Chapter

Light-Dependent Regulation of Circadian Clocks in Vertebrates

Izawa Junko, Yoshimi Okamoto-Uchida, Akari Nishimura and Jun Hirayama

Abstract

Circadian clocks are intrinsic time-tracking systems that endow organisms with a survival advantage. The core of the circadian clock mechanism is a cell-autonomous and self-sustained oscillator called a cellular clock, which operates via a transcription-/translation-based negative feedback loop. Under natural conditions, circadian clocks are entrained to a 24-hour day by environmental time cues, most commonly light. In mammals, circadian clocks are regulated by cellular clocks located in the central nervous system, such as the suprachiasmatic nucleus (SCN), and in other peripheral tissues. Importantly, mammals have no photoreceptors in the peripheral tissues; therefore the effect of light on peripheral clocks is indirect. By striking contrast, zebrafish peripheral cellular clocks are directly light responsive. This characteristic of the zebrafish cellular clock has contributed to the identification of molecules and signaling pathways that are involved in the light-dependent regulation of the cellular clock. Here, selected light-dependent regulatory mechanisms of circadian clocks in mammals and zebrafish are described.

Keywords: circadian clock, cellular clock, zebrafish, light, photolyase

1. Introduction

Circadian clocks constitute ubiquitous processes that regulate various biochemical and physiological events that occur with 24-hour periodicity, even in the absence of external cues [1]. The exact timing of this rhythm is established by cell-autonomous mechanisms, called cellular clocks, which are controlled by a transcription-/translation-based negative feedback loop [2, 3]. In both vertebrates and invertebrates, cellular clocks are scattered throughout their bodies; thus, the circadian system comprises both central and peripheral oscillators [4].

To guarantee that an organism's behavior remains tied to the rhythms of its environment, the circadian clock must respond to environmental stimuli to be reset [5]. The main cue for animals is light, which is provided by the day-night cycle. It has been proposed that in mammals the light-induced resetting of the circadian clock is dependent on transcription activation in the suprachiasmatic nucleus (SCN), where the central clock is located [6]. The mammalian route for the regulation of the circadian clock by light uses the retinohypothalamic tract (RHT), which connects directly to the central clock located in the SCN [7]. This makes it difficult to understand the mechanisms underlying light regulation of the circadian clock at a cellular level. Thus, although changes in gene expression have been implicated

in the light-induced phase shift of the circadian clock [6, 8], the induction of the expression of clock genes by light and the exact mechanism by which these gene products work remain to be elucidated at the cellular level.

Zebrafish peripheral clocks display a striking characteristic in that they are directly light responsive [9, 10]. Light induces the expression of clock genes and the circadian expression of several clock-related genes in zebrafish peripheral cells [11]. In addition, zebrafish embryonic cell lines can recapitulate the light-response characteristics of a vertebrate clock. In these cell lines, the oscillations of clock gene expression can be entrained to a new light-dark cycle, showing that cultured zebrafish cells have the clock components required for light-induced circadian clock resetting, and the cultured cell system thus provides a valuable tool for studying the light-dependent regulation of the circadian clock at a cellular level [12, 13]. Zebrafish cellular clocks can be studied in cultured cells, which facilitate the study of the photic responses of clock genes encoding cellular-clock regulators, and have revealed cellular signaling pathways that are involved in the light-dependent regulation of the cellular clock [14–18]. Additionally, an increased understanding of light-dependent cellular-clock regulation in zebrafish has suggested intriguing associations among the circadian clock, DNA repair, and cell cycle control [19–23].

Here we describe selected light-dependent regulatory aspects of vertebrate circadian machinery.

2. Cellular-clock regulation in mammals

In mammals, the cellular clock comprises the CLOCK, NPAS2, BMAL1, BMAL2, PER1, PER2, CRY1, and CRY2 proteins [1, 24]. These cellular clock components are called clock proteins. CLOCK or NPAS2 proteins heterodimerize with BMALs to form an active transcription complex that transactivates clock-controlled genes, including *Crys* and *Pers*. Once the CRY and PER proteins have been translated, they are translocated to the nucleus, where they inhibit CLOCK (NPAS2):BMAL-mediated transcription through a direct protein-protein interaction, setting up the rhythmic gene expression that drives the circadian clock. The CLOCK(NPAS2):BMAL complex also stimulates expression of the clock-controlled genes (Ccgs) to regulate various elements of physiology. This accounts in part for the presence of circadian rhythms in a variety of physiological processes [25]. Although the relatively straightforward mechanism of positive and negative feedback loops is necessary to establish and maintain circadian clocks, cellular clocks have further levels of complexity, including posttranscriptional regulation, posttranslational modification, chromatin remodeling, availability and stability of clock proteins, and regulation of intracellular localization. These regulatory mechanisms provide an interface that can be used as an entry point for stimuli that can reset or control the clock. In addition, genetic studies of genes encoding cellular-clock regulators have revealed distinct roles for clock proteins in regulating circadian clocks, as well as direct links between the circadian clock and various pathologies [26–28].

