechanism [13]. This

review will focus on our current understanding of the molecular and cellular mechanism of the insect circadian system.

The circadian clock network

Cellular organization of the clock has been extensively studied in *Drosophila* and other insects, using molecular probes, such as antibodies against clock gene products and reporter genes driven by clock gene promoter, etc. In *Drosophila*, there are about 150 cells in the brain that express the clock genes, and they are divided into seven subgroups [10, 14] (Fig. 1a). Three of them are located laterally between the optic lobe and the central brain, and are called lateral neurons (LNs). The ventrally located LNs consist of two subgroups of neurons with larger and smaller cell bodies and are called large-LNv (l-LNv) and small-LNv (s-LNv), respectively. The dorsally located LNs are called LNd. There are three groups of neurons in the dorsal region of the central brain called DN1, DN2, and DN3, respectively. The other group is located on the lateral posterior side of the central brain, and is called lateral posterior neurons (LPNs). Detailed analysis of the role of the cells revealed that s-LNvs drive the morning peak and a part of the evening activity, and the LNds regulate the evening peak [15–17]. Recently, l-LNv cells have been revealed to be involved in light-induced arousal and phase-shifting in the late night [18]. DNs, however, have some roles in locomotor rhythms, since the flies lacking LNs still show a circadian locomotor rhythm for at least a few days in constant conditions [19–21]. In larval brain, DN2 cells have the principal role in temperature entrainment and might regulate the phase of LNs, while under LD, the larval DN2 s are controlled by LNs via PDF signaling [22].

Virtually similar clock structures are reported for other insects, including the blow flies, crickets, and cockroaches [23–25]. In the cockroaches and crickets, some of the PER-immunoreactive neurons are located in the optic lobe (Fig. 1b): These probably correspond to the LNvs in *Drosophila*. Their importance for overt activity rhythm generation is implicated by various surgical and electrophysiological experiments [4, 26–28]. Like in DNs of

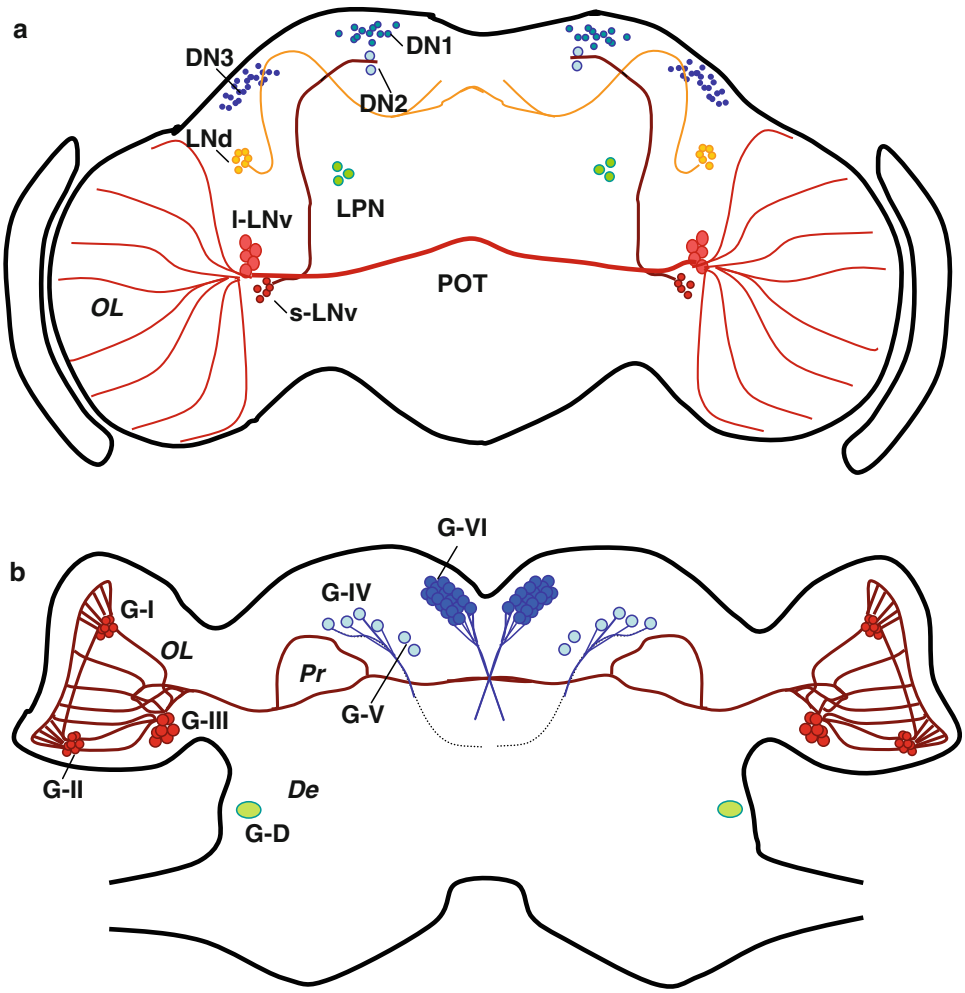

Fig. 1 Schematic diagrams showing *Drosophila* (**a**) and cockroach (**b**) cerebral cells expressing PERIOD (redrawn from [23, 125]). **a** In *Drosophila*, there are seven main groups of neurons. Three groups (*DN1*, *DN2*, and *DN3*) are located in the dorsal region, and the remaining four (*LNd*, *l-LNv*, *s-LNv*, and *LPN*) are located laterally. The *s-LNv* and *l-LNv* express a neuropeptide, PDF. *l-LNvs* have their processes in the optic lobe and send their axonal projection to the contralateral optic lobe through the posterior optic tract (*POT*). *s-LNvs* have axonal projection to the dorsomedial region of the protocerebrum. **b** In the cockroach, *Blattera germanica*, three groups of neurons are located in the optic lobe (*G-I*, *G-II*, and *G-III*) and three groups in the dorsal protocerebrum (*G-IV*, *G-V*, and *G-VI*). There is another group (*G-D*) in the deutocerebrum. PDF is coexpressed in *G-I*, *-II*, *-III*, *-IV* and *-D* cells. *OL* Optic lobe, *Pr* protocerebrum, *De* deutocerebrum

Drosophila, the neurons located in the dorsal protocerebrum may also play a role in the control of overt activity rhythms, because some residual rhythms are often observed even after optic lobe removal [29], and temperature or light cycles often induce a rhythm with circadian properties [30–32].

Molecular machinery of the circadian clock

Irrespective of the similarity in the neuronal architecture of the circadian clock system, recent studies have revealed considerable differences in the molecular mechanisms among insects.

