

Chapter 2

Oxidative Stress and Its Role in the Synchronization of Circadian Rhythms in Crustaceans: An Ecological Perspective

María Luisa Fanjul-Moles and Julio Prieto-Sagredo

Abstract This work reviews concepts regarding the endogenous circadian clock and the relationship between oxidative stress (OS), light, and entrainment in different organisms, particularly in crayfish. In the first section, the molecular control of circadian rhythms in invertebrates, particularly in *Drosophila*, is reviewed, and this model is contrasted with recent reports on the circadian genes and proteins in crayfish. Second, the redox mechanisms and signaling pathways that participate in the entrainment of the circadian clock in different organisms such as zebrafish, its relationship with the redox state, and synchronization by cryptochromes is discussed. Finally, the relationship between metabolism, ROS signals, and transcription factors, such as HIF-1- α in crayfish, as well as the possibility that HIF-1- α participates in the regulation of circadian control genes in crustaceans, particularly in crayfish, is discussed.

2.1 Redox State as an Entrainment Pathway

Different environmental cyclic cues, or *zeitgeber*, are able to synchronize the circadian rhythms, with light being one of the most important. Circadian rhythms are entrained by light to adapt to the daily solar cycles, and the 24-h light–dark cycle (LD) is considered the most important *zeitgeber* for synchronization. In nature, the intensity and spectrum of light change seasonally throughout the daily cycle, specifically near dawn and dusk. The photo-entrainment of the circadian clock depends on these two light parameters, producing physiological and behavioral rhythms that match the particular features of the environmental cycles, such as the time of sunrise and sunset, organizing the internal temporal order of the animal according to external time (Bradshaw and Holzapfel 2010).

M.L. Fanjul-Moles (✉) • J. Prieto-Sagredo
Lab. Neurofisiología Comparada de Invertebrados, Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México,
Av. Universidad 3000, 04510 México, D.F., México
e-mail: mfajul@gmail.com

In addition to light, other extrinsic factors, including temperature, activity, and food availability, can advance or delay (phase shift) circadian rhythms, thereby synchronizing the clock with the external environment (Pittendrigh 1981). This coupling of environmental cues to the clock is termed entrainment. Another class of stimuli based on arousal or activity can also reset an animal's circadian clock in a manner distinct from light. The mechanism underlying these nonphotic phase shifts is unknown, although suppression of canonical clock genes and immediate early genes has been implicated (Antle et al. 2008).

Thus, functioning as an endogenous clock, the circadian system entrains the overt rhythms to the seasonal photoperiodic changes. Daily exogenous fluctuations in the external environment such as light and temperature also produce oxidative stress (OS) in a predictable manner. Meanwhile, daily and endogenous fluctuations produce reactive oxygen species (ROS) such as superoxide anions and hydroxide peroxide as a consequence of metabolism and behavior. These ROS may function as intracellular second messengers (Finkel 1998). In animals, circadian and exogenous daily variations in metabolism, locomotor, and brain activities result in corresponding oscillations of the redox state. External and internal induced oxidative stresses may perturb overt rhythms and the proper internal synchronization, that is, the internal temporal order of physiological processes, and thus alter their physical fitness. In addition, animals are able to defend themselves against the periodic rise in ROS by means of compensatory antioxidative rhythms (Hardeland et al. 2003).

2.2 Circadian Photopigments and Their Response to Light

Notably, in some animals peripheral clocks are directly light responsive (Whitmore et al. 2002). In a variety of organisms, photopigments undergo forward light-induced reactions involving electron transfer to the excited state, flavin, to generate radical intermediates, which correlate with the biological activity of the intermediates (Sancar 2003).

The light-dependent signaling state in cryptochrome (CRY), flavin, and folate-containing blue-light photoreceptors, proposed as circadian extra-retinal photoreceptors in invertebrates such as *Drosophila* (Stanewsky 2003), is also present in crustaceans. In the crayfish *Procambarus clarkii* and *Cherax destructor* (Fanjul-Moles et al. 2004; Sullivan et al. 2009), CRY is involved in the entrainment of the clock (Fig. 2.1). Cryptochromes are found throughout the biological kingdom, ranging from protists to plants, animals, and humans (Stanewsky 2003; Lin and Todo 2005). They are evolutionarily derived from photolyases, or DNA-repair enzymes. Both cryptochromes and photolyases exhibit a high degree of structural homology and bind the same flavin adenine dinucleotide (FAD) and folate light-absorbing cofactors. Although belonging to the DNA photolyase/cryptochrome protein family and being highly similar in amino acid sequence (Todo 1999; Sancar 2000), they differ in their biological function. Cryptochromes have lost the ability to repair DNA, but they play a key role in controlling the circadian clock in plants and animals.

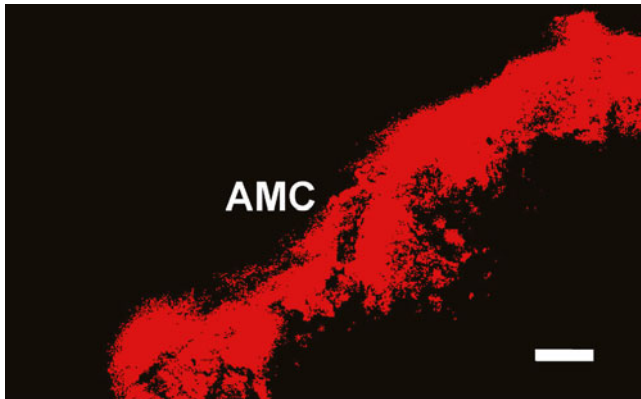


Fig. 2.1 Confocal image of cryptochrome (CRY) immunoreactivity in the *Procamburus clarkii* brain at 19:00. The image corresponds to optical sections of a whole-mount preparation. Neurons of the protocebral anterior medial cluster (AMC) express strong CRY immunoreactivity. Bars 100 μ m. (Modified from Fanjul-Moles et al. 2004)

The photochemistry and the signaling pathways of cryptochromes are just beginning to be understood. Recent attention has focused primarily on the pathway of flavin photoreduction as a possible mechanism of photoreceptor activation (Müller and Ahmad 2011). Studies with isolated proteins have shown that these can be photoreduced from flavin in the oxidized state to the radical state by light in the presence of a reducing agent. The photoreduction of flavin correlates with its biological activity, as shown by an action spectra with maximal activity at a 450-nm peak with no activity above 500 nm (Hoang et al. 2008; VanVickle-Chavez and Van Gelder 2007; Kay et al. 2003; Yu et al. 2010). In the presence of flavin-containing oxidases, light drives the production of intracellular ROS, such as H_2O_2 (Uchida et al. 2010). The excess production of ROS has deleterious effects because ROS can react with various cellular targets to cause OS (Dalton et al. 1999). However, as already mentioned, light-induced ROS can also take on a signaling role by stimulating intracellular signal pathways that lead to transcriptional activation, including the transactivation of genes.