3. Photoreceptors for circadian-clock regulation in mammals

Circadian clocks regulate various biochemical, physiological, and behavioral processes with a periodicity of approximately 24 hours. Under natural conditions, circadian rhythms are entrained to this 24-hour day by environmental time cues,

with light level being the most important [5]. The eye is the principal mediator of light input to the central clock in mammals. Rods and cones receive visual information within the retina [29, 30] (**Figure 1**). These cells, however, are dispensable for photoreception of circadian clocks. Indeed, rodents that lack classical visual responses are still capable of circadian photoentrainment [31]. Retrograde tracing experiments have identified retinal cells projecting to the SCN through the RHT, but not to the visual centers of the brain [32]. These cells constitute a small subset of retinal ganglion cells (RGCs) localized in the ganglion cell layer (GCL), and they have been shown to display intrinsic phototransduction abilities, with photic properties matching those of clock entrainment [33]. The main candidate for the circadian photoreceptor is melanopsin, which is an opsin found in the eye and other photoreceptive structures in amphibians and exclusively in retinal RGCs in primates and rodents. Photic information received by RGCs is conveyed through the retinohypothalamic tract to the SCN central clock in mammals [33, 34].

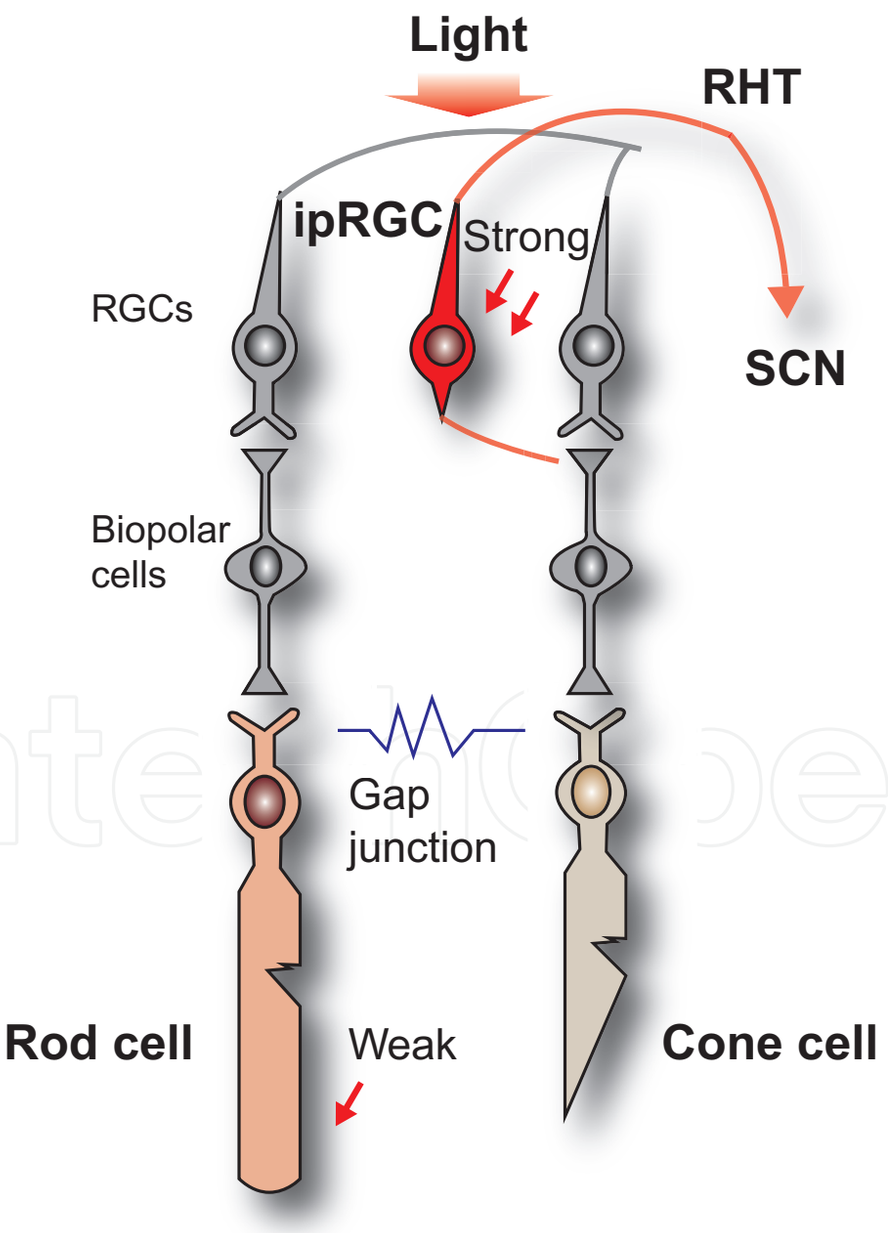

Figure 1.
Retinal cells responsible for vision and photoreception for circadian clock regulation.

4. Light-dependent transcription in the mammalian retina

Dopamine is the major catecholamine in the vertebrate retina and plays a central role in neural adaptation to light. Indeed, light stimulates the synthesis, turnover, and release of retinal dopamine, which makes dopamine an important mediator of light signaling to retinal cellular clocks [35–37]. Among the members of the dopamine-receptor family, the dopamine D2 receptor (D2R) has been shown to control light-induced reset of the circadian clock in the mouse retina [38–40]. At the molecular level, it has been reported that signaling mediated by the D2R enhances the transcriptional capacity of the CLOCK:BMAL complex. This effect involves the extracellular signal-regulated kinase (ERK)/MAPK transduction cascade and is associated with a D2R-induced increase in phosphorylation of the transcriptional coactivator, cAMP-responsive element-binding protein (CREB) and its recruitment to the CLOCK:BMAL complex [40]. Importantly, this activation of CLOCK:BMAL1-dependent transcription is responsible for the induction of the *Per1* gene by light in the retina, which is in turn responsible for the reset of the retinal cellular clock. These findings provide evidence for the physiological links among the ERK/MAPK signaling pathway, dopamine, and the light input pathway of circadian clocks.