Drosophila's clock machinery

The molecular machinery of the circadian clock has been extensively studied in the fruit fly, *Drosophila melanogaster*. There are several so-called clock genes involved in the generation of the rhythmicity [9, 13]. At least three interdependent feedback loops are thought to constitute the rhythm-generating machinery (Fig. 2). One major loop is that formed by *period* (*per*), *timeless* (*tim*), *Clock* (*Clk*), and *cycle* (*cyc*) (cf. [9, 33]). The transcription of *per* and *tim* is activated by product proteins of *Clk* (CLK) and *cyc* (CYC). CLK and CYC form a heterodimer and bind to a promoter region, called E-box, of *per* and *tim* and promote their transcription during late day to early night. The product proteins, PER and TIM, increase during the night to peak at late night, when their heterodimeric complex enters into the nucleus to repress their own transcription through inhibitory action on CLK-CYC. The repression of *per* and *tim* transcription results in reduced level of PER and TIM, which eventually releases CLK-CYC from the repression to reactivate the transcription of *per* and *tim*. This reactivation leads the loop to the next round.

The second loop is for a circadian oscillation of *Clk* [34, 35]. Besides *per* and *tim*, CLK-CYC activates transcription of *vrille* (*vri*) and *PAR domain protein 1ε* (*Pdp1ε*) during late day to early night. The *vri* mRNA is soon translated to its product protein VRI, which enters the nucleus and inhibits transcription of CLK by binding to a promoter region, V/P-box. Thus the *Clk* mRNA is reduced during the night. The translation of *Pdp1ε* occurs in a rather delayed manner and PDP1ε protein increases during late night to early day. PDP1ε is thought to bind to V/P-box competitively with VRI and activate the transcription of *Clk*. Thus, the *Clk* transcripts increase during the day, also leading to an increase of CLK protein during the day. But a recent study revealed that PDP1ε is not essential for the operation of the loop [36].

The third loop includes *clockwork orange* (*cwo*), which is a transcriptional repressor belonging to the basic helix-loop-helix ORANGE family [37–40]. *cwo* is rhythmically expressed to peak under the regulation by CLK-CYC and forms its own negative feedback loop. CWO represses the expression of other clock genes, such as *per* and *tim*, through E-box elements. This negative feedback loop contributes to sustaining a high-amplitude circadian oscillation.

For precise temporal control of the molecular oscillation and for function of the molecular oscillator, posttranslational regulation of clock proteins seems crucial [9, 41, 42]. For example, the cyclic activation and inhibition of CLK/CYC is paralleled by cyclic phosphorylation levels [43]. Phosphorylation may regulate the timing of nuclear entry of PER and TIM [44, 45], as well as the degradation of TIM [46]. These posttranslational modifications are

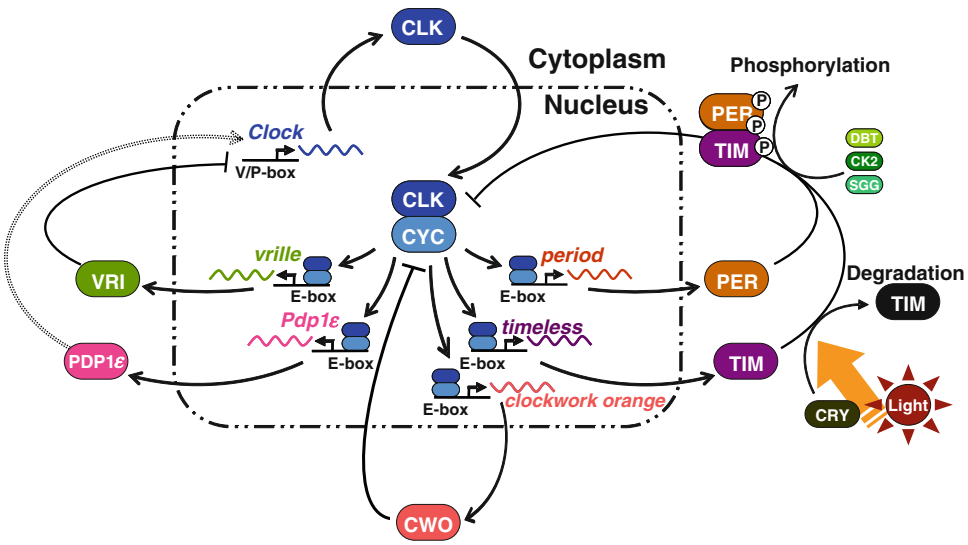

Fig. 2 A scheme illustrating current knowledge about molecular machinery of the *Drosophila* circadian clock. The circadian clock consists of three feedback loops that are interlocked to oscillate with a period of about 24 h. See text for details

suggested to regulate the period, phase, and amplitude of the circadian clock [41].

Recent studies showed that in *Drosophila*, electrical silencing of clock neurons stops the circadian locomotor rhythm free-running in constant darkness (DD) [47]. Immunohistochemical studies revealed that this results from a cessation of the clock itself caused by absence of nighttime nuclear transport of TIM protein from the cytoplasm [47]. A similar stop of the clock was found in larvae [48]. Thus, the electrical activity is thought to be an essential element of the molecular clock in the cerebral pacemaker neurons. However, the molecular oscillation persists under LD in the flies with electrical silencing of clock neurons, suggesting that there is a light-dependent drive on the molecular clock that at least partially compensates electrical activity in LD [47].

Results in other insects do not fully support the *Drosophila* hypothesis

The progress of clock study in *Drosophila* has promoted research on molecular machinery of the clock in other insect species [13, 49]. The studies, however, revealed some significant discrepancies between *Drosophila* and others. In the silkmoth, *Antheraea pernyi*, and the cockroach, *Blattera germanica*, immunohistochemisty with anti-PER and anti-TIM revealed that these proteins always stay in the cytoplasm and never enter into the nucleus [23, 50]. Instead of negative feedback of product proteins, rhythmically expressed *per* antisense RNA is proposed to produce the rhythmicity in *A. pernyi* [51]. However, immunohistochemistry may only reveal large amounts of the proteins, and a trace amount may enter into the nucleus and repress transcription [52]. Another explanation may be that, instead of PER-TIM, CRY2, which is a mammalian type CRY [53], enters the nucleus and works as a transcriptional repressor [54, 55]. These still need to be experimentally examined.