Cryptochromes were originally identified in plants and have orthologues and paralogues among insects and vertebrates (Yu et al. 2010). The role of cryptochromes in the circadian clock differs among species. In mammals, CRY1 and CRY2 represent the core of the circadian oscillator with a light-independent function (Lin and Todo 2005); however, in *Drosophila* and other insects, CRYs were originally thought to act as circadian photoreceptors. Recently, in non-drosophilid insects, a second cry (cry2) gene has been identified in some species that encodes for a light-insensitive protein; this is more similar in sequence to the mammalian CRYs that act as transcriptional repressors (Zhu et al. 2005). Other insects, such as bees and beetles, possess only a mammalian-like CRY (Yuan et al. 2007).

Drosophila CRY also appears to have a tissue-specific pleiotropic role, because in clock neurons that generate rhythmic locomotor behavior it acts as a photoreceptor

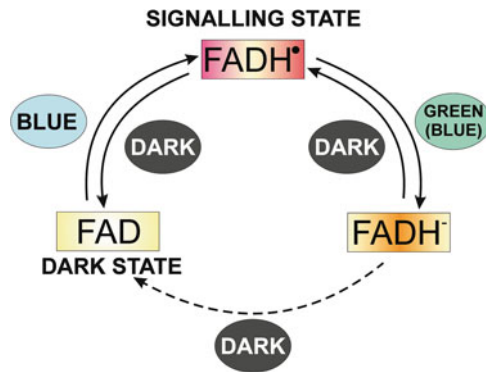


Fig. 2.2 The photocycle of plant cryptochromes. In the dark, the flavin chromophore is in its oxidized state. *Blue light* induces conversion to a metastable semi-quinone redox state that is the activated signaling state. *Green light* causes further reduction to the fully reduced redox state of flavin, which is inactive in signaling. In the *dark*, fully reduced flavin reoxidizes to the fully oxidized form and can be reactivated by *blue light*. The photocycle of plant cryptochromes is different from DNA photolyases, in which only the fully reduced redox state is catalytically active. (Reproduced with permission from Hoang et al. 2008)

and mediates light synchronization of the circadian clock by promoting the light-dependent degradation of dTIM (Emery et al. 1998; Peschel et al. 2009). After absorbing a photon, dCRY undergoes a conformational change involving its C-terminal domain and binds to dTIM, which is then tagged for ubiquitination and proteasomal degradation. The mechanism by which dCRY initiates the cascade of events that leads to dTIM degradation remains unclear (Dubruille et al. 2009). However, the homology between *Drosophila* CRY and photolyases, or light-dependent enzymes utilizing a flavin cofactor to repair DNA, suggests that flavin-dependent reduction/oxidation redox reactions may be involved in dCRY functions (Froy et al. 2002). Cryptochrome-bound flavin is found in an oxidized redox state in vivo, and light activation results in flavin photoreduction to a radical intermediate that represents the likely signaling state. The derived photocycle of animal cryptochromes is therefore similar to the reaction mechanism of plant cryptochromes (Fig. 2.2). Both photocycles involve the reduction of flavin, leading to a cycling between the reduced (active) and oxidized (inactive) redox forms (Berndt et al. 2007; Hoang et al. 2008).

In *Drosophila* it has been proposed that CRY activation involves intramolecular electron transfer and presumably subsequent conformational changes; thus, the cellular redox status also regulates the transfer of photic information and CRY stability (Lin et al. 2001). Using a microarray analysis, Sathyanarayanan et al. (2008) identified three thioredoxin domain-containing redox molecules (GstE7, Tx1, and CG11790) and a cytochrome p450, CYP49a1, that modulate light-dependent CRY and TIM degradation. These results suggest that cellular redox status and electron transfer modulate the light-dependent activation of CRY, which in turn affects the subsequent transmission of the light signal to TIM and the degradation of CRY itself, with the subsequent clock resetting.

In zebrafish cells, the light-induced redox changes stimulating intracellular mitogen-activated protein kinase (MAPK) signaling that transduces photic signals to zCry1a gene transactivation (Hirayama et al. 2009). Importantly, light also drives the production of intracellular ROS, such as H_2O_2 , that leads to an altered redox status and increases intracellular catalase activity by stimulating catalase transcription, an event which occurs after the maximum expression of the zCry1a gene has been reached (Uchida et al. 2010). This increased catalase activity diminishes light-induced cellular ROS levels, resulting in decreased zCry1a transcription and creating a negative feedback loop. Thus, this altered redox state triggers the transduction of photic signals that regulate and synchronize the circadian clock (Uchida et al. 2010) (Fig. 2.3).

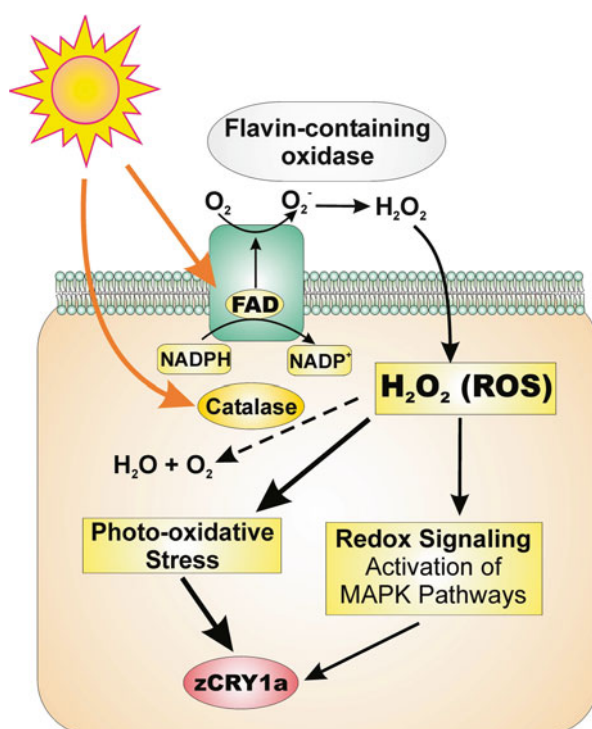


Fig. 2.3 Potential molecular mechanism underlying light-dependent redox signaling in zebrafish. In the presence of flavin-containing oxidases, light drives the production of intracellular reactive oxygen species (ROS) such as H_2O_2 . Excess ROS production has deleterious effects because ROS can react with various cellular targets to cause photo-oxidative stress. However, light-induced ROS can also take on a signaling role by stimulating MAPK pathways that lead to transcriptional activation, including transactivation of the zCry1a gene. Light also increases catalase transcription and thus intracellular catalase activity, resulting in H_2O_2 degradation and decreased photo-oxidative stress. This reduction in ROS also leads to decreased zCry1a expression, thus creating a negative feedback loop that directly impinges on the circadian clock. (Reproduced with permission from Hirayama et al. 2009)