5. Light-dependent transcription in mammalian SCN

Light resets the circadian clock by its phase-shifting properties. In particular, the phase-shifting effects of light only occur during the nighttime period of the circadian cycle. In nocturnal mammals kept in darkness, a light pulse during the subjective night (that is, the time of day corresponding to the dark period in a normal light-dark cycle) can reset the clock by evoking changes in the SCN-controlled rhythms [41–43]. If the light pulse is given at an early point in time during the subjective night, it induces a shift in SCN-controlled rhythms to a later time (phase delay). Conversely, if the light pulse is provided at the end of the subjective night, the SCN-controlled rhythms will be shifted to an earlier position in the circadian cycle (phase advance). Photic signals perceived by the retina are conveyed to the SCN through the RHT [32]. Glutamate has been identified as the major neurotransmitter responsible for transducing the photic information to the SCN along the RHT [44] (**Figure 2**). Once glutamate is released by the SCN, it binds to N-methyl-D-aspartate (NMDA) receptors, which in turn leads to the Ca^{2+} influx, that is, finally responsible for the activation of calcium-/calmodulin-dependent protein kinase (CaMK).

The involvement of the ERK/MAPK pathway in the light-input system of the circadian clock in the SCN has been well established. Mice exposed to light pulses during their subjective night display rapid ERK upregulation (phosphorylation) in the SCN [45]. Furthermore, disruption of the MAPK pathway has been shown to block light-induced phase shifting of the circadian clock at the behavioral level [46]. This finding suggests that the ERK cascade is integrally involved in photic entrainment of mammalian circadian rhythms. Events downstream of the light-induced signaling pathway in the SCN lead to the phosphorylation of cAMP-response element-binding protein (CREB), which then stimulates expression of Per1 and Per2 genes, which contain a calcium-/cAMP-response element (CRE) in their promoters [6, 47, 48]. Although the exact mechanism by which light induces early gene expression remains to be elucidated, it has been shown that a single light pulse engenders chromatin remodeling via the phosphorylation of histone H3 at Ser10 [49].