Recently, reverse genetic approaches have been applied to those non-model insects. In many of them, cDNAs of canonical clock genes have been cloned and their expressions have been examined. In most of the tested insects, the clock genes, *per, tim,* and *Clk*, are shown to cycle in a daily or circadian fashion [50, 55–57]. Functions of some clock genes have been examined in a cultured cell system in the monarch butterfly, *Danaus plexippus* [55], and CRY2 seems to repress the transcriptional activity of CLK-CYC in vitro [55]. *cry* belongs to a photolyase family and plays a role in photic entrainment in *Drosophila* [58, 59]. Molecular evolutionary studies have revealed that gene duplication and loss have resulted in three modes of *cry* gene expression [54]; in some insects, either *cry1* (*Drosophila* type *cry*) or *cry2* (mammalian type *cry*) is expressed, and in others, both *cry1* and *cry2* are expressed (Table 1). In the honey bee, *Apis merifella, tim* does not exist in the genome, and *cry2* is thought to act as a

Table 1 Two types of *cryptochrome* in insects

Order	Species	*Drosophila* type (*cry1*)	Mammalian type (*cry2*)
Lepidoptera	*Antheraea pernyi*	AF333998	EF117812
	Danaus plexippus	AY860425	DQ184682
	Mamestra brassicae	AY947639	
	Sesamia nonagrioides	DQ243704	DQ243705
	Spodoptera littoralis	EF364035	EF396286
Diptera	*Anopheles gambiae*	DQ219482	DQ219483
	Culex quinquefasciatus	B0WRR9	XM_001869421
	Bactrocera tryoni	AY708049	
	Drosophila melanogster	NM_169852	DNE
	Sarcophaga bullata	FJ373353	
	Sarcophaga crassipalpis	AB079536	
Hymenoptera	*Apis mellifera*	DNE	EF117814
	Bombus impatiens		EF110521
	Nasonia vitripennis		XM_001606355
Coleoptera	*Tribolium castaneum*	DNE	EF117815
Hemiptera	*Acyrthosiphon pisum*	XM_001944367	XM_001950658
	Riptortus pedestris		AB379863
Orthoptera	*Dianemobius nigrofasciatus*	AB291231	

GenBank accession number is indicated. *DNE* Does not exist

transcriptional repressor like in *Danaus* [60]. Thus, there seem to be considerable differences in the molecular oscillatory mechanism among insects.

In crickets, the role of clock genes has been examined in a more direct way using RNA interference (RNAi) [61–63]. Like other insects, the expression of cricket's *per* gene cycles in a daily fashion in both LD and DD, and peaks at early night. Double stranded RNA (dsRNA) of *per* knocks down *per* mRNA levels, and disrupts both its cycling and the locomotor activity rhythm [61]. Functional analysis with RNAi of other clock genes will clarify the molecular oscillatory mechanism in the cricket. However, so far the molecular dissection of the oscillatory mechanism has not been performed in enough species to illustrate its divergence.

Input pathways

Light entrainment

Light is the most important zeitgeber for the circadian clock to synchronize to the environmental cycles. Photoreceptors necessary for the entrainment, or resetting, of the clock have been localized in some insects. In hemimetabolous insects such as crickets and cockroaches, the compound eye is the most important photoreceptor [28, 64], but ocelli also sometimes have minor roles in the photic entrainment [65, 66]. In *Drosophila*, in addition to these external eyes, H–B eyelet that is a remnant of larval eye, and the blue light receptor molecule CRY1, are known for circadian photoreceptors [66].

In *Drosophila,* CRY1 is expressed in a circadian fashion in some of the cerebral clock neurons, and shifts the phase of the clock light-dependently [58, 67, 68]. It is known to bind TIM in a light-dependent manner and to lead to TIM's degradation [68]. This light-dependent degradation of TIM results in a phase shift of the clock in a phase-dependent manner: A reduced level of TIM in the evening leads to a delay of the clock at certain times until TIM increases to a required level, while it causes an advance of the clock in the morning to the time at which the TIM's level coincides [69, 70]. The phase-dependent phase shifts provide a base of non-parametric entrainment. *Danaus* CRY1 partially rescued light-induced phase shiftability in *Drosophila* cry^b mutant flies, while CRY2 did not [55]. Experiments using RNA interference revealed that, in DpN1 cells, the light-induced decrease in TIM abundance is mediated through *Danaus* CRY1 [53]. Thus, CRY1 seems to mediate the light entrainment in the monarch butterfly.

Besides CRY1, rhodopsins (Rhs) are also involved in photic entrainment. In *Drosophila*, Rh1 and Rh6 were implicated in entrainment to red light [71], and Rh1, Rh5, and Rh6 to green and yellow light [72]. Although histamine is suggested to be an important neurotransmitter for the Rh pathway [73], other neurotransmitters and the intracellular signal pathways have yet to be elucidated. Details of what role photoreceptor molecules play in photic entrainment of other insects and their divergence among insect species deserve to be investigated.

Temperature entrainment

Temperature is also an important time cue to synchronize the clock, especially in dark places, such as eggs laid underground or insects that pupate in darkness. Temperature cycle or temperature steps are known to cause phase shifts of the clock to adjust to the time of environmental cycles [74, 75]. The molecular mechanism for the temperature synchronization is less understood. In *Drosophila*, the effects of temperature step-up and -down were examined at transcript levels [76]. Under constant light, temperature step-up up-regulates the *Clk* gene, and step-down down-regulates it. In contrast, *per, tim, vri,* and *Pdp1ε* genes are down-regulated and up-regulated by step-up and step-down, respectively. After temperature step-down, all these clock genes showed an oscillation in constant conditions. These temperature responses are virtually eliminated in Clk^{Jrk} mutant flies, suggesting that the *Clk* gene is the principal component for temperature entrainment [76]. Although the temperature sensing system is still largely unclear, it might involve *nocte* and PLC, mutation of which disrupts temperature entrainment [77]. PLC was shown to have a role in temperature-dependent *per* splicing [78, 79]: Under low temperature conditions, splicing of an intron in the 3′ untranslated region of *per* is enhanced, leading to a rapid accumulation of PER protein and an advance of the phase of the evening activity [80]. In addition, *tim* mRNA levels are increased earlier at low temperatures through photic stimulation of *tim* expression [81].

Existence of temperature entrainable oscillators has been reported for other insects including crickets [75], cockroaches [31], and mosquitoes [82]. Unfortunately, no detailed investigation on molecular and cellular mechanisms of temperature entrainment is available for insects other than *Drosophila*.

