[Frontiers in Bioscience, Landmark, 18, 665-675, January 1, 2013]

ROS signaling pathways and biological rhythms: perspectives in crustaceans

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1. ABSTRACT

This work reviews concepts regarding the endogenous circadian clock and the relationship between oxidative stress (OS), light and entrainment in different organisms including crustaceans, particularly 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 are reviewed. Finally, the light signals and transduction pathways involved in the entrainment of the circadian clock, specifically in relation to cryptochromes (CRYs) and their dual role in the circadian clock of different animal groups and their possible relationship to the circadian clock and redox mechanisms in crustaceans is discussed. The relationship between metabolism, ROS signals and transcription factors, such as HIF-1 alpha in crayfish, as well as the possibility that HIF-1 alpha participates in the regulation of circadian control genes (ccgs) in crustaceans is discussed.

2. INTRODUCTION

The reactive oxygen species (ROS) are the by-products of aerobic respiration and metabolism. Superoxide anion, which is converted to hydrogen peroxide by the enzyme superoxide dismutase, the hydroxyl radical a by-product of the Fenton reaction, nitric oxide and singlet oxygen are examples of these reactive species. Over millions of years of evolution, organisms have developed diverse protective systems to address excess ROS. Conversely, ROS can act as secondary messengers, traducing messages from extracellular signals to generate specific cell responses. Proteins and other molecules that participate in signaling pathways can be modified by redox changes (1). ROS are suited to be signaling molecules because they are small and can easily diffuse short distances within a cell. In addition, the mechanisms for ROS production (such as via flavin-containing oxidases) and its rapid removal (such as via catalase) are present in almost all cell types including those of crustaceans. In a variety of organisms, light impinging on different photo pigments, such as flavin-based cryptochromes, induces ROS production that leads to an altered redox status. This

light-induced redox change stimulates intracellular signal cascades, such as mitogen-activated protein kinase (MAPK) that transduces photic signals to circadian genes (2). Recently it has been proposed that the regulation of the circadian clock by redox signaling is due to a crosstalk between the biological clock and cellular processes through shared intracellular signal cascades. An exciting new area of physiological redox signaling in the circadian biology is the recent discovery that the oxidation state of peroxiredoxin proteins can provide a way for cells to keep time without the canonical transcription or translation loop. Although the molecular basis responsible for these redox fluctuations are yet to be identified, the correlations between the fluctuations in ATP and NADPH suggest a link between peroxiredoxin oxidation, central metabolism and the circadian rhythm (3).

Recently, the activities of most of the antioxidant enzymes, including catalase (CAT), superoxide-dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST), have been described in different tissues and life stages of crustaceans (for a review, see 4). In addition, the relationship between these enzymes, metabolic parameters and ROS modulation, as well as its relation to the circadian clock in crayfish have been investigated (5, 6). Simultaneously, new insights regarding the circadian system of crustaceans are emerging (7, in this issue). Thus, this review revises the signaling pathways related to the redox balance in the cell and proposes a connection with the circadian clocks of different groups to infer possible connections with the circadian systems of crustaceans, focusing on crayfish.

3. CIRCADIAN CLOCKS

Circadian rhythmicity is a fundamental biological phenomenon of ubiquitous importance. This endogenous, innate oscillation with a period of approximately one day is present in all the organisms studied, from bacteria to eukaryotes. Temporal variations, driven by a circadian oscillator, are evident in many cellular functions, including gene expression, metabolic flux rates, concentration of signal molecules and even cell substructure. In multicelullar organisms, circadian rhythms can be studied at different integration levels, from cell-to-cell interactions, organ physiology, endocrine and neural communications and behavior. Although the control and coordination of the circadian rhythms in metazoans are typically organized by specialized pacemaker structures, primary oscillations are generated at the cellular level. It has widely been proven that these rhythms are genetically determined and that several gene clocks have been found in different organisms, including protists (8).