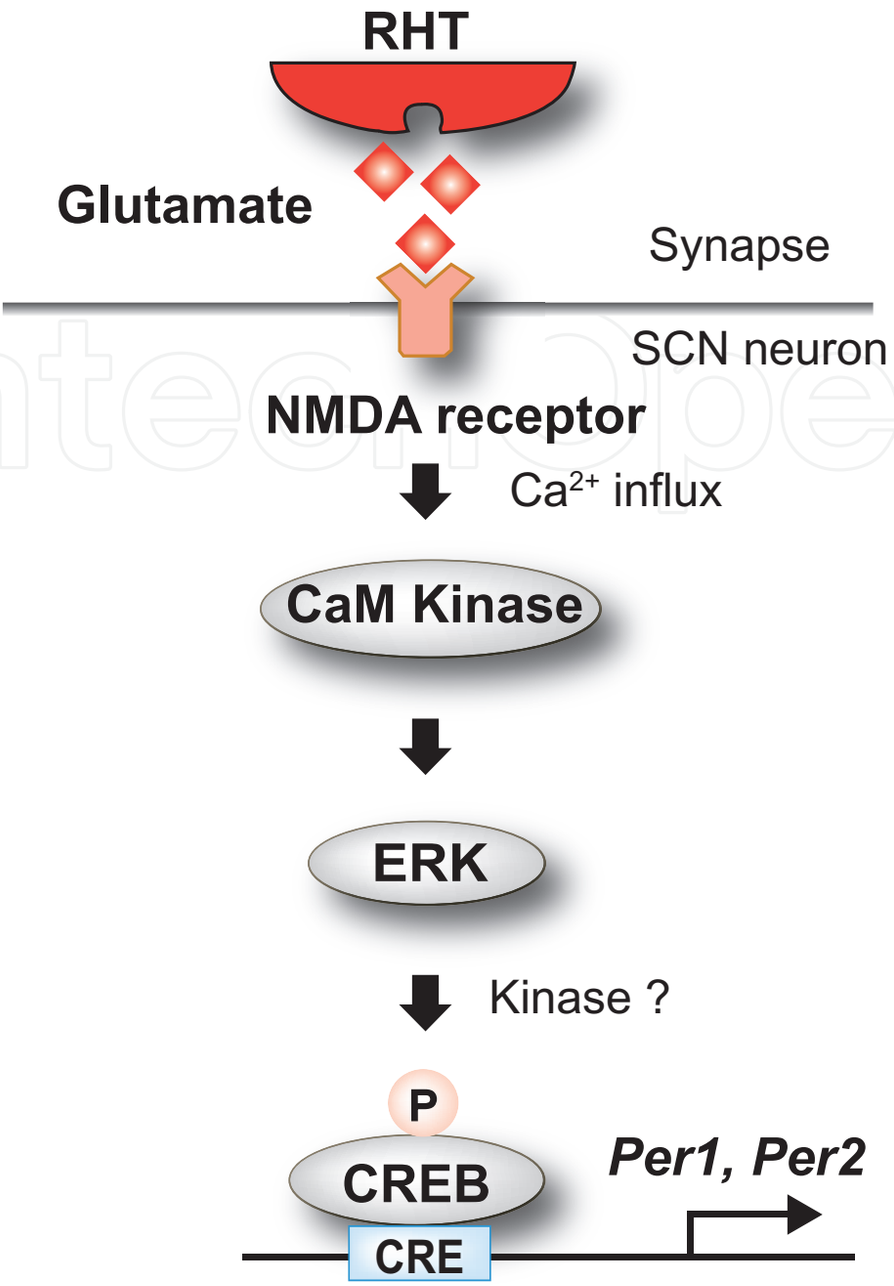

Figure 2.
Signaling cascade transducing photic signal perceived by the retina to the transcription of clock genes in SCN.

6. Light-dependent synchronization of cellular clocks in the mammalian SCN

The appropriate synchronization of cellular clocks in tissues and organs is required for the generation of circadian rhythms in a variety of physiological processes, such as sleep and metabolism [50]. In addition, the light-dependent induction of *Per1* and *Per2* is thought to contribute to the synchronization of cellular clocks in the SCN [6, 8]. However, this idea has not been fully elucidated using adequate genetically modified mice. Mouse *Per1* and *Per2* genes are induced by the CLOCK (NPAS2):BMAL complex and by light. In particular, the CLOCK (NPAS2):BMAL-dependent regulation of *Per1* and *Per2* is essential for establishment of the circadian clock's rhythmicity. Thus, genetic inhibition of both mouse *Per1* and *Per2* genes disrupts the cellular clock, preventing the analysis of synchronization [51, 52]. This problem has been solved by using zebrafish models, as described below.

7. Cellular-clock regulation in zebrafish

The zebrafish constitutes an attractive alternative to the mammalian system with which to study the complexity of circadian clock machinery and light's influence on it [9]. Characterization of the molecular components of the zebrafish circadian oscillator has revealed that the negative feedback loop in zebrafish consists of components similar to those of mammals [11]. Organ- and tissue-culture explant experiments have demonstrated that peripheral circadian oscillators are present throughout the tissues and organs of the zebrafish and that they display the remarkable feature of being light responsive [10, 13].

The characterization of components of the zebrafish cellular clock has revealed duplication of most clock genes. There are two, three, four, and eight homologues of the *Clock*, *Bmal*, *Per*, and *Cry* genes, respectively. Their circadian expression profiles and light inducibility are different, indicating the differential contribution of various clock components in the regulation of cellular clocks [28, 53–55]. For example, an investigation into the in vitro functions of the protein products of zebrafish *Cry* genes revealed that they fall into one of two groups: one group inhibits CLOCK:BMAL-mediated transcription (repressor-type CRYs: zCRY1a, zCRY1b, zCRY2a, and zCRY2b), while the other group does not inhibit transcription (non-repressor type CRYs: zCRY3, zCRY4, zCRY Dash, and plant-type zCRY).

The CLOCK (NPAS2):BMAL complex and/or light regulates the expression of zebrafish repressor types of *Crys* and *Pers* [50, 56]. The *zCry2a* and *zCry2b* genes are induced both by the CLOCK (NPAS2):BMAL complex and by light; *zCry1b*, *zPer1a*, *zPer1b*, and *zPer3* are induced by the CLOCK (NPAS2):BMAL complex but not by light; and *zCry1a* and z*Per2* are induced by light but not by the CLOCK (NPAS2):BMAL complex. These distinct dependencies of *zPer* and *zCry* gene expressions recently enabled us to uncover the role of light-induced zPER2, zCRY1a, and zCRY2a in the light-dependent synchronization of cellular clocks.

8. zPER2, zCRY1a, and zCRY2a are required for the light-dependent ontology of circadian clocks during development

In vertebrates, cellular clocks in zygotes and early embryos are not functional and become gradually set in motion during development [57, 58]. In mammals, it is quite difficult to analyze the processes of cellular-clock formation during development because embryogenesis proceeds inside the maternal uterus. Thus, the molecular mechanisms underlying the establishment of cellular clocks during vertebrate development are not well understood. Zebrafish eggs are externally fertilized and are transparent [11, 54]. In addition, zebrafish embryos develop rapidly from fertilized eggs to larvae that swim, making them an excellent model for studies investigating the ontology of vertebrate clocks.

During zebrafish development, organogenesis is completed within 2 days post-fertilization (dpf) [59]. Zebrafish larvae hatch within four dpf and start to display locomotor behavior. Zebrafish cellular clocks are autonomously set in motion during development within 1–4 dpf but are out of phase with each other in tissues and organs. Light synchronizes the phases of the cellular clocks to establish behavioral rhythms [50, 60]. Our recent study generated $zCry1a^{-/-}$ $zPer2^{-/-}$ $zCry2a^{-/-}$ triple knockout (TKO) zebrafish and used these TKO animals to show that light-induced zPER2, zCRY1a, and zCRY2a help to synchronize cellular clocks in early embryos and larvae in a light-dependent manner, thus contributing to behavioral rhythm formation in zebrafish larva [50]. Notably, these findings provide evidence that

light-dependent-induced PER1 and PER2 contribute to the synchronization of cellular clocks in the SCN of mammals.