Output pathways

The pathways through which the clock regulates overt rhythms are not well understood. Pigment-dispersing factor (PDF) is the best characterized output molecule of the circadian clock system. Its involvement was first notified when immunohistochemistry revealed that PDF

colocalized with PER in some clock neurons, s-LNv and l-LNv, in the *Drosophila* brain (Fig.1) [83], and is now thought to be an output neurotransmitter of the circadian clock in a wide variety of insects. It is a member of the pigment dispersing hormone (PDH), an octadeca peptide that was first found in crustacea as a hormone that disperses pigments in the epithelium [84]. *pdf* gene expression shows no circadian rhythm in *Drosophila* [85], while the dorsomedial termini of PDF-expressing neurons show daily morphological changes probably controlled by the circadian clock [86]. pdf^{01} mutant flies that lack PDF show a rhythm with advanced onset of the evening activity and become arrhythmic within a few days in DD [87]. PDF is required to adjust cycling amplitude, period, and phase in a variety of clock neurons in the brain [88–90] in accordance with the expression of PDF receptors in those neurons [91–93]. Also, like in *Drosophila*, PDF in cockroaches is both a phase regulator and a locomotor rhythm driver [94, 95]. In crickets, however, since the partial destruction of the optic lobe leads to arrhythmic locomotor activity without elimination of the PDF neurons, PDF may play only minor roles in the behavioral rhythm [96]. Besides regulation of the activity rhythms, PDF plays an important role in the sensory system of crickets. When injected into the optic lobe, PDF is revealed to increase the photoresponsiveness of the optic lobe visual interneurons during early night [97]. In fact, the optic lobe content of PDF increases during the evening [98]. The PDF-induced increase of photoresponsiveness might be associated with the morphological changes reported for flies [99], because the rate of impulse propagation depends on the axonal diameter in unmielinated neurons.

Pdp1ε and *takeout* (*to*) have been located downstream of the circadian clock in *Drosophila*. When the PDP1ε level was kept constantly higher or lower in clock neurons, the clock runs normally but locomotor activity rhythms are disrupted [36]. Thus, PDP1ε seems to drive behavioral rhythms when some specific genes are controlled by its binding to V/P-box. *to* is expressed in the inner part of cardia and the crop, under the regulation by the circadian clock, in response to starvation. The *to* expression is arrhythmic in arrhythmic mutants, per^{01}, tim^{01}, Clk^{Jrk} and cyc^{01}. *to* mutant flies show aberrant locomotor activity and die quickly upon starvation. Based on these results, *to* is concluded to be a clock-controlled output gene controlling feeding behavior [100]. Recently, this link between TO and feeding is suggested to occur through juvenile hormones [101]. Involvement of *Pdp1ε* and *to* deserves to be examined in other insects. Besides these, mRNA levels of hundreds of other genes have been shown to oscillate [39, 102]. Some of these genes may be involved in the output pathways of the clock, inducing a structural daily remodeling of the output circuit [86].

Peripheral oscillators

It has been reported many times that circadian oscillators exist in various tissues of insects (see [2]). For example, physiological studies revealed that sperm release from testis to the associated vas deferens, as well as ecdysone secretion from the prothoracic gland, was controlled in a circadian manner by peripheral oscillators in moths (see [103]).

In *Drosophila*, *per*-driven luciferase activity in a transgenic reporter strain showed that *per* is rhythmically expressed in cells of the compound eye, antennae, proboscis, wings, and legs [104]. Basically, similar results were observed in cells of the ring gland and Malpighian tubules (Mts), which are tissues for endocrine and excretory activity in flies, respectively [105, 106]. Rhythmic expression of *per* in these cells continues in vitro and is able to entrain to the light–dark regime of the culture condition. Transplanted Mts maintain their original phase of oscillation even in the host flies which have been entrained to the reverse light–dark cycle to the donor [107]. Thus, these peripheral oscillators seem to function as a stand-alone pacemaker. The same is true for the olfaction rhythm as an output of the antennal clock [108], and for the circadian synaptic plasticity rhythm, which was recently found as the rhythmic change of size in synaptic boutons [86, 109].

These peripheral tissues are thought to have a similar but not identical molecular machinery of circadian oscillation shown in the central pacemaker described above. One remarkable difference is CRY1's function. A loss of function mutant of CRY1, cry^{b}, showed intact molecular oscillations of PER and TIM in s-LNv, but lost the molecular oscillation in Mts [110]. In the antennae, the rhythmic electroantennographic (EAG) responses to odorants were also abolished in the mutants [111]. These results indicate that, instead of a photoreceptor in the central pacemaker, CRY1 functions as a core component in these peripheral oscillators [112]. Unlike these, however, another peripheral rhythm, which was recently found in the cuticle deposition, persisted in *cry* mutant flies [113]. Thus, there are at least two kinds of circadian molecular machinery in peripheral oscillators: one that requires CRY1 as an essential component, and the other that includes it just as a photoreceptor.

The *Drosophila* antennal clock seems to be one of the best models to investigate how peripheral oscillators control output phenomena at the molecular and behavioral levels. Rhythmic expressions of clock gene products have been observed in the antenna of tephritid fruit flies [114] and moths [115–117]. Thus, the molecular machinery of antennal oscillator seems common principally in insects. Detailed studies in *Drosophila* have revealed that rhythmic

expression of G-protein receptor kinase, regulated by the circadian clock, controls the EAG amplitude in antennae [108, 118, 119]. Although circadian EAG rhythms have also been reported for cockroaches [120] and moths [121, 122], the regulatory mechanism differs substantially from that of *Drosophila* described above. The EAG rhythms in cockroaches are driven by the central pacemaker, even though individual olfactory receptor neurons exhibit circadian rhythms independent of the central pacemaker [123]. Circadian pheromonal responses in male turnip moths are not controlled at the antennal level [121]. In hawkmoths, octopamine and tyramine rhythmically modulate the sensitivity of olfactory sensilla for pheromone-sensitivity, but have no effect on EAG amplitudes [124]. Thus, it is noteworthy that the scenario illustrated in *Drosophila* cannot always be applied in other insects.

Conclusions

Recent physiological and molecular studies on insect circadian rhythms considerably promoted our understanding of the underlying cellular and molecular mechanisms. The cellular network of circadian clock systems shows virtually similar structures among insects, and PDF is commonly involved as an output neuromodulator/neurotransmitter of the system. The molecular oscillatory mechanism is considered to consist of autoregulatory negative feedback loops based mainly on the study of *Drosophila*. However, with the recent knowledge on other insect species, the structure of the feedback loop should be reconsidered. Although most insects examined possess common canonical clock genes, some have additional genes and some lose certain genes. Perhaps the most striking finding is the involvement of *cry2* as a transcriptional repressor [53, 54]. In this context, CRY2 might enter the nucleus in insects where nuclear entry of PER and TIM was not detected by immunocytochemistry, and this deserves to be addressed in future studies. In addition, CRY1 may play a major role in photic entrainment in dipteran and lepidopteran species, but rhodopsins in the external photoreceptors seems to be the major photoreceptor in orthopteran and dyctiopteran species. The molecular mechanisms for photic entrainment through the external photoreceptor pathway remain to be examined. The insect world is estimated to include nearly one million species with highly diverged morphology and physiology to adapt to various environments. Thus, it is likely that the circadian clock system also diverged from an ancestral one, in respect to the core oscillatory mechanism, as well as the photic entrainment pathways. Although information is only available for quite limited orders of the class insecta, future comparative studies should provide an insight for the general understanding of the insect clock mechanism as well as how the variety of clocks have diversified from an ancestral one.