The core of the circadian clock is based on the intracellular time-tracking system that enables organisms to anticipate environmental changes and thereby adapt their behavior and physiology to the appropriate time of day (9). In some animals, such as insects and mammals, it is well known that a specific set of transcription factors constitutes the molecular architecture of the circadian clock. These are organized in regulatory positive-negative feedback loops, which function in a cell-autonomous manner (10) and are rhythmically controlled by a master oscillatory system, which coordinates tissue-specific rhythms according to the input it receives from the outside rhythmic world.

At the core of the circadian system are one or more endogenous oscillators that function to generate a free-running period that is close to 24-h when the organism is kept in constant environmental conditions. At the molecular level the oscillators are based both on the products of “clock genes” that are organized in transcriptional-translational feedback loops (TTL) and on the oscillating post-translational modification of the proteins that contribute significantly to the circadian oscillation (11). Some of the clock genes encode transcription activators, while others encode negative elements that feedback to inhibit their own expression by disrupting the activity of the activators. Meanwhile, kinases and phosphatases regulate the speed and precision of the clock (12). Components of the oscillators receive environmental information through input pathways, allowing the oscillators to remain synchronized to the 24-h solar day. The time-of-day information from the oscillator(s) is then relayed through output pathways to regulate the expression of circadian clock-controlled genes (ccgs) and overt rhythmicity. One mechanism by which the output pathways are predicted to be rhythmically controlled is through transcription factors or signaling molecules that are themselves components of the oscillator. These factors, activated by the circadian clock, may in turn regulate the downstream ccgs in a time-of-day-specific manner.

The molecular control of circadian rhythms in invertebrates is best understood in the fruit fly *Drosophila melanogaste*r. It involves interactions among the transcription factors Period (dPER), Timeless (dTIM), Clock (dCLK), Cycle (dCYC), Par Domain Protein 1 (Pdp1), Vrille (Vri), as well as the kinases Double-Time (Dbt), Shaggy (Sgg), Casein Kinase 2 (CK2)and protein phosphatase 2a (PP2a) and the protein degradation protein Supernumerary Limbs (Slimb) (13). The proteins dCLK and dCyc interact and form a complex that binds E-box elements (CACGTG) in regulatory sequences of the *Per* and *Tim promoter* regions to activate their transcription. The mRNA transcripts of these genes accumulate in the cytoplasm of pacemaker cells, where they are translated into proteins. The protein products dPER and dTIM accumulate during the night, entering the nucleus and binding to the CLK/CYC complex. The binding of dPER and or dTIM to the CLK/CYC complex interferes with the binding of the complex to the E-box and results in a cessation of transcriptional activity (14). Via this feedback loop, dPER and dTIM inhibit their own transcription. Degradation of dTIM in the late night renders dPER unstable and leads to its degradation later in the morning. These events release the inhibition from CLK/CYC and enable a new cycle of *Per, Tim, Vri* and *dPdp* transcription. The negative feedback loop is tuned by the action of the photopigment cryptochrome (dCRY), kinases and phosphatases (for review see 11; 15). dCRY allows the synchronization of the clock. The CLK/CYC complex is involved in a second autoregulatory loop in the fly pacemaker that controls the cycling levels of dCLK. Interestingly, *in*

vitro phosphorylation analysis showed a direct phosphorylation of CLK by CaMK II and p42 MAPK [extracellular signal-regulated kinase (ERK) 2], suggesting that these kinases regulate CLK/CYC-dependent transcription by direct phosphorylation of CLK (16). Although the molecular mechanism responsible for the tick of the clock is unknown in crustaceans, recently Yang *et al.* (17) have reported the presence of the gene *clk* in the prawn *Macrobranquium rosembergii* (*Mar-clock*). Other studies have demonstrated the presence and circadian functionality of circadian proteins such as CRY, PER, TIM AND CLK in the putative pacemakers of the crayfish *Procambarus clarkii* (18, 19, 20). These and other works performed on the gene of the crustacean pigment dispersing hormone (PDH), a hormone that shares multiple putatively homologous characters with the insect pigment dispersing factor (PDF), which is recognized as a ccg in insects (21, 22, 23, 24), indicate that there are similar circadian components in crustacean and insects. These findings could support the hypothesis that the intracellular pathways in the core of the clock of both animal groups are similar.