9. Light signaling pathway regulating cellular clocks in zebrafish cells

Studies using cultured zebrafish cells have identified cellular signaling cascades involved in the light-dependent regulation of cellular clocks. In several organisms, external stimuli are connected to a cell's nucleus via MAPK signaling pathways [61]. There are three major MAPKs: c-JUN N-terminal kinase (JNK), p38, and ERK. Light has been reported to activate these signaling cascades in zebrafish cells. Using a pharmacological approach, it was established that light-induced *zPer2* transactivation requires the ERK signaling pathway [15]. It has also been proposed that light-induced ERK activation triggers *zCry1a* transcription, whereas light-induced p38 activation suppresses it, highlighting a MAPK-mediated cross-regulatory mechanism for the expression of circadian-clock genes [21]. As mentioned above, evidence strongly suggests the involvement of the ERK pathway in the light-input system of the mammalian circadian clock. Thus, these findings are consistent with the idea that several aspects of the complex mammalian photo-signal transduction pathway involved in the regulation of circadian clocks are more easily investigated, both pharmacologically and molecularly, using cultured zebrafish cells. In addition, it was reported that the light-activated JNK signaling pathway induces expression of *zCry1a* and *zPer2* [62]. Notably, in contrast to these studies, it has recently been reported that the light-activated p38 pathway facilitates the expression of *zCry1a* and *zPer2* and that the ERK/MAPK signaling pathway is not involved in the light-induced expression of *zCry1a* and *zPer2* [62, 63]. The reason for these contradictory results is unknown.

10. Role of redox signaling in cellular-clock regulation by light in zebrafish

It has been proposed that the light-dependent transcription of *zCry1a* and *zPer2* is controlled through the production and removal of cellular reactive oxygen species (ROS) [16]. ROS were originally thought to act solely as toxic metabolites, because they react with components of DNA, proteins, and lipids and exert oxidative stress [64]. However, ROS are also ideally suited to be signaling molecules because they are small and can easily diffuse over short distances within a cell. In addition, mechanisms for ROS production and their rapid removal (for example, via catalase) are present in almost all cell types [64, 65]. In various organisms, light induces ROS production, which leads to an altered redox status in cells [28]. In zebrafish cells, this light-induced redox change transduces photic signals and leads to the transactivation of *zCry1a* and *zPer2* [16, 62, 66]. Importantly, light increases intracellular catalase activity by increasing the expression of *catalase*, an event that occurs after the maximum expression of the *zCry1a* and *zPer2* genes has been reached [16]. This increased catalase activity diminishes light-induced cellular ROS levels, resulting in decreased expression of the *zCry1a* and *zPer2* genes.

The toxic effects of oxidative stress have been linked to cellular ROS production induced by light-activated flavin-containing oxidases [67]. The absorbance of light in the near violet-blue region by these enzymes activates them and induces photoreduction of the flavin adenine dinucleotide (FAD) moiety, leading to ROS production. Accordingly, signaling by flavoproteins frequently induces a change in

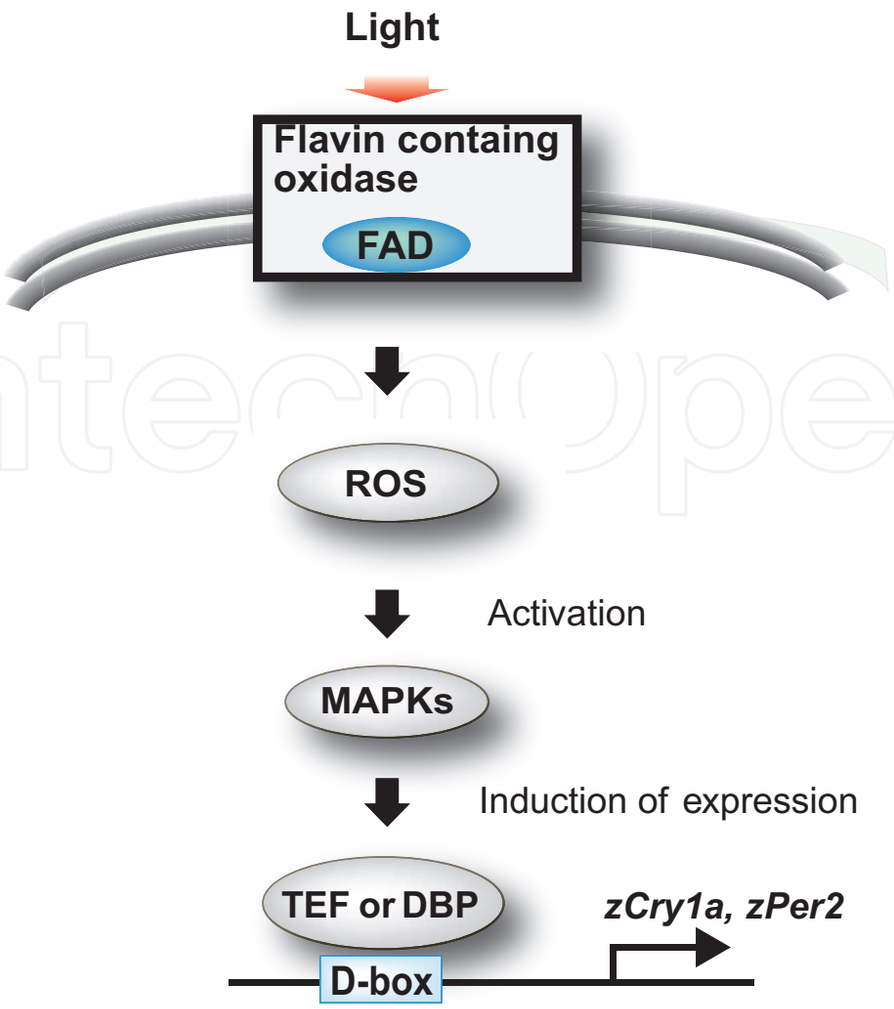

Figure 3.
Light signaling pathway regulating clock gene induction in zebrafish.