Acknowledgments We thank Dr. Kouji Yasuyama at Kawasaki Medical School for discussion and reading the earlier version of the manuscript, and Miss Tiffanie Chan for linguistic corrections. We also thank anonymous reviewers for their critical reading of the manuscript.

References

1. Dunlap JC, Loros J, DeCoursey PJ (2004) Chronobiology: biological timekeeping. Sinauer, Sunderland
2. Saunders DS (2002) Insect clocks. Elsevier, Amsterdam
3. Page TL (1982) Transplantation of the cockroach circadian pacemaker. Science 216:73–75
4. Tomioka K, Chiba Y (1992) Characterization of optic lobe circadian pacemaker by in situ and in vitro recording of neuronal activity in the cricket *Gryllus bimaculatus*. J Comp Physiol A 171:1–7
5. Fleissner G (1982) Isolation of an insect circadian clock. J Comp Physiol 149:311–316
6. Truman JW (1974) Physiology of insect rhythms, IV: role of the brain in the regulation of the flight rhythm of the giant silkmoths. J Comp Physiol A 95:281–296
7. Helfrich-Förster C (2005) Neurobiology of the fruit fly's circadian clock. Genes Brain Behav 4:65–76
8. Kasai M, Chiba Y (1987) Effects of optic lobe ablation on circadian activity in the mosquito, *Culex pipiens pallens*. Physiol Entomol 12:59–65
9. Hardin PE (2006) Essential and expendable features of the circadian timekeeping mechanism. Curr Opin Neurobiol 16:686–692
10. Helfrich-Förster C (2006) The neural basis of *Drosophila*'s circadian clock. Sleep Biol Rhythms 4:224–234
11. Sheeba V (2008) The *Drosophila melanogaster* circadian pacemaker circuit. J Genet 87:485–493
12. Boothroyd CE, Young MW (2008) The in(put)s and out(put)s of the *Drosophila* circadian clock. N Y Acad Sci 1129:350–357
13. Sandrelli F, Costa R, Kyriacou CP, Rosato E (2008) Comparative analysis of circadian clock genes in insects. Insect Mol Biol 17:447–463
14. Tomioka K, Yoshii T (2006) Entrainment of *Drosophila* circadian rhythms by temperature cycles. Sleep Biol Rhythms 4:240–247
15. Grima B, Chélot E, Xia R, Rouyer F (2004) Morning and evening peaks of activity rely on different clock neurons of the *Drosophila* brain. Nature 431:869–873
16. Stoleru D, Peng Y, Agosto J, Rosbash M (2004) Coupled oscillators control morning and evening locomotor behaviour of *Drosophila*. Nature 431:862–868
17. Rieger D, Shafer OT, Tomioka K, Helfrich-Förster C (2006) Functional analysis of circadian pacemaker neurons in *Drosophila melanogaster*. J Neurosci 26:2531–2543
18. Shang Y, Griffith LC, Rosbash M (2008) Light-arousal and circadian photoreception circuits intersect at the large PDF cells of the *Drosophila* brain. Proc Natl Acad Sci USA 105:19587–19594
19. Dushay MS, Rosbash M, Hall JC (1989) The *disconnected* visual system mutations in *Drosophila* drastically disrupt circadian rhythms. J Biol Rhythms 4:1–27
20. Helfrich-Förster C (1998) Robust circadian rhythmicity of *Drosophila melanogaster* requires the presence of lateral