2.3 Cryptochromes, Circadian Rhythms, and Redox Mechanisms in Crustaceans

In crustaceans, some behavioral studies have reported rhythmic activity–rest changes as well as synchronization or entrainment of circadian rhythms by blue light (Fanjul-Moles et al. 1992; Bernal-Moreno et al. 1996; Miranda-Anaya and Fanjul-Moles 1997; Aguzzi et al. 2009, 2011). Recently, it has been reported that positive phototactic circadian rhythmicity is stronger under blue light in the parasite *Argulus japonicus* (Branchiura) (Yoshizawa and Nogami 2008), suggesting the presence of cryptochromes in the circadian photoreceptor system of different groups of crustaceans. The circadian system of genus *Procambarus* is considered to be distributed throughout a multioscillatory system in a hierarchical nature (Fanjul-Moles and Prieto-Sagredo 2003). It is a complex model, which includes three pairs of coupled oscillators such as the retina, the eyestalk X-organ–sinus gland complex (XOSG), putative brain pacemakers, and probably a pair of extra-retinal photoreceptors in the caudal abdominal ganglion. A pair of extra-retinal photoreceptors in the brain (BPR) are involved in light-dependent entrainment (Strauss and Dirksen 2010) (Fig. 2.4), which in crayfish, similar to insects, appears to be mediated by CRYs (Miranda-Anaya and Fanjul-Moles 1997) (Fig. 2.5).

Immunochemical, biochemical, and behavioral studies using a *Drosophila* anti-CRY antibody (Fanjul-Moles et al. 2004; Escamilla-Chimal and Fanjul-Moles 2008; Sullivan et al. 2009) have shown the presence and circadian rhythm of CRY in the brain of crayfish, as well as its role in this organism in the activity of rhythm synchronization. The molecular characteristic of this protein has not been characterized.

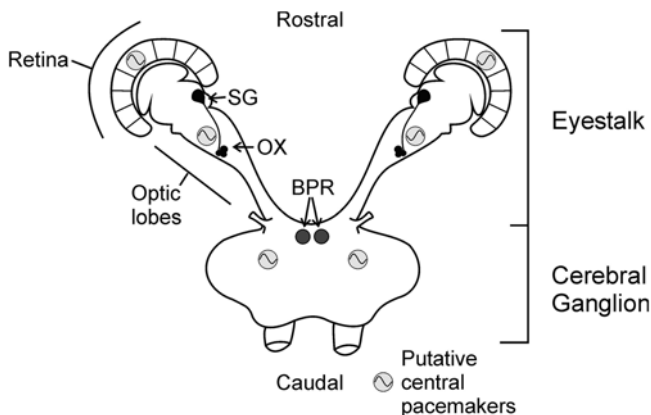


Fig. 2.4 Schematic summary of known circadian oscillators and their functional correlations in crayfish. Established physiological oscillators or pacemakers (grey circles) exist in the retina, the X-organ–sinus gland complex, and likely the brain, whereas the brain photoreceptor (BPR) is not an oscillator but is responsible for light-driven entrainment of locomotory rhythms. BPR brain photoreceptor, SG sinus gland, XO X organ