4. SYNCHRONIZING MECHANISMS AND SIGNALING PATHWAYS

4.1 Redox state as an entrainment pathway

Different environmental cyclic cues, or zeitgebers, are able to synchronize the circadian clock, with light being one of the most important. Importantly, 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 quality of light changes seasonally through the daily cycle, specifically near dawn and dusk. The photo entrainment of the circadian clock depends on these two luminous factors, 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 the external time (25).

In addition, to light, other extrinsic factors, including temperature, activity, and food, can advance or delay the system, thereby synchronizing the intrinsic clock with the external environment (26). This coupling of environmental cues to the intrinsic clock is termed entrainment. A second 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 non-photic phase shifts is unknown, although suppression of canonical clock genes and immediate early genes has been implicated (27).

Thus, functioning as an endogenous clock, the circadian system entrains the circadian rhythms to the seasonal photoperiodic changes. Daily exogenous fluctuations in the external environment such as lighting and temperature produce OS in a predictable manner, meanwhile daily and endogenous fluctuations produce ROS such as superoxide anions and hydroxide peroxide, as a consequence of metabolism and behavior .This reactive species may function as intracellular second messengers (28) .In nimals, 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, altering the internal temporal order of the animals and thus their physical fitness. Hence, animals are able to defend themselves against the periodic rise in ROS by means of compensatory anti-oxidative rhythms (29).

Notably, zebra fish peripheral clocks are directly light-responsive (30). In zebra fish cells, the light-induced redox changes stimulate intracellular MAPK signaling that transduces photic signals to zCry1a gene trans-activation (31). 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 that occurs after the maximum expression of the zCry1a gene has been reached (32). 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 (32) (Figures 1, 2).

Another stimulus able to reset the phase of the circadian system depends on the modulation of the redox state through metabolism (33). 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 emanating from the SCN or through local oscillators in peripheral tissues. A relationship between both processes by means the AMP/ATP ratio [which major sensor is adenosine monophosphate-dependent protein kinase (AMPK)] or glucose and fatty acid sensors have been shown (34). 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 (35). Interestingly AMPK impacts mammal's 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 modulates PER2 protein stability via an indirect mechanism involving casein kinase 1e (CK1e). (36) Then AMPK appears to be another potential regulator of the coupling and interaction between metabolism and the circadian clock (34).

Thus, it is plausible to hypothesize that essentially any parameter changing in a reliable way as a function of metabolic activity, such as ROS, is a candidate for the direct entrainment of the genetic oscillatory core of the clock. ROS and antioxidants, influence the expression of a number of genes in eukaryotes (1).Transcription pathway studies in different organisms have demonstrated that the direct effect of the redox state depends on NAD

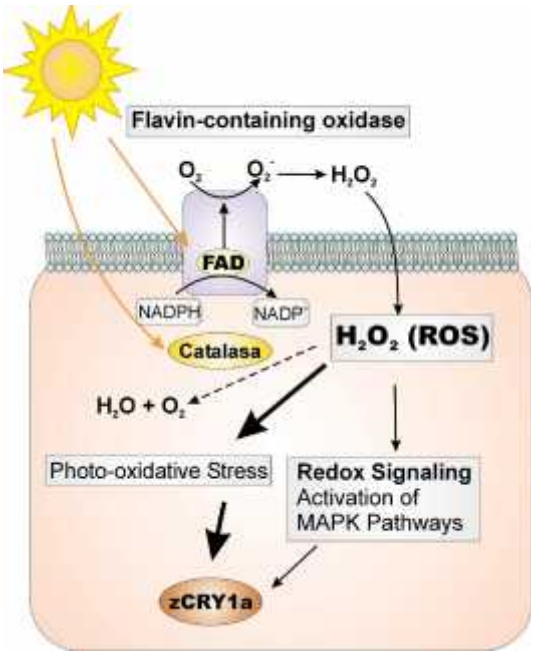

Figure 1. Potential Molecular Mechanism Underlying Light-Dependent Redox Signaling in Zebrafish. In the presence of flavin-containing oxidases, light drives the production of intracellular ROS such as H_2O_2. Excess ROS production has deleterious effects because ROS can react with various cellular targets 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 trans activation of the *zCry1a* gene. Light also increases *catalase* transcription and thus intracellular catalase activity, results in H_2O_2 degradation of 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, ref 31

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 the their cognate E-Box sequences, while the oxidized forms NAD+ and NADP+ strongly inhibited binding (37). The reduced cofactors NADH and NADPH have daily rhythms in plants (35) and regulate the activity of clock-like transcription factors in animals, strongly enhancing their DNA binding (37).