neurons: a brain-behavioral study of *disconnected* mutants. J Comp Physiol A 182:435–453
21. Murad A, Emery-Le M, Emery P (2007) A subset of dorsal neurons modulates circadian behavior and light responses in *Drosophila*. Neuron 53:689–701
22. Picot M, Klarsfeld A, Chélot E, Malpel S, Rouyer F (2009) A role for blind DN2 clock neurons in temperature entrainment of the *Drosophila* larval brain. J Neurosci 29:8312–8320
23. Wen C-J, Lee H-J (2008) Mapping the cellular network of the circadian clock in two cockroach species. Arch Insect Biochem Physiol 68:215–231
24. Shiga S, Numata H (2009) Roles of PER immunoreactive neurons in circadian rhythms and photoperiodism in the blow fly, *Protophormia terraenovae*. J Exp Biol 212:867–877
25. Lupien M, Marshall S, Leser W, Pollack GS, Honegger H-W (2003) Antibodies against the PER protein of *Drosophila* label neurons in the optic lobe, central brain, and thoracic ganglia of the crickets *Teleogryllus commodus* and *Teleogryllus oceanics*. Cell Tissue Res 312:377–391
26. Page TL (1978) Interaction between bilaterally paired components of the cockroach circadian system. J Comp Physiol 124:225–236
27. Colwell CS, Page TL (1990) A circadian rhythm in neural activity can be recorded from the central nervous system of the cockroach. J Comp Physiol A 166:643–649
28. Tomioka K, Chiba Y (1984) Effects of nymphal stage optic nerve severance or optic lobe removal on the circadian locomotor rhythm of the cricket, *Gryllus bimaculatus*. Zool Sci 1:385–394
29. Tomioka K (1985) Residual circadian rhythmicity after bilateral lamina-medulla removal or optic stalk transection in the cricket, *Gryllus bimaculatus*. J Insect Physiol 31:653–657
30. Rence BG, Loher W (1975) Arrhythmically singing crickets: thermoperiodic reentrainment after bilobectomy. Science 190:385–387
31. Page TL (1985) Circadian organization in cockroaches: effects of temperature cycles on locomotor activity. J Insect Physiol 31:235–243
32. Tomioka K, Chiba Y (1989) Photoperiodic entrainment of locomotor activity in crickets (*Gryllus bimaculatus*) lacking the optic lobe pacemaker. J Insect Physiol 35:827–835
33. Stanewsky R (2002) Clock mechanisms in *Drosophila*. Cell Tissue Res 309:11–26
34. Cyran SA, Buchsbaum AM, Reddy KL, Lin M-C, Glossop NRJ, Hardin PE, Young MW, Storti RV, Blau J (2003) *vrille, Pdp1* and *dClock* form a second feedback loop in the *Drosophila* circadian clock. Cell 112:329–341
35. Glossop NR, Houl JH, Zheng H, Ng FS, Dudek SM, Hardin PE (2003) VRILLE feeds back to control circadian transcription of clock in the *Drosophila* circadian oscillator. Neuron 37:249–261
36. Benito J, Zheng H, Hardin PE (2007) PDP1ε functions downstream of the circadian oscillator to mediate behavioral rhythms. J Neurosci 27:2539–2547
37. Kadener S, Stoleru D, McDonald M, Nawathean P, Rosbash M (2007) *Clockwork orange* is a transcriptional repressor and a new *Drosophila* circadian pacemaker component. Genes Dev 21:1675–1686
38. Lim C, Chung BY, Pitman JL, McGill JJ, Pradhan S, Lee J, Keegan KP, Choe J, Allada R (2007) *Clockwork orange* encodes a transcriptional repressor important for circadian-clock amplitude in *Drosophila*. Curr Biol 17:1082–1089
39. Matsumoto A, Ukai-Tadenuma M, Yamada RG, Houl J, Uno KD, Kasukawa T, Dauwalder B, Itoh TQ, Takahashi K, Ueda R, Hardin PE, Tanimura T, Ueda HR (2007) A functional genomics strategy reveals *clockwork orange* as a transcriptional regulator in the *Drosophila* circadian clock. Gene Dev 21:1687–1700
40. Richier B, Michard-Vanhée C, Lamouroux A, Papin C, Rouyer F (2008) The clockwork orange *Drosophila* protein functions as both an activator and a repressor of clock gene expression. J Biol Rhythms 23:103–116
41. Bae K, Edery I (2006) Regulating a circadian clock's period, phase and amplitude by phosphorylation: insights from *Drosophila*. J Biochem 140:609–617
42. Weber F (2009) Remodeling the clock: coactivators and signal transduction in the cricadian clockworks. Naturwissenschaften 96:321–337
43. Weber F, Hung HC, Maurer C, Kay SA (2006) Second messenger and Ras/MAPK signalling pathways regulate CLOCK/CYCLE-dependent transcription. J Neurochem 98:248–257
44. Akten B, Jauch E, Genova GK, Kim EY, Edery I, Raabe T, Jackson FR (2003) A role for CK2 in the *Drosophila* circadian oscillator. Nat Neurosci 6:251–257
45. Meyer P, Saez L, Young MW (2006) PER-TIM interactions in living *Drosophila* cells: an interval timer for the circadian clock. Science 311:226–229
46. Ashmore LJ, Sehgal A (2003) A fly's eye view of circadian entrainment. J Biol Rhythms 18:206–216
47. Nitabach MN, Blau J, Holmes TC (2002) Electrical silencing of *Drosophila* pacemaker neurons stops the free-running circadian clock. Cell 109:485–495
48. Nitabach MN, Sheeva V, Vera DA, Blau J, Holmes TC (2005) Membrane electrical excitability is necessary for the free-running larval *Drosophila* circadian clock. J Neurobiol 62:1–13
49. Zavodska R, Sehadova H, Sauman I, Sehnal F (2005) Light-dependent PER-like proteins in the cephalic ganglia of an apterygote and a pterygote insect species. Histochem Cell Biol 123:407–418
50. Sauman I, Reppert SM (1996) Circadian clock neurons in the silkmoth *Antheraea pernyi*: novel mechanisms of period protein regulation. Neuron 17:889–900
51. Sauman I, Reppert SM (1996) Molecular characterization of prothoracicotropic hormone (PTTH) from the giant silkmoth *Antheraea pernyi*: developmental appearance of PTTH-expressing cells and relationship to circadian clock cells in central brain. Dev Biol 178:418–429
52. Chang DC, McWatters HG, Williams JA, Gotter AL, Levine JD, Reppert SM (2003) Constructing a feedback loop with circadian clock molecules from the silkmoth, *Antheraea pernyi*. J Biol Chem 278:38149–38158
53. Zhu H, Yuan Q, Briscoe AD, Froy O, Casselman A, Reppert SM (2005) The two CRYs of the butterfly. Curr Biol 15:R953–R954
54. Yuan Q, Metterville D, Briscoe AD, Reppert SM (2007) Insect cryptochromes: gene duplication and loss define diverse ways to construct insect circadian clocks. Mol Biol Evol 24:948–955
55. Zhu H, Sauman I, Yuan Q, Casselman A, Emery-Le M, Emery P, Reppert SM (2008) Cryptochromes define a novel circadian clock mechanism in monarch butterflies that may underlie sun compass navigation. PLoS Biol 6:138–155
56. Goto SG, Denlinger DL (2002) Short-day and long-day expression patterns of genes involved in the flesh fly clock mechanism: *period, timeless, cycle* and *cryptochrome*. J Insect Physiol 48:803–816
57. Miyatake T, Matsumoto A, Matsuyama T, Ueda HR, Toyosato T, Tanimura T (2002) The *period* gene and allochronic reproductive isolation in *Bactrocera cucurbitae*. Proc R Soc Lond B 269:2467–2472
58. Stanewsky R, Kaneko M, Emery P, Beretta B, Wager-Smith K, Kay SA, Rosbash M, Hall JC (1998) The cry^b mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. Cell 95:681–692
59. Ishikawa T, Matsumoto A, Kato T Jr, Togashi S, Ryo H, Ikenaga M, Todo T, Ueda R, Tanimura T (1999) DCRY is a