the redox state of cells [67]. Recent studies have provided evidence that flavin-containing oxidases are responsible for the light-dependent production of ROS that are second messengers coupling photoreception to photoreactivation and the circadian clock in zebrafish [62, 66] (**Figure 3**).

11. Link between circadian clocks and light-dependent DNA repair in zebrafish

Solar radiation has both beneficial and harmful effects for most species. Beneficial aspects include its role in photosynthesis and the entrainment of circadian clocks [28]. However, the UV component of solar radiation can produce cytotoxic, mutagenic, and carcinogenic lesions in DNA, which can transform or kill cells. In particular, the UV component of solar radiation produces cytotoxic and mutagenic lesions in DNA called cyclobutane pyrimidine dimers (CPDs) and pyrimidine [6-4] pyrimidone photoproducts. Photoreactivation is a light-dependent DNA repair mechanism mediated by DNA photolyases (PHRs), which bind to and repair UV-induced DNA damage using visible light as an energy source [43, 68]. Two classes of PHRs have been identified, one specific for CPDs (CPD PHRs) and the other specific for [6-4] photoproducts (64PHRs). Importantly, both the induction of PHRs in response to light and the subsequent light-dependent repair of DNA by PHRs are essential for successful photoreactivation in zebrafish

cells [21]. Notably, the expression level of the *z64Phr* gene is regulated by the same light-induced MAPK cascades as those controlling the expression of the clock gene *zCry1a*, which is associated with the light-dependent regulation of the circadian clock [21, 66]. Light-induced ERK activation triggers the expression of *z64Phr*, whereas light-induced p38 activation inhibits it. Thus, both light-dependent DNA repair and regulation of the circadian clock are governed by shared regulatory pathways. Both CRYs and PHRs belong to the DNA photolyase/cryptochrome protein family and have highly similar amino acid sequences [43, 68]. Evolutionary studies have shown that the animal CRY protein functionally diverged first from the CPD photolyase and then further to generate 64PHR [69]. These facts, together with the observation that *zCry1a* and *z64Phr* share regulatory pathways, strongly indicate an evolutionary link between the circadian clock and DNA repair.

12. Conclusion

In mammals, light signals are received by the retina and then integrated with the SCN cellular clocks [7]. The SCN cellular clocks then transmit light information to peripheral cellular clocks via humoral signals and synchronize them. Recent studies have reported that factors other than cellular clocks in the SCN can synchronize peripheral cellular clocks in a light-dependent manner [42]. In contrast, in zebrafish, light directly synchronizes peripheral cellular clocks in addition to central cellular clocks [9]. Despite the differences between the light-dependent regulation of peripheral cellular clocks in mammals and zebrafish, both require similar MAPK signaling pathways and light induction of clock genes to regulate cellular clocks in a light-dependent manner.

The development of circadian clocks would be one way to segregate daytime from nighttime processes, with light-dark cycles acting as selective pressures [28]. In this scenario, increasing levels of oxygen free radicals during the daytime may have been a decisive factor in relegating the anabolic processes of mitosis, growth, and consolidation to the dark hours. Thus, it is reasonable to propose that redox signaling and stress responding pathways such as MAPKs are utilized in the light-dependent regulation of the circadian clock.

Acknowledgements

This work was supported in part by a Japan Society for the Promotion of Science (JSPS) Grant-in-Aid for Scientific Research [19K12900 (J.I.) and 18KT0068 (J.H.)]. This work was also supported by grants from the Watanabe Foundation and the Smoking Research Foundation (J.H.).

Author details

Izawa Junko[1], Yoshimi Okamoto-Uchida[2], Akari Nishimura[1] and Jun Hirayama[1*]

1 Department of Clinical Engineering, Faculty of Health Sciences, Komatsu University, Ishikawa, Japan

2 Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan

*Address all correspondence to: jun.hirayama@komatsu-u.ac.jp

References

[1] Takahashi JS. Transcriptional architecture of the mammalian circadian clock. Nature Reviews. Genetics. 2017;**18**:164-179

[2] Dunlap JC. Molecular bases for circadian clocks. Cell. 1999;**96**:271-290

[3] Hirayama J, Sassone-Corsi P. Structural and functional features of transcription factors controlling the circadian clock. Current Opinion in Genetics & Development. 2005;**15**:548-556

[4] Schibler U, Sassone-Corsi P. A web of circadian pacemakers. Cell. 2002;**111**:919-922

[5] Okamura H. Clock genes in cell clocks: Roles, actions, and mysteries. Journal of Biological Rhythms. 2004;**19**:388-399