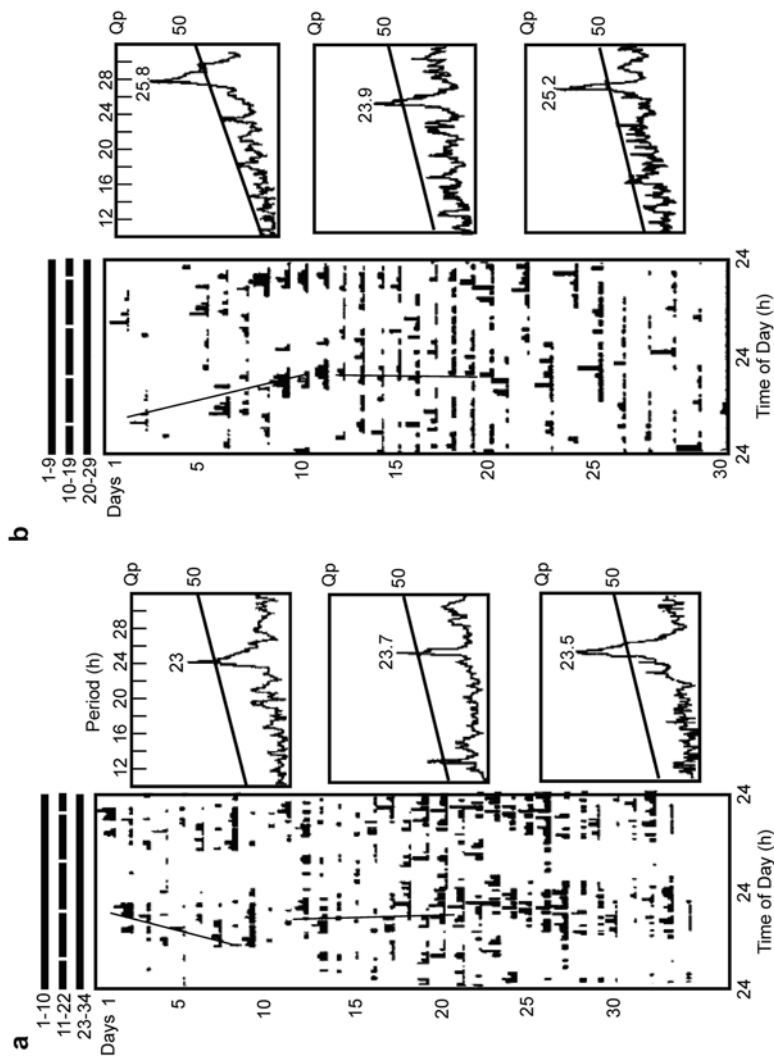


Fig. 2.5 Locomotor activity record and its associate periodograms of two juvenile crayfish, intact (a) and retina ablated (b), that were exposed to red Skeleton photoperiod (SP) (two 30-min pulses of 640 nm and 25 W m⁻²). Note the “lights-on” peak during the entrainment condition in the intact crayfish is not present in retina-deprived crayfish. Note the rhythm splitting as well as the change of the rhythm period value during the DD post entrainment. (a) A 19:00 pulse light occurring at CT 14 during the 11th day evokes a phase delay. (b) A 19:00 light pulse occurring at CT 13 produces an advance. *Regression line* periods are 22.8 and 24.1 h (a) and 25.3 and 23.88 h (b), respectively. (Modified from Miranda-Anaya and Fanjul-Moles 1997)

Based on both the homologies and the molecular mass detected by Western blot analysis (60 kDa), and considering that the peptide sequence within the C-terminus of *Drosophila* sp. used to generate the antibody was specific to the *Drosophila melanogaster* and *Drosophila pseudoobscura* CRY 1, this protein seems to function as a photopigment in the brain of crayfish (Escamilla-Chimal and Fanjul-moles 2008; Sullivan et al. 2009). Recently, Mazzotta et al. (2010) isolated and cloned a new CRY gene from the Antarctic krill (*Euphausia superba*). The EsCRY gene appears to be an orthologue of mammalian-like CRYs and clusters with the insect CRY2 subfamily. Importantly, these results suggest the presence of two CRYs in crustaceans, similar to mammals and some insects. Both CRYs could act either as a photopigment or as a transcription factor participating in the transcription–translation loop (TTL) of the core of the clock.

Both the mechanisms of the photo-activation of CRY and the different pathways involved in the resetting of the clock are unknown in crustaceans; however, the results of some experiments provide hints toward ROS and redox mechanisms underlying these processes. Fanjul-Moles et al. (2009) demonstrated bi- and unimodal daily and circadian rhythms in all glutathione (GSH) parameters, substrates, and enzymes in the putative pacemakers of crayfish, including the optic lobe, brain, and retina, as well as an apparent direct effect of light on these rhythm conditions, especially in the retina.

In a series of experiments we used two different species, *Procambarus clarkii* and *Procambarus digueti*, adapted with a different geographical distribution and different latitudes, 27° and 17° N, with different luminosity and daylength throughout the year. During the summer *P. clarkii* is exposed to photoperiods of as long as 16 h whereas in southern latitudes the maximal daylight for *P. digueti* is about 13 h. Using animals of both species collected in the field, we performed different eco-physiological studies in our laboratory (Prieto-Sagredo et al. 2000; Fanjul-Moles and Prieto-Sagredo 2003). The results of different experiments showed daily and circadian changes in OS produced by different light conditions are counterbalanced by antioxidant circadian rhythms of glutathione and related enzymes detected in liver and hemolymph. The robust antioxidant circadian rhythms of *P. clarkii* are able to entrain to all conditions, resetting to lights on or off. However, the weak circadian glutathione system of *P. digueti* did not entrain to LD cycles, showing a random distribution of phases (Fig. 2.6). This observation suggests that the photoperiodic history of each species determines the adaptive abilities of entrainment to extreme light conditions, probably by means of the OS control of circadian antioxidative mechanisms. This finding, of course, indicates interesting ecological implications in the process of light synchronization. Moreover, we found circadian differences between liver and hemolymph glutathione (GSH) oscillatory systems that indicate differences in the coupling strength of both compartments to the crayfish circadian system. These results led us to propose the hemolymph as a passive system responding to an oscillatory driving force depending on various self-sustaining oscillators, one of which could be the liver, proposed elsewhere as a peripheral clock in mammals (Stokkan et al. 2001).

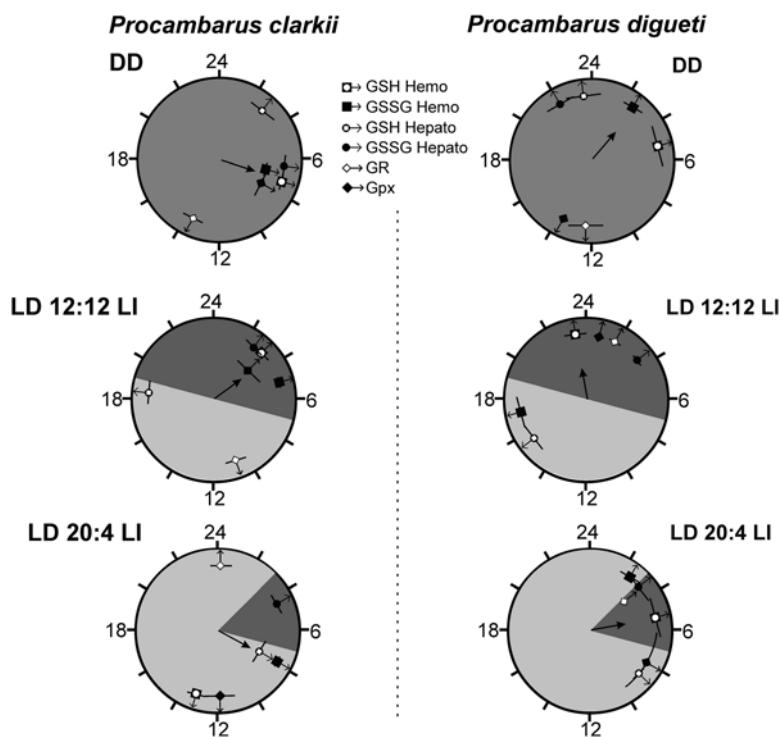


Fig. 2.6 Estimated acrophases of the glutathione daily rhythms of *Procamburus clarkii* and *P. digueti* entrained to the control and experimental conditions. The mean vector indicates the clustering direction of the rhythms acrophase. Horizontal bars indicate the mean $\pm$ 6 SE. V test indicated, for *P. clarkia*, $P < 0.05$ in LD 12:12 LI and LD 20:4 LI. (Modified from Fanjul-Moles and Prieto-Sagredo 2003)

Herein we investigated the retina and optic lobe–brain (OL-B) circadian GSH system and its ability to handle reactive oxygen species (ROS) produced as a consequence of metabolic rhythms and light variations. We characterized daily and antioxidant circadian variations of the different parameters of the glutathione system, including GSH, oxidized glutathione (GSSG), glutathione reductase (GR), and glutathione peroxidase (GPx), as well as metabolic and lipoperoxidative circadian oscillations in the retina and optic lobe–brain complex (OL-B) proposed elsewhere as central pacemakers of crayfish, determining internal and external GSH system synchrony. The results of our experiments demonstrated bi- and unimodal daily and circadian rhythms in all GSH cycle parameters, substrates, and enzymes in OL-B and retina, as well as an apparent direct effect of light on these rhythms, especially in the retina. The luminous condition appears to stimulate the GSH system to antagonize ROS and lipid peroxidation (LPO) daily and circadian rhythms occurring in both structures, oscillating with higher LPO under dark conditions.