Drosophila photoreceptor protein implicated in light entrainment of circadian rhythm. Genes Cells 4:57–65
60. Rubin EB, Shemesh Y, Cohen M, Elgavish S, Robertson HM, Bloch G (2006) Molecular and phylogenetic analyses reveal mammalian-like clockwork in the honey bee (*Apis mellifera*) and shed new light on the molecular evolution of the circadian clock. Genome Res 16:1352–1365
61. Moriyama Y, Sakamoto T, Karpova SG, Matsumoto A, Noji S, Tomioka K (2008) RNA interference of the clock gene *period* disrupts circadian rhythms in the cricket *Gryllus bimaculatus*. J Biol Rhythms 23:308–318
62. Moriyama Y, Sakamoto T, Matsumoto A, Noji S, Tomioka K (2009) Functional analysis of the circadian clock gene *period* by RNA interference in nymphal crickets *Gryllus bimaculatus*. J Insect Physiol 55:396–400
63. Sakamoto T, Uryu O, Tomioka K (2009) The clock gene *period* plays an essential role in photoperiodic control of nymphal development in the cricket *Modicogryllus siamensis*. J Biol Rhythms 24:379–390
64. Loher W (1972) Circadian control of stridulation in the cricket *Teleogryllus commodus* Walker. J Comp Physiol 79:173–190
65. Rence BG, Lisy MT, Garves BR, Quilan BJ (1988) The role of ocelli in circadian singing rhythms of crickets. Physiol Entomol 13:201–212
66. Helfrich-Förster C, Edwards T, Yasuyama K, Wisotzki B, Schneuwly S, Stanewsky R, Meinertzhagen IA, Hofbauer A (2002) The extraretinal eyelet of *Drosophila*: development, ultrastracture, and putative circadian function. J Neurosci 22:9255–9266
67. Yoshii T, Todo T, Wülbeck C, Stanewsky R, Helfrich-Förster C (2008) Cryptochrome is present in the compound eyes and a subset of *Drosophila*'s clock neurons. J Comp Neurol 508:952–966
68. Ceriani MF, Darlington TK, Staknis D, Mas P, Petti AA, Weitz CJ, Kay SA (1999) Light-dependent sequentation of TIMELESS by CRYPTOCHROME. Science 285:553–556
69. Myers MP, Wager-Smith K, Rothenfluh-Hilfiker A, Young MW (1996) Light-induced degradation of TIMELESS and entrainment of the *Drosophila* circadian clock. Science 271:1736–1740
70. Lee C, Parikh V, Itsukaichi T, Bae K, Edery I (1996) Resetting the *Drosophila* clock by photic regulation of PER and a PER-TIM complex. Science 271:1740–1744
71. Hanai S, Hamasaka Y, Ishida N (2008) Circadian entrainment to red light in *Drosophila*: requirement of *Rhodopsin 1* and *Rhodopsin 6*. NeuroReport 19:1441–1444
72. Hanai S, Ishida N (2009) Entrainment of *Drosophila* circadian clock to green and yellow light by Rh1, Rh5, Rh6 and CRY. NeuroReport 20:755–758
73. Rieger D, Stanewsky R, Helfrich-Förster C (2003) Cryptochrome, compound eyes, Hofbauer-Buchner eyelets, and ocelli play different roles in the entrainment and masking pathway of the locomotor activity rhythm in the fruit fly *Drosophila melanogaster*. J Biol Rhythms 18:377–391
74. Zimmerman WF, Pittendrigh CS, Pavlidis T (1968) Temperature compensation of the circadian oscillation in *Drosophila pseudoobscura* and its entrainment by temperature cycles. J Insect Physiol 14:669–684
75. Ikeda M, Tomioka K (1993) Temperature dependency of the circadian locomotor rhythm in the cricket *Gryllus bimaculatus*. Zool Sci 10:597–604
76. Yoshii T, Fujii K, Tomioka K (2007) Induction of *Drosophila* behavioral and molecular circadian rhythms by temperature steps in constant light. J Biol Rhythms 22:103–114
77. Glaser FT, Stanewsky R (2005) Temperature synchronization of the *Drosophila* circadian clock. Curr Biol 15:1352–1363
78. Collins BH, Rosato E, Kyriacou CP (2004) Seasonal behavior in *Drosophila melanogaster* requires the photoreceptors, the circadian clock, and phospholipase C. Proc Natl Acad Sci USA 101:1945–1950
79. Majercak J, Chen W-F, Edery I (2004) Splicing of the *period* gene 3′-terminal intron is regulated by light, circadian clock factors, and phospholipase C. Mol Cell Biol 24:3359–3372
80. Majercak J, Sidote D, Hardin PE, Edery I (1999) How a circadian clock adapts to seasonal decreases in temperature and day length. Neuron 24:219–230
81. Chen W-F, Majercak J, Edery I (2006) Clock-gated photic stimulation of *timeless* expression at cold temperatures and seasonal adaptation in *Drosophila*. J Biol Rhythms 21:256–271
82. Chiba Y, Uki M, Kawasaki Y, Matsumoto A, Tomioka K (1993) Entrainability of circadian activity of the mosquito *Culex pipiens pallens* to 24-h temperature cycles, with special reference to involvement of multiple oscillators. J Biol Rhythms 8:211–220
83. Helfrich-Förster C (1995) The *period* clock gene is expressed in central nervous system neurons which also produce a neuropeptide that reveals the projections of circadian pacemaker cells within the brain of *Drosophila melanogaster*. Proc Natl Acad Sci USA 92:612–616
84. Rao KR, Riehm JP (1988) Pigment-dispersing hormones: a novel family of neuropeptides from arthropods. Peptides 9(Suppl.):153–159
85. Park JH, Helfrich-Förster C, Lee G, Liu L, Rosbash M, Hall JC (2000) Differential regulation of circadian pacemaker output by separate clock genes in *Drosophila*. Proc Natl Acad Sci USA 97:3608–3613
86. Fernández MP, Berni J, Ceriani MF (2008) Circadian remodeling of neuronal circuits involved in rhythmic behavior. PLoS Biol 6:e69
87. Renn SCP, Park JH, Rosbash M, Hall JC, Taghert PH (1999) A *pdf* neuropeptide gene mutation and ablation of PDF neurons each cause severe abnormalities of behavioral circadian rhythms in *Drosophila*. Cell 99:791–802
88. Yoshii T, Wullbeck C, Sehadova H, Veleri S, Bichler D, Stanewsky R, Helfrich-Förster C (2009) The neuropeptide pigment-dispersing factor adjusts period and phase of *Drosophila*'s clock. J Neurosci 29:2579–2610
89. Tomioka K, Miyasako Y, Umezaki Y (2008) PDF as a coupling mediator between the light-entrainable and temperature-entrainable clocks in *Drosophila melanogaster*. Acta Biol Hung 59(Suppl.):149–155
90. Miyasako Y, Umezaki Y, Tomioka K (2007) Separate sets of cerebral clock neurons are responsible for light and temperature entrainment of *Drosophila* circadian locomotor rhythms. J Biol Rhythms 22:115–126
91. Mertens I, Vandingenen A, Johnson EC, Shafer OT, Li W, Trigg JS, Loof AD, Schoofs L, Taghert PH (2005) PDF receptor signaling in *Drosophila* contributes to both circadian and geotactic behaviors. Neuron 48:213–219
92. Hyun S, Lee Y, Hong S-T, Bang S, Paik D, Kang J, Shin J, Lee J, Jeon K, Hwang S (2005) *Drosophila* GPCR Han is a receptor for the circadian clock neuropeptide PDF. Neuron 48:267–278
93. Lear BC, Merrill CE, Lin J-M, Schroeder A, Zhang L, Allada R (2005) A G protein-coupled receptor, groom-of-PDF is required for PDF neuron action in circadian behavior. Neuron 48:221–227
94. Petri B, Stengl M (1997) Pigment-dispersing hormone shifts the phase of the circadian pacemaker of the cockroach *Leucophaea maderae*. J Neurosci 17:4087–4093
95. Lee C-M, Su M-T, Lee H-J (2009) Pigment dispersing factor: an output regulator of the circadian clock in the german cockroach. J Biol Rhythms 24:35–43