5. CRYPTOCHROMES, ROS LIGHT SIGNAL AND TRANSDUCTION PATHWAYS

In a variety of organisms, photopigments undergo forward light-induced reactions involving the electron transfer to the excited state flavin to generate radical intermediates, which correlate with the biological activity of the intermediates (38).

The light-dependent signaling state in CRY, flavin and folate-containing blue-light photoreceptors, proposed as circadian extraretinals in invertebrates such as *Drosophila* (39) , the crayfish *P. clarkii* and *Cherax destructor* (18, 40), is involved in the entrainment of the clock. Cryptochromes are blue light-absorbing photoreceptors found throughout the biological kingdom, ranging from protists to plants, animals, and humans (39, 41). 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 (42, 43), 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 (39). The photochemistry and the signaling pathways of cryptochromes are just beginning to be understood. Recent attention has focused primarily on the pathway of flavin photo-reduction as a possible mechanism of photoreceptor activation (44). 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 450-nm peak with no activity above 500 nm (45, 46, 47).In the presence of flavin-containing oxidases, light drives the production of intracellular ROS, such as H_2O_2 (32). The excess production of ROS has deleterious effects because ROS can react with various cellular targets to cause OS (1). However as mentioned above, light induced ROS can also take on a signaling role by stimulating intracellular signal pathways that lead to transcriptional activation, including the trans activation of genes..

Cryptochromes were originally identified in plants and have orthologs and paralogs among insects and

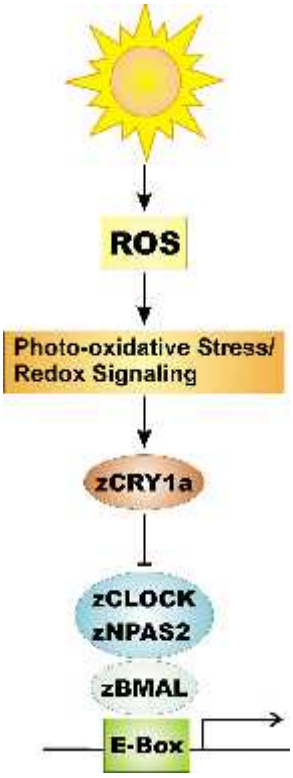

Figure 2. ROS induced by the sun trigger expression of zCRY1a. This transcriptional repressor interacts directly with the zCLOCK (zNPAS2):zBMAL complex and inhibits its transcriptional capacity, thereby entraining the circadian clock. (Reproduced with permission from,31).

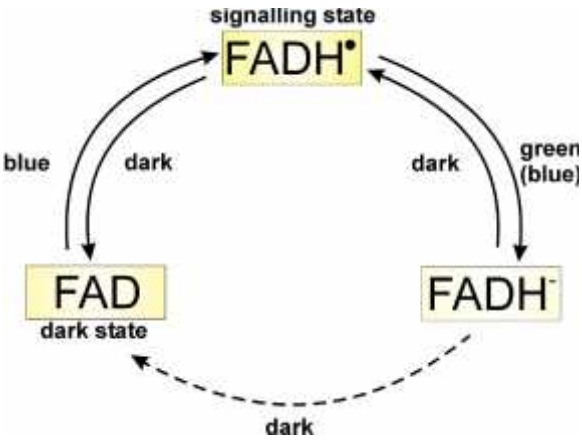

Figure **3.** The photocycle of plant cryptochromes. In the dark, the flavin chromophore is in its oxidized redox state. Blue light induces conversion to a metastable semi-quinone redox state that is the activated signaling state. Green light causes further reduction to the fully reduced redox state 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, ref 45).

vertebrates (48) 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 (41); 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 (49). Other insects, such as bees and beetles, possess only a mammalian-like CRY (50).