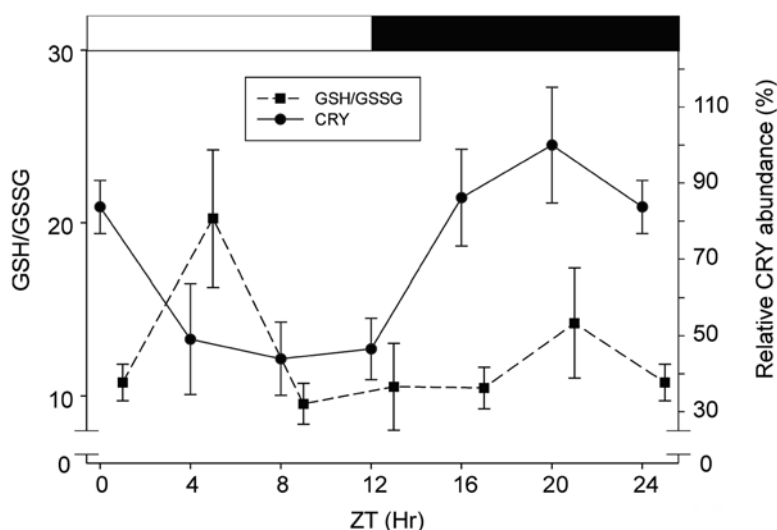


Fig. 2.7 Relationship between GSH/GSSG ratio and CRY abundance in the brain of *P. clarkii* submitted to a 24-h LD cycle. Light drives photo-oxidative stress, as is shown by the low GSH/GSSG ratio, and the decrease in CRY abundance suggests CRY degradation. However, importantly light seems to upregulate glutathione reductase (not shown), which increases GSH and the GSH/GSSG ratio. This reduction in reactive oxygen species (ROS) leads to greater CRY degradation. The abundance increments of CRY occur in the dark phase (from ZT 12 to ZT 20) when the brain showed maximal oxidation (not shown). ZT zeitgeber time. (Modified from Escamilla-Chimal and Fanjul-Moles 2008 and Fanjul-Moles et al. 2009)

These results confirmed that light producing photo-oxidation is a factor that determines ROS in crayfish, as was previously reported (Duran-Lizarraga et al. 2001). Putative pacemakers in the retina and eyestalk–brain complex showed higher GSH/GSSG ratio mean values in LD than in DD. The increment in this ratio in the retina and eyestalk coincides with the photophase, indicating that OS produced by light is antagonized by the rapid transformation of GSSG into GSH. This result suggests that the antioxidant defense system (ADS) is upregulated at the mid-photophase level, between ZT4 and ZT6 at the same time of the CRY higher decrement (Fanjul-Moles et al. 2004, 2009; Escamilla-Chimal and Fanjul-Moles 2008) (Fig. 2.7). This response suggests a photo-oxidative redox stress signal similar to those of the model of light-induced signaling cascades potentially involved in the control of the circadian clock of zebrafish (Uchida et al. 2010). In crayfish, the redox signal might be the result of the direct effect of ROS produced by the light onset of the LD cycle on CRY to reset the clock. This ROS increment seems activate the glutathione system, especially glutathione reductase (GR), directly or indirectly, through an unknown signaling pathway (Fanjul-Moles et al. 2009). It has been proposed that light-induced ROS, especially in response to UVB, is involved in the activation of mitogen-activated protein kinase (MAPK) downstream antioxidant-response effectors (Assefa et al. 2005), and certain MAPK signal regulation by GSH has been

described recently in the mouse (Limón-Pacheco et al. 2007). Many reports have indicated that the GSH/GSSG ratio is an important “sensor” for ADS regulation (Dalton et al. 1999; Dalle-Donne et al. 2009; Maciel et al. 2010), occurring in parallel with ROS increment. These changes promote the oxidation of protein cysteinyl thiols, which activate or deactivate specific enzymes in the signaling cascades (for a review, see Maciel et al. 2010).

As already mentioned, the photic input to the clock directly activates MAPK signaling cascades in zebrafish cells. The light-induced activation of these pathways controls the expression of two evolutionarily related genes, *z64Phr* and *zCry1a*, revealing that light-dependent DNA repair and entrainment of the circadian clock share common regulatory pathways (Hirayama et al. 2009). We are exploring similar possibilities in crayfish, where we have already found the presence and cycling of MAPK proteins, such as extracellular-signal-regulated kinases (ERK), in some of the putative pacemakers of this animal, such as brain and eyestalk (unpublished data), as well as the presence of PKA and its possible participation in the circadian regulation of cAMP response element-mediated circadian gene expression (unpublished data).

2.4 Synchronization and Metabolism

Another stimulus able to reset the phase of the circadian system depends on the modulation of the redox state through metabolism (Bianchi 2008). Experimental evidence supports the link between metabolism and circadian rhythms in mammals. The timing of metabolism can be influenced by the circadian system via systemic cues from the suprachiasmatic nuclei (SCN) or peripheral oscillators. A relationship between both processes by means of the AMP/ATP ratio, sensed mainly by adenosine monophosphate-dependent protein kinase (AMPK) or glucose and fatty acid sensors, has been shown (Albrecht 2012). Furthermore, it is well documented that the circadian clock controls the level of many cellular and circulating metabolites, and it is accepted that there is a cyclic relationship between the circadian clock and metabolism wherein the rhythm impacts metabolism and metabolism feeds back to impinge upon the rhythm (Roennnenberg and Mero 1999). Interestingly, AMPK impacts the mammalian circadian clock mechanisms in various ways. It can directly phosphorylate CRY1, leading to destabilization and degradation of this core clock protein, and consequently affecting the negative limb of the circadian clock mechanism, or it may modulate PER2 protein stability via an indirect mechanism involving casein kinase ϵ (CK1 ϵ). (Lamia et al. 2009) Thus, AMPK appears to be another potential regulator of the coupling and interaction between metabolism and the circadian clock, as it is in the case of light.