[6] Shigeyoshi Y, Taguchi K, Yamamoto S, Takekida S, Yan L, Tei H, et al. Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript. Cell. 1997;**91**:1043-1053

[7] Paul KN, Saafir TB, Tosini G. The role of retinal photoreceptors in the regulation of circadian rhythms. Reviews in Endocrine & Metabolic Disorders. 2009;**10**:271-278

[8] Okamura H, Miyake S, Sumi Y, Yamaguchi S, Yasui A, Muijtjens M, et al. Photic induction of mPer1 and mPer2 in cry-deficient mice lacking a biological clock. Science. 1999;**286**:2531-2534

[9] Tamai TK, Carr AJ, Whitmore D. Zebrafish circadian clocks: Cells that see light. Biochemical Society Transactions. 2005;**33**:962-966

[10] Whitmore D, Foulkes NS, Strahle U, Sassone-Corsi P. Zebrafish clock rhythmic expression reveals independent peripheral circadian oscillators. Nature Neuroscience. 1998;**1**:701-707

[11] Idda ML, Bertolucci C, Vallone D, Gothilf Y, Sanchez-Vazquez FJ, Foulkes NS. Circadian clocks: Lessons from fish. Progress in Brain Research. 2012;**199**:41-57

[12] Pando MP, Pinchak AB, Cermakian N, Sassone-Corsi P. A cell-based system that recapitulates the dynamic light-dependent regulation of the vertebrate clock. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**:10178-10183

[13] Whitmore D, Foulkes NS, Sassone-Corsi P. Light acts directly on organs and cells in culture to set the vertebrate circadian clock. Nature. 2000;**404**:87-91

[14] Ben-Moshe Z, Alon S, Mracek P, Faigenbloom L, Tovin A, Vatine GD, et al. The light-induced transcriptome of the zebrafish pineal gland reveals complex regulation of the circadian clockwork by light. Nucleic Acids Research. 2014;**42**:3750-3767

[15] Cermakian N, Pando MP, Thompson CL, Pinchak AB, Selby CP, Gutierrez L, et al. Light induction of a vertebrate clock gene involves signaling through blue-light receptors and MAP kinases. Current Biology: CB. 2002;**12**:844-848

[16] Hirayama J, Cho S, Sassone-Corsi P. Circadian control by the reduction/oxidation pathway: Catalase represses light-dependent clock gene expression in the zebrafish. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**:15747-15752

[17] Vallone D, Gondi SB, Whitmore D, Foulkes NS. E-box function in a period gene repressed by light. Proceedings

of the National Academy of Sciences of the United States of America. 2004;**101**:4106-4111

[18] Vatine G, Vallone D, Appelbaum L, Mracek P, Ben-Moshe Z, Lahiri K, et al. Light directs zebrafish period 2 expression via conserved D and E boxes. PLoS Biology. 2009;**7**:e1000223

[19] Dekens MP, Santoriello C, Vallone D, Grassi G, Whitmore D, Foulkes NS. Light regulates the cell cycle in zebrafish. Current Biology: CB. 2003;**13**:2051-2057

[20] Dickmeis T, Lahiri K, Nica G, Vallone D, Santoriello C, Neumann CJ, et al. Glucocorticoids play a key role in circadian cell cycle rhythms. PLoS Biology. 2007;**5**:e78

[21] Hirayama J, Miyamura N, Uchida Y, Asaoka Y, Honda R, Sawanobori K, et al. Common light signaling pathways controlling DNA repair and circadian clock entrainment in zebrafish. Cell Cycle. 2009;**8**:2794-2801

[22] Laranjeiro R, Tamai TK, Peyric E, Krusche P, Ott S, Whitmore D. Cyclin-dependent kinase inhibitor p20 controls circadian cell-cycle timing. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**:6835-6840

[23] Zhao H, Di Mauro G, Lungu-Mitea S, Negrini P, Guarino AM, Frigato E, et al. Modulation of DNA repair systems in blind cavefish during evolution in constant darkness. Current Biology: CB. 2018;**28**:3229-3243.e4

[24] King DP, Takahashi JS. Molecular genetics of circadian rhythms in mammals. Annual Review of Neuroscience. 2000;**23**:713-742

[25] Sahar S, Sassone-Corsi P. Metabolism and cancer: The circadian clock connection. Nature Reviews. Cancer. 2009;**9**:886-896

[26] Gallego M, Virshup DM. Post-translational modifications regulate the ticking of the circadian clock. Nature Reviews. Molecular Cell Biology. 2007;**8**:139-148

[27] Grimaldi B, Nakahata Y, Kaluzova M, Masubuchi S, Sassone-Corsi P. Chromatin remodeling, metabolism and circadian clocks: The interplay of CLOCK and SIRT1. The International Journal of Biochemistry & Cell Biology. 2009;**41**:81-86

[28] Uchida Y, Hirayama J, Nishina H. A common origin: Signaling similarities in the regulation of the circadian clock and DNA damage responses. Biological & Pharmaceutical Bulletin. 2010;**33**:535-544

[29] Lee HS, Nelms JL, Nguyen M, Silver R, Lehman MN. The eye is necessary for a circadian rhythm in the suprachiasmatic nucleus. Nature Neuroscience. 2003;**6**:111-112

[30] Pando MP, Sassone-Corsi P. Signaling to the mammalian circadian clocks: In pursuit of the primary mammalian circadian photoreceptor. Science's STKE. 2001;**2001**:RE16