Drosophila CRY also appears to play a tissue specific pleiotropic role, because in clock neurons that generate rhythmic locomotor behavior, it acts as a photoreceptor and mediates light synchronization of the circadian clock by promoting the light-dependent degradation of dTIM (51, 52). 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 (53). But 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 (54). Cryptochrome-bound flavin is found in an oxidized redox state *in vivo* and light activation results in flavin photo-reduction to a radical intermediate that represents the likely signaling state. The derived photo-cycle of animal cryptochromes is therefore similar to the reaction mechanism of plant cryptochromes (Figure 3). Both photocycles involve the reduction of flavin, leading to a cycling between the reduced (active) and oxidized (inactive) redox forms (55, 56)

Then 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 (57). Using a microarray analysis, Sathyanarayanan, *et al.* (58) identified three thioredoxin domain-containing redox molecules (GstE7, Txl and CG11790) and a cytochrome p450, CYP49a1, that modulate light-dependent CRY and TIM degradation. These results suggest that the cellular redox status and electron transfer modulates 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 crustacean, some behavioral studies have reported rhythmic activity rest changes as well as synchronization or entrainment of circadian rhythms by blue light (59, 60, 61, 62, 63). Recently, it has been reported that positive phototaxic circadian rhythmicity is stronger under blue light in the parasite *Argulus japonicus* (Branchiura) (64), suggesting the presence of cryptochromes in the circadian photoreceptor system of different groups of crustaceans.

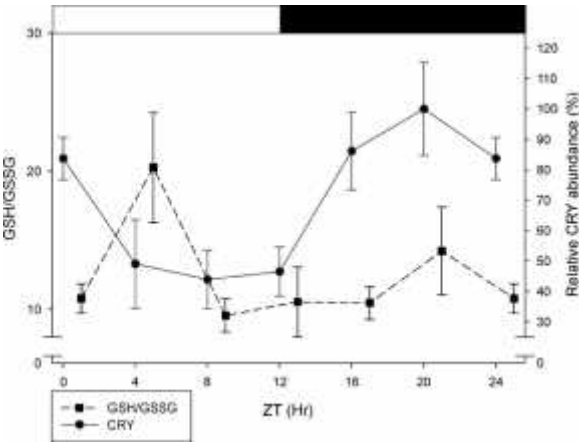

Figure 4. Relationship between GSH/GSSG ratio and CRY abundance in the brain of *P. clarkii* submitted to a 24hLD cycle. Light drives photo-oxidative stress, as it is shown by the low GSH/GSSG ratio, and CRY's abundance decrement suggesting CRY´s degradation. But importantly light seems up regulate glutathione reductase (not show) which increases GSH and GSH/GSSG ratio. This reduction in ROS leads to higher CRY degradation. CRY´s abundance increments occur in at dark phase (from ZT 12-ZT 20) when the brain showed maximal-oxidation (not shown). ZT:(zetgeber time). (modify from 5 and 19. See text for further information.

The circadian system of *P. clarkii* is considered to be distributed throughout the multi-oscillatory system in a hierarchical nature (65). It is a complex model, which includes three to four pairs of coupled oscillators such as the retina, the eyestalk X-O sinus gland complex (XOSG), putative brain pacemakers and the caudal abdominal ganglion (CPR). A pair of extra-retinal photoreceptors in the brain (BPR) are involved in light-dependent entrainment (7, in this Issue), that in crayfish, similar to insects, appears to be mediated by CRYs.