Thus, it is plausible to hypothesize that essentially any parameter reliably changing as a function of metabolic activity, such as ROS, is a candidate for the direct entrainment of the molecular core clock. ROS and antioxidants influence the expression of a number of genes in eukaryotes. Studies from different organisms have

demonstrated that the direct effect of the redox state depends on NAD cofactors for the transcriptional and translational control of the molecular clock. Experiments in mammalian cell culture revealed that the reduced forms NADH and NADPH stimulated the binding of CLOCK/BMAL1 and NPAS2/BMAL1 to their cognate E-box sequences, although the oxidized forms NAD⁺ and NADP⁺ strongly inhibited its binding (Rutter et al. 2001). The reduced cofactors NADH and NADPH have daily rhythms in plants (Roennnenberg and Merrow 1999) and regulate the activity of clock-like transcription factors in animals, strongly enhancing their DNA binding (Rutter et al. 2001). In crayfish the presence and oscillation of glutathione, and of the related enzymes in the circadian system of crayfish as already mentioned, indicate the presence and cycling of NADPH as an electron donor to reduce the system and perhaps to regulate the clockwork in this crustacean.

One mechanism by which the clock's output pathways are predicted to be rhythmically controlled is through transcription factors or signaling molecules that are themselves components of the oscillator. These direct outputs may in turn regulate downstream clock-controlled genes (CCGs) in a complex web of events. For example, in invertebrates, such as the fly, the positive oscillator components dCLK and CYC bind to E-box elements in gene promoters and mediate rhythmic transcription of negative components of the oscillator PER and TIM, as well as some clock outputs, such as the distal pigment hormone (PDH) gene. A similar situation could exist in crustaceans, where a CCG, such as PDH (Farca-Luna et al. 2010; Strauss et al. 2011) or the crustacean hyperglycemic hormone (CHH), have been proposed as putative clock outputs (Fanjul-Moles and Prieto-Sagredo 2003). In crayfish, metabolic changes, such as in the production of CHH and its relationship to glucose and lactate rhythmic concentrations, show evidence that circadian rhythms correlate with the expression of the hypoxia-inducible factor- α (HIF-1- α) at dawn and dusk (Velázquez-Amado et al. 2012).

The transcription factor HIF-1- α is a heterodimer composed of a regulated protein, HIF-1- α , and its constitutive partner, HIF-1- α -b. This protein is the most prominent and best described transcription factor that activates the hypoxia-induced expression of target genes involved in cellular and physiological responses such as oxygen transport, iron metabolism, glycolysis, glucose uptake, and growth factor signaling (Bozek et al. 2010). The gene coding for HIF-1- α is found to be clock controlled in the mouse (Panda et al. 2002); furthermore, HIF-1- α participates in metabolism (Bozek et al. 2007; Bozek et al. 2009). Thus, it has been proposed that this transcription factor is connected to the circadian clock. In mammals, HIF-1- α has been described as a factor with an overrepresented number of binding sites in the promoters of CCG (Reinke et al. 2008). Novel target genes activated by HIF-1- α are being constantly identified, and targets include genes with protein products involved in many functions including energy metabolism and hormonal control (Dröge 2002). However, in addition to regulating the response of different animals to hypoxia, HIF-1- α is considered part of the mechanisms that regulate the transcriptional output of the circadian clock in several organisms. In mammals, HIF-1- α has been described as a factor with a number of overrepresented binding sites in the promoters of CCGs, and its mRNA and promoter activity in human cells has recently been reported (Fujita et al. 2010).

HIF-1-alpha regulates the metabolic adjustment to hypoxia in many crustacean species, such as crabs (Head 2010), crayfish (Velázquez-Amado et al. 2012), shrimp (Kodama et al. 2012), and *Daphnia* (Gorr et al. 2004). Hypoxia and hyperoxia present during physiological states of the life cycle in crustaceans, as well as extreme changes in the environment that challenge these organisms, generating an excess of ROS; these physiological states as dormancy are sometimes rhythmic. Thus, the ability to reduce the metabolic rate during exposure to environmental stress, termed metabolic rate suppression, is thought to be an important component to enhance survival in many crustaceans and related to both HIF-1-alpha and the output of the clock (Gorr et al. 2010). Recently, it has been suggested that the oscillation of HIF-1 is involved in the circadian pacemaker of *P. clarkii* (Velázquez-Amado et al. 2012). Light/dark 12:12 cycles induced greater HIF-1 expression at ZT 13 than at ZT 0100. Thus, these cyclic values persist in darkness and are inversely related with the minimal and maximal ROS concentration at both hours, oxidative stress resulting from light-dark cycles with a long photoperiod (20:4 LD) that activated HIF-1a in all the putative pacemakers of crayfish notwithstanding. HIF-1 has a rhythmic expression in the retina and eyestalk and seems to be related to metabolism and antioxidant rhythms (Fanjul-Moles et al. 2009; Duran-Lizarraga et al. 2001), suggesting that HIF-1-alpha is a possible mediator between hypoxic and circadian pathways that may regulate target genes in crustaceans in a manner that is similar to its effects in mammals.

2.5 Conclusions and Perspectives

Evidence indicates that the circadian rhythms of crayfish are controlled by a distributed circadian system that includes four pairs of coupled oscillators (the retina, the eyestalk, XO-SG, brain pacemakers, and the six abdominal ganglia) and two extra-retinal circadian photoreceptors sensitive to blue light, which are involved in photic entrainment. In the crayfish *P. clarkii*, histochemical and biochemical studies have demonstrated that these oscillators express clock proteins similar to those found in the *Drosophila* TTL. In addition, the brain and the sixth abdominal ganglion extra-retinal receptors express CRY. It has been proposed that the blue light-induced photo-entrainment of some rhythms in crayfish, particularly ERG amplitude and activity rhythms, are mediated by CRY. The changes produced by light intensity and photoperiod length in ROS and the antioxidant rhythms of crayfish inhabiting different latitudes suggest that a photo-oxidative redox signal may be contributing to this animal's entrainment to latitudinal photoperiodic changes using similar signaling pathways as aquatic vertebrates to activate CCG transcription. A necessary step is to design further experiments to characterize both clock genes and transcription factors involved in the expression and synchronization of circadian rhythms in crustaceans, particularly in crayfish, exploring the relationship between redox status and light entrainment to determine the signaling pathways involved in the mechanisms of the metabolic and luminous synchronization in this interesting animal group. Undoubtedly, new and ecological comparative experiments will help to clarify this phenomenon.