[31] Selby CP, Thompson C, Schmitz TM, Van Gelder RN, Sancar A. Functional redundancy of cryptochromes and classical photoreceptors for nonvisual ocular photoreception in mice. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**:14697-14702

[32] Gooley JJ, Lu J, Chou TC, Scammell TE, Saper CB. Melanopsin in cells of origin of the retinohypothalamic tract. Nature Neuroscience. 2001;**4**:1165

[33] Gooley JJ, Lu J, Fischer D, Saper CB. A broad role for melanopsin in nonvisual photoreception. The Journal of Neuroscience: The Official Journal

of the Society for Neuroscience. 2003;**23**:7093-7106

[34] Viney TJ, Balint K, Hillier D, Siegert S, Boldogkoi Z, Enquist LW, et al. Local retinal circuits of melanopsin-containing ganglion cells identified by transsynaptic viral tracing. Current Biology: CB. 2007;**17**:981-988

[35] Iuvone PM, Galli CL, Garrison-Gund CK, Neff NH. Light stimulates tyrosine hydroxylase activity and dopamine synthesis in retinal amacrine neurons. Science. 1978;**202**:901-902

[36] Kramer SG. Dopamine: A retinal neurotransmitter. I. Retinal uptake, storage, and light-stimulated release of H3-dopamine in vivo. Investigative Ophthalmology. 1971;**10**:438-452

[37] Nir I, Haque R, Iuvone PM. Diurnal metabolism of dopamine in the mouse retina. Brain Research. 2000;**870**:118-125

[38] Doi M, Yujnovsky I, Hirayama J, Malerba M, Tirotta E, Sassone-Corsi P, et al. Impaired light masking in dopamine D2 receptor-null mice. Nature Neuroscience. 2006;**9**:732-734

[39] Vallone D, Picetti R, Borrelli E. Structure and function of dopamine receptors. Neuroscience and Biobehavioral Reviews. 2000;**24**:125-132

[40] Yujnovsky I, Hirayama J, Doi M, Borrelli E, Sassone-Corsi P. Signaling mediated by the dopamine D2 receptor potentiates circadian regulation by CLOCK:BMAL1. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**:6386-6391

[41] Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. Annual Review of Physiology. 2010;**72**:517-549

[42] Husse J, Eichele G, Oster H. Synchronization of the mammalian circadian timing system: Light can control peripheral clocks independently of the SCN clock: Alternate routes of entrainment optimize the alignment of the body's circadian clock network with external time. Bio essays: News and reviews in molecular, cellular and. Developmental Biology. 2015;**37**:1119-1128

[43] Sancar A. Cryptochrome: The second photoactive pigment in the eye and its role in circadian photoreception. Annual Review of Biochemistry. 2000;**69**:31-67

[44] Albers HE, Walton JC, Gamble KL, McNeill JK, Hummer DL. The dynamics of GABA signaling: Revelations from the circadian pacemaker in the suprachiasmatic nucleus. Frontiers in Neuroendocrinology. 2017;**44**:35-82

[45] Obrietan K, Impey S, Storm DR. Light and circadian rhythmicity regulate MAP kinase activation in the suprachiasmatic nuclei. Nature Neuroscience. 1998;**1**:693-700

[46] Antoun G, Bouchard-Cannon P, Cheng HY. Regulation of MAPK/ERK signaling and photic entrainment of the suprachiasmatic nucleus circadian clock by Raf kinase inhibitor protein. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 2012;**32**:4867-4877

[47] Serchov T, Heumann R. Ras activity tunes the period and modulates the entrainment of the Suprachiasmatic clock. Frontiers in Neurology. 2017;**8**:264

[48] Travnickova-Bendova Z, Cermakian N, Reppert SM, Sassone-Corsi P. Bimodal regulation of mPeriod promoters by CREB-dependent signaling and CLOCK/BMAL1 activity. Proceedings of the National Academy

of Sciences of the United States of America. 2002;**99**:7728-7733

[49] Crosio C, Cermakian N, Allis CD, Sassone-Corsi P. Light induces chromatin modification in cells of the mammalian circadian clock. Nature Neuroscience. 2000;**3**:1241-1247

[50] Hirayama J, Alifu Y, Hamabe R, Yamaguchi S, Tomita J, Maruyama Y, et al. The clock components period 2, Cryptochrome 1a, and Cryptochrome 2a function in establishing light-dependent behavioral rhythms and/or total activity levels in zebrafish. Scientific Reports. 2019;**9**:196

[51] Bae K, Jin X, Maywood ES, Hastings MH, Reppert SM, Weaver DR. Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock. Neuron. 2001;**30**:525-536

[52] Zheng B, Albrecht U, Kaasik K, Sage M, Lu W, Vaishnav S, et al. Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock. Cell. 2001;**105**:683-694

[53] Daiyasu H, Ishikawa T, Kuma K, Iwai S, Todo T, Toh H. Identification of cryptochrome DASH from vertebrates. Genes to Cells. 2004;**9**:479-495

[54] Hirayama J, Kaneko M, Cardone L, Cahill G, Sassone-Corsi P. Analysis of circadian rhythms in zebrafish. Methods in Enzymology. 2005;**393**:186-204

[55] Vatine G, Vallone D, Gothilf Y, Foulkes NS. It's time to swim! Zebrafish and the