Immunochemical, biochemical and behavioral studies using a *Drosophila* anti-CRY antibody (18, 19, 40) 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 (41). The molecular characteristic of this protein has not been characterized. Based both on the homologies and the molecular mass detected by Western blot analysis (60 kd), and considering that the peptide sequence within the C-terminus of *Drosophila* sp. used to generate the antibody was specific to the *Drosophila melanogaste*r and *Drosophila pseud*obscura CRY 1, this protein seems to function as a photopigment in the lateral protocerebrum of crayfish (19,40). Recently, Mazzotta *et al.* (66) isolated and cloned a new CRY gene from the antartic krill (*Euphausia superba*). The EsCRY gene appears to be an ortholog 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 CRY could act either as a photopigment or as a transcription factor participating in the 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 crustacean; however, the results of experiments provide hints towards ROS and redox mechanisms underlying these processes. Fanjul-Moles *et al.* (5) demonstrated statistically significant 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 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 occur in both structures and oscillate showing a higher LPO under dark conditions. These results suggest that the difference in the effect of light on GSH rhythmic mechanisms of both structures for antagonizing ROS could be due to differences in glutathione-system coupling strength with the circadian clock´s synchronizing mechanisms. Light irradiation producing photo-oxidation is a factor that determines ROS in crayfish (67). Putative pacemakers in the retina and eyestalk-brain complex had higher GSH/GSSG ratio mean values in LD than in DD. The increment in this parameter in the retina and eyestalk coincides with the photo-phase, indicating that OS produced by light is antagonized by the rapid transformation of GSSG into GSH. This suggests that the antioxidant defense system (ADS) is up-regulated at the mid photo-phase level, between ZT4 and ZT6 at the same time that CRY higher decrement , (5, 18, 19) (Figure 4) . 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 zebra fish (32). In crayfish, the redox signal might be the result of the direct effect of ROS produced by the light onset of the L/D 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 (5). It has been proposed that light, especially UVB-induced ROS, is involved in the activation of mitogen-activated protein kinase (MAPK) downstream antioxidant-response effectors (68) and that certain MAPK signal-regulation regulations by GSH have been described recently in the mouse (69). In my laboratory, unpublished immunochemical studies have detected the expression of MAPK in the eyestalk and brain of *P. clarkii*. Many reports have indicated that the GSH/GSSG ratio is an important “sensor” for ADS regulation (70, 71, 72), 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 72 in this issue).

As mentioned above, 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 evolutionary-related genes, z64Phr and zCry1a, revealing that light-dependent DNA repair and the entrainment of circadian clock share

common regulatory pathways (31). We should explore similar possibilities in crayfish.

6. CLOCK OUTPUT AND ROS

One mechanism by which the 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 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 (21, 22) or the crustacean hyperglycemic hormone (CHH), have been proposed as putative clock outputs (73). In crayfish, metabolic changes, such as in the production of CHH and its relation to glucose and lactate concentrations, show evidence that circadian rhythms correlate with the expression of the Hypoxia-inducible factor (HIF-1 alpha at dawn and dusk (6).

The transcription factor HIF-1 alpha is a heterodimer composed by a regulated protein HIF-1 alpha and its constitutive partner, HIF-1 alpha-b. This protein is the most prominent and well-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 (74). The gene coding for HIF-1 alpha is found to be clock controlled in the mouse (75); furthermore, HIF-1alpha participates in metabolism (76, 77). Thus, this transcription factor has been proposed as connected to the circadian clock. In mammals, HIF-1 alpha has been described as a factor with an overrepresented number of binding sites in the promoters of ccg (78). Novel target genes activated by HIF-1 alpha are being constantly identified, and targets include genes with protein products involved in many functions including energy metabolism and hormonal control (79). However, in addition to regulating the response of different animals to hypoxia, HIF-1 alpha is considered part of the mechanisms that regulate the transcriptional output of the circadian clock in several organisms. In mammals, HIF-1alpha has been described as a factor with a number of overrepresented binding sites in the promoters of clock controlled genes (CCGs) and its mRNA and promoter activity in human cells has recently been reported (80).

HIF-1 alpha regulates the metabolic adjustment to hypoxia in many crustacean species, such as crabs (81), crayfish (6), shrimp (82) and Daphnia (83). Hypoxia and hyperoxia present during physiological states of the life cycle in crustacean, as well as extreme changes in the environment that challenge these organisms generating an excess of ROS. Thus, the ability to reduce the metabolic rate during the exposure to environmental stress, termed metabolic rate suppression, is thought to be an important component to enhance survival in many crustaceans and it is related to both HIF-1 alpha and the output of the clock (84). Recently, it has been suggested that the oscillation of HIF-1 is involved in the circadian pacemaker of *P. clarkii* (6). Light /dark 12:12 cycles induced greater HIF-1 expression at ZT 13, 1 h after lights off (20:00), than ZT 0100, 1 h after lights on (08:00). Thus, these cyclic values persist in darkness and are inversely related with the minimal and maximal ROS abundance expression at both hours. The HIF-1 has a rhythmic expression in the retina and eyestalk and is related to metabolism and antioxidant rhythms has been reported in the same work and elsewhere (5, 67), suggesting that HIF-1alpha 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.