Acknowledgments This work was partially supported by PAPIIT IN 218811 and CONACYT 178526.

References

- Aguzzi J, Sanchez-Pardo J, García JA et al (2009) Day-night and depth differences in haemolymph melatonin of the Norway lobster, *Nephrops norvegicus*. Deep Sea Res Part I Oceanogr Res Pap 56:1894–1905
- Aguzzi J, Company JB et al (2011) Activity rhythms in the deep-sea: a chronobiological approach. Front Biosci 16:131–150
- Albrecht U (2012) Timing to perfection: the biology of central and peripheral circadian clocks. Neuron 74:246–260
- Antle MC, Tse F, Koke SJ et al (2008) Non-photic phase shifting of the circadian clock: role of the extracellular signal-responsive kinases I/II/mitogen activated protein kinase pathway. Eur J Neurosci 28:2511–2518
- Assefa Z, Van Laethem A, Garmyn M, Agostinis P (2005) Ultraviolet radiation-induced apoptosis in keratinocytes on the role of cytosolic factors. Biochim Biophys Acta 1755:90–106
- Bernal-Moreno JA, Miranda-Anaya M, Fanjul-Moles ML (1996) Phase shifting the ERG amplitude circadian rhythm of juvenile crayfish by caudal monochromatic illumination. Biol Rhythm Res 27:299–301
- Berndt A, Kottke T, Breitkreuz H et al (2007) A novel photoreaction mechanism for the circadian blue light photoreceptor Drosophila cryptochrome. J Biol Chem 282(17):13011–13021
- Bianchi MM (2008) Collective behavior in gene regulation: metabolic clocks and cross-talking. FEBS J 275:2356–2363
- Bozek K, Kielbasa SM, Kramer A et al (2007) Promoter analysis of mammalian clock controlled genes. Genome Inform 18:65–74
- Bozek K, Relogio A, Kielbasa M et al (2009) Regulation of clock-controlled genes in mammals. PLoS One 4(3):e4882
- Bozek K, Rosahl AL, Gaub S et al (2010) Circadian transcription in liver. Biosystems 102:61–69
- Bradshaw WE, Holzapfel CM (2010) What season is it anyway? Circadian tracking vs photoperiodic anticipation in insects. J Biol Rhythms 25(3):155–165
- Dalle-Donne I, Rossi R, Colombo G et al (2009) Protein glutathionylation: a regulatory device from bacteria to humans. Trends Biochem Sci 34:85–96
- Dalton P, Shertzer HG, Puga A (1999) Regulation of gene expression by reactive oxygen. Annu Rev Pharmacol Toxicol 39:67–101
- Dröge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82:47–95
- Dubruille R, Murad A, Rosbash M et al (2009) A constant light-genetic screen identifies KISMET as a regulator of circadian photoresponses. PLoS Genet 5(12):e1000787. doi:10.1371/journal.pgen.1000787
- Duran-Lizarraga ME, Prieto-Sagredo J, Gonsebatt M et al (2001) Crayfish *Procambarus clarkii* shows circadian variations in different parameters of the GSH cycle. Photochem Photobiol 74:350–355
- Emery P, So WV, Kaneko M et al (1998) CRY, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell 95:669–679
- Escamilla-Chimal EG, Fanjul-Moles ML (2008) Daily and circadian expression of cryptochrome during the ontogeny of crayfish. Comp Biochem Physiol A Mol Integr Physiol 151:461–470
- Fanjul-Moles ML, Prieto-Sagredo J (2003) The circadian system of crayfish: a developmental approach. J Microsc Res Tech 60:291–301
- Fanjul-Moles ML, Miranda-Anaya M, Fuentes-Pardo B (1992) Effect of monochromatic light upon the ERG circadian rhythm during ontogeny in crayfish *Procambarus clarkii*. Comp Biochem Physiol A 102:99–106