96. Okamoto A, Mori H, Tomioka K (2001) The role of optic lobe in generation of circadian rhythms with special reference to the PDH immunoreactive neurons. J Insect Physiol 47:889–895
97. Saifullah ASM, Tomioka K (2003) Pigment-dispersing factor sets the night state of the medulla bilateral neurons in the optic lobe of the cricket, *Gryllus bimaculatus*. J Insect Physiol 49:231–239
98. Abdelsalam S, Uemura H, Umezaki Y, Saifullah ASM, Shimohigashi M, Tomioka K (2008) Characterization of PDF-immunoreactive neurons in the optic lobe and cerebral lobe of the cricket, *Gryllus bimaculatus*. J Insect Physiol 54:1205–1212
99. Pyza E, Meinertzhagen IA (1996) Neurotransmitters regulate rhythmic size changes amongst cells in the fly's optic lobe. J Comp Physiol A 178:33–45
100. Sarov-Blat L, So WV, Liu L, Rosbash M (2000) The *Drosophila takeout* gene is a novel molecular link between circadian rhythm and feeding behavior. Cell 101:647–656
101. Meunier N, Belgacem YH, Martin J-R (2007) Regulation of feeding behaviour and locomotor activity by *takeout* in *Drosophila*. J Exp Biol 210:1424–1434
102. Matsumoto A (2006) Genome-wide screenings for circadian clock genes in *Drosophila*. Sleep Biol Rhythms 4:248–254
103. Giebultowicz JM (1999) Insect circadian clocks: is it all in their heads? J Insect Physiol 45:791–800
104. Plautz JD, Kaneko M, Hall JC, Kay SA (1997) Independent photoreceptive circadian clocks throughout *Drosophila*. Science 278:1632–1635
105. Emery IF, Noveral JM, Jamison CF, Siwickii KK (1997) Rhythms of *Drosophila period* gene in culture. Proc Natl Acad Sci USA 94:4092–4096
106. Giebultowicz JM, Hege DM (1997) Circadian clock in Malphigian tubules. Nature 386:664
107. Giebultowicz JW, Stanewsky R, Hall JC, Hege DM (2000) Transplanted *Drosophila* excretory tubules maintain circadian clock cycling out of phase with the host. Curr Biol 10:107–110
108. Tanoue S, Krishnan P, Chatterjee A, Hardin PE (2008) G protein-coupled receptor kinase 2 is required for rhythmic olfactory responses in *Drosophila*. Curr Biol 18:787–794
109. Mehnert KI, Cantera R (2008) A peripheral pacemaker drives the circadian rhythm of synaptic boutons in *Drosophila* independently of synaptic activity. Cell Tiss Res 334:103–109
110. Ivanchenko M, Stanewsky R, Giebultowicz JM (2001) Circadian photoreception in *Drosophila*: functions of cryptochrome in periopheral and central clocks. J Biol Rhythms 16:205–215
111. Krishnan B, Levine JD, Lynch MK, Dowse HB, Funes P, Hall JC, Hardin PE, Dryer SE (2001) A new role for cryptochrome in a *Drosophila* circadian oscillator. Nature 411:313–317
112. Emery P, Stanewsky R, Hall JC, Rosbash M (2000) *Drosophila* cryptochromes: a unique circadian-rhythm photoreceptor. Nature 404:456–457
113. Ito C, Goto SG, Shiga S, Tomioka K, Numata H (2008) Peripheral circadian clock for the cuticle deposition rhythm in *Drosophila melanogaster*. Proc Natl Acad Sci USA 105:8446–8451
114. An X, Tebo M, Song S, Frommer M, Raphael KA (2004) The *cryptochrome* (*cry*) gene and a mating isolation mechanism in tephritid fruit flies. Genetics 168:2025–2036
115. Merlin C, François MC, Queguiner I, Maïbèche-Coisné M, Jacquin-Joly E (2006) Evidence for a putative antennal clock in *Mamestra brassicae*: Molecular cloning and characterization of two clock genes—*period* and *cryptochrome*—in antennae. Insect Mol Biol 15:137–145
116. Schuckel J, Siwicki KK, Stengl M (2007) Putative circadian pacemaker cells in the antenna of the hawkmoth *Manuca sexta*. Cell Tiss Res 330:271–278
117. Merlin C, Lucas P, Rochat D, François MC, Maïbèche-Coisné M, Jacquin-Joly E (2007) An antennal circadian clock and circadian rhythms in peripheral pheromone reception in the moth *Spodoptera littoralis*. J Biol Rhythms 22:502–514
118. Krishnan P, Dryer SE, Hardin PE (2005) Measuring circadian rhythms in olfaction using electroantennograms. Meth Enzymol 393:495–508
119. Krishnan P, Chatterjee A, Tanoue S, Hardin PE (2008) Spike amplitude of single-unit responses in antennal sensillae is controlled by the *Drosophila* circadian clock. Curr Biol 18:803–807
120. Page TL, Koelling E (2003) Circadian rhythm in olfactory response in the antennae controlled by the optic lobe in the cockroach. J Insect Physiol 49:697–707
121. Rosén WQ, Han GB, Lofstedt C (2003) The circadian rhythm of the sex-pheromone-mediated behavioral response in the tunip moth, *Agrotis segetum*, is not controlled at the peripheral level. J Biol Rhythms 18:402–408
122. Flecke C, Dolzer J, Krannich S, Stengl M (2006) Perfusion with cGMP analogue adapts the action potential response of pheromone-sensitive sensilla trichoidea of the hawkmoth *Manduca sexta* in a daytime-dependent manner. J Exp Biol 209:3898–3912
123. Saifullah ASM, Page TL (2009) Circadian regulation of olfactory receptor neurons in the cockroach antenna. J Biol Rhythms 24:144–152
124. Flecke C, Stengl M (2009) Octopamine and tyramine modulate pheromone-sensitive olfactory sensilla of the hawkmoth *Manduca sexta* in a time-dependent manner. J Comp Physiol A 195:529–545
125. Helfrich-Förster C (2002) The circadian system of *Drosophila melanogaster* and its light input pathways. Zoology 105:297–312