7. CONCLUSIONS AND PERSPECTIVES

Evidence strongly suggests that the circadian rhythms of decapod crustaceans are controlled by a distributed system that includes four pairs of coupled oscillators (the retina, the eyestalk, XO-SG, brain pacemakers and the six abdominal ganglion) and two extra-retinal circadian photoreceptors sensitive to blue light, which are undoubtedly involved in photic entrainment. In the crayfish *P. clarkii*, histochemical and biochemical studies have demonstrated that these oscillators express some clock proteins similar to those found in the *Drosophila* TTL. In addition, the brain and the sixth abdominal ganglion extraretinal receptors express CRY. It has been proposed that the blue light-induced photo-entrainment of some rhythms in crayfish must be mediated by CRY´s. However, the changes produced by light on the antioxidant system of crayfish indicate that a photo oxidative redox signal may be participating in this animal´s entrainment.

In vertebrates, light and metabolism drive the production of intracellular ROS, such as H_2O_2 production that leads to an altered redox status (85). This altered redox state triggers the transduction of photic or metabolic signals that regulate the circadian clock, both the input to the TTL and the ccgs. In the last few years, the activity of most of the antioxidant enzymes, such as CAT, SOD, GPx, GRx, and GST, have been described in different tissues and life stages of crustaceans. In addition, the modulation of the activity of these enzymes and expression of the protein has been studied (for review, see 4). A necessary step is to design further experiments to explore the relation between the redox status and light entrainment in crustaceans, as well as the signaling pathways involved, to disclose the metabolic and luminous synchronization mechanisms in interesting animal groups. Undoubtedly, new and comparative experiments will clarify this phenomenon.

8. ACKNOWLEDGEMENTS

I thank Julio Prieto-Sagredo for his support with the production of the figures. I am deeply grateful to Professors Hirayama, Uchida and Ahmad for allowing me to use their original figures. Financial support was provided by UNAM- PAPIIT IN218811-3 and CONACYT 178526.

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Abbreviations:OS: oxidative stress; CRY´s: cryptochromes; ccgs: circadian control genes; ROS: reactive oxygen species ; MAPK: mitogen-activated protein kinase; CAT: catalase; SOD: superoxide-dismutase; GPx: glutathione peroxidase; GR: glutathione reductase; GST: glutathione S-transferase; TTL: transcriptional-translational feedback loops; ccgs: circadian clock-controlled genes; PER Period; TIM: Timeless; CLK: Clock; CYC:Cycle; pdp1:Par Domain Protein 1; vri: Vrille, dbt: kinases Double-Time; Sgg: Shaggy: CK2: Casein Kinase 2; PP2a: protein phosphatase 2a; Slimb: Supernumerary Limbs; LD: light-dark cycle; GSH: glutathione; CHH: crustacean hyperglycemic hormone; PDH: distal pigment hormone; HIF-1 alpha: Hypoxia-inducible factor

Key Words: Circadian rhythms, Signaling Pathways, Cryptochromes, Crustaceans, Entrainment, ROS, Light, Crayfish, Review

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cue that can set the circadian rhythm ïµ Some circadian rhythms are endogenous (do not require light) suggesting the existence of an internal (biological) clock ï¬ Monthly rhythms ïµ ï¬ Menstrual cycle Seasonal rhythms Aggression, sexual activity in male deer ïµ Hibernation ïµ 9.21 Suprachiasmatic Nucleus ï® The suprachiasmatic nucleus (SCN) contains a biological clock that governs some circadian rhythms ï¬ SCN receives input from ïµ amacrine/ganglion cells in the. retina, a pathway that may account for the ability of light to reset the biological clock (zeitgeber function) ïµ the intergeniculate leafle