- Fanjul-Moles ML, Escamilla-Chimal EG, Gloria-Soria A et al (2004) The crayfish *Procambarus clarkii* CRY shows daily and circadian variation. *J Exp Biol* 207:1453–1460
- Fanjul-Moles ML, Prieto-Sagredo J, López DS et al (2009) Crayfish *Procambarus clarkii* retina and nervous system exhibit antioxidant circadian rhythms coupled with metabolic and luminous daily cycles. *Photochem Photobiol* 85:78–87
- Farca-Luna AJ, Heinrich R, Reischig T (2010) The circadian biology of the marbled crayfish. *Front Biosci* 2:1414–1431
- Finkel T (1998) Oxygen radicals and signaling. *Curr Opin Cell Biol* 10:248–253
- Froy O, Chang DC, Reppert SM (2002) Redox potential. Differential roles in dCRY and mCRY1 functions. *Curr Biol* 12(2):147–152
- Fujita S, Koyama Y, Higashimoto M et al (2010) Regulation of circadian rhythm of human vascular endothelial growth factor by circadian rhythm of hypoxia inducible factor-1: implication for clinical use as anti-angiogenic therapy. *Ann Cancer Res Ther* 18:28–36
- Gorr TA, Cahn JD, Yamagata H et al (2004) Hypoxia-induced synthesis of hemoglobin in the crustacean *Daphnia magna* is hypoxia inducible factor-dependent. *J Biol Chem* 34: 36038–36047
- Gorr TA, Wichmann D, Hu J et al (2010) Hypoxia tolerance in animals: biology and application. *Physiol Biochem Zool* 83:733–752
- Hardeland R, Coto-Montes A, Poeggeler B (2003) Circadian rhythms, oxidative stress, and antioxidative defense mechanisms. *Chronobiol Int* 20:921–962
- Head JM (2010) The effects of hypoxia on hemocyanin regulation in *Cancer magister*: possible role of hypoxia-inducible factor. *J Exp Mar Biol Ecol* 386:77–85
- Hirayama J, Miyamura N, Uchida Y et al (2009) Common light signaling pathways controlling DNA repair and circadian clock entrainment in zebrafish. *Cell* 8:2794–2801
- Hoang N, Schleicher E, Kacprzak S et al (2008) Human and *Drosophila* cryptochromes are light activated by flavin photoreduction in living cells. *PLoS Biol* 6:e160
- Kay CW, Schleicher E, Kuppig A et al (2003) Blue light perception in plants. Detection and characterization of a light-induced neutral flavin radical in a C450A mutant of phototropin. *J Biol Chem* 278:10973–10982
- Kodama K, Rahman MS, Horiguchi T et al (2012) Assessment of hypoxia inducible factor-1 α mRNA expression in mantis shrimp as a biomarker of environmental hypoxia exposure. *Biol Lett* 8:278–281
- Lamia KA, Sachdeva UM, DiTacchio L et al (2009) AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science* 326:437–440
- Limón-Pacheco JH, Hernández NA, Fanjul-Moles ML et al (2007) Glutathione depletion activates mitogen-activated protein kinase (MAPK) pathways that display organ-specific responses and brain protection in mice. *Free Radic Biol Med* 43:1335–1347
- Lin C, Todo T (2005) The cryptochromes. *Genome Biol* 6:220. doi:10.1186/gb-2005-6-5-220
- Lin FJ, Song W, Meyer-Bernstein E et al (2001) Photic signaling by cryptochrome in the *Drosophila* circadian system. *Mol Cell Biol* 21:7287–7294
- Maciel FE, Geijs MA, Monserrat JM et al (2010) Antioxidant defense system rhythms in crustaceans and possible roles for melatonin. *Front Biosci* 12:1448–1459
- Mazzotta GM, De Pittà C, Benna C et al (2010) A cry from the krill. *Chronobiol Int* 27:425–445
- Miranda-Anaya M, Fanjul-Moles ML (1997) Nonparametric effects of monochromatic light on the activity rhythm of juvenile crayfish. *Chronobiol Int* 14:25–34
- Müller P, Ahmad M (2011) Light-activated cryptochrome reacts with molecular oxygen to form a flavin-superoxide radical pair consistent with magnetoreception. *J Biol Chem* 286:21033–21040
- Panda S, Antoch MP, Miller BH et al (2002) Coordinate transcription of key pathways in the mouse by the circadian clock. *Cell* 109:307–320
- Peschel N, Chen KF, Szabo G et al (2009) Light dependent interactions between the *Drosophila* circadian clock factors cryptochrome, jetlag, and timeless. *Curr Biol* 19:241–247
- Pittendrigh C (1981) Circadian rhythms and entrainment. In: Aschoff J (ed) *Handbook of behavioral neurobiology*, vol 4, Biological rhythms. Plenum, New York, pp 95–124

- Prieto-Sagredo J, Ricalde-Recchia I, Durán-Lizarraga ME et al (2000) Changes in hemolymph glutathione status after variation in photoperiod and light-irradiance in crayfish *Procambarus clarkii* and *Procambarus digueti*. *Photochem Photobiol* 71(4):487–492
- Reinke H, Saini C, Fleury-Olela F et al (2008) Differential display of DNA binding proteins reveals heat-shock factor 1 as a circadian transcription factor. *Genes Dev* 22:331–345
- Roennnberg T, Mellow M (1999) Circadian clocks and metabolism. *J Biol Rhythms* 14: 449–459
- Rutter J, Reick M, Wu LC et al (2001) Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* 293:510–514
- Sancar A (2000) Cryptochrome: the second photoactive pigment in the eye and its role in circadian photoreception. *Annu Rev Biochem* 69:31–37
- Sancar A (2003) Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. *Chem Rev* 103:2203–2237
- Sathyanarayanan S, Zheng X, Kumar S et al (2008) Identification of novel genes involved in light-dependent CRY degradation through a genome-wide RNA screen. *Genes Dev* 22:1522–1533
- Stanewsky R (2003) Genetic analysis of the circadian system in *Drosophila melanogaster* and mammals. *J Neurobiol* 54:111–147
- Stokkan KA, Yamazaki S, Tei H et al (2001) Entrainment of the circadian clock in the liver by feeding. *Science* 291(5503):490–493
- Strauss J, Dirksen H (2010) Circadian clocks in crustaceans: identified neuronal and cellular systems. *Front Biosci* 15:1040–1074
- Strauss J, Zhang Q, Verleyen P et al (2011) Pigment dispersing hormone in *Daphnia* interneurons, one type homologous to insect clock neurons displaying circadian rhythmicity. *Cell Mol Life Sci* 68:3403–3423
- Sullivan JM, Genco MC, Marlow ED et al (2009) Brain photoreceptor pathways contributing to circadian rhythmicity in crayfish. *Chronobiol Int* 26:1136–1168
- Todo T (1999) Functional diversity of the DNA photolyase/blue light receptor family. *Mutat Res* 434:89–97
- Uchida Y, Hirayama J, Nishina H (2010) Common origin: signaling similarities in the regulation of the circadian clock and DNA damage responses. *Biol Pharm Bull* 33:535–544
- VanVickle-Chavez SJ, Van Gelder RN (2007) Action spectrum of *Drosophila* cryptochrome. *J Biol Chem* 282:10561–10566
- Velázquez-Amado RM, Escamilla-Chimal EG, Fanjul-Moles ML (2012) Daily light-dark cycles influence hypoxia-inducible factor-1 and heat shock protein levels in the pacemakers of crayfish. *Photochem Photobiol* 88:81–89
- Whitmore D, Foulkes NS, Sassone-Corsi P (2002) Light acts directly on organs and cells in culture to set the vertebrate circadian clock. *Nature* 404:87–91
- Yoshizawa K, Nogami S (2008) The first report of phototaxis of fish ectoparasite *Argulus japonicus*. *Res Vet Sci* 85:128–130
- Yu X, Liu H, Klejnot J et al (2010) The cryptochrome blue light receptors. *Arabidopsis Book* 8:e0135
- Yuan Q, Metterville D, Briscoe AD et al (2007) Insect cryptochromes: gene duplication and loss define diverse ways to construct insect circadian clocks. *Mol Biol Evol* 24:948–955
- Zhu H, Yuan Q, Briscoe AD et al (2005) The two CRYs of the butterfly. *Curr Biol* 15:R953–R954

Mechanisms of Circadian Systems in Animals and Their
Clinical Relevance

Aguilar-Roblero, R.; Díaz-Muñoz, M.; Fanjul-Moles, M.L.
(Eds.)

2015, XXIV, 380 p. 83 illus., 47 illus. in color., Hardcover

ISBN: 978-3-319-08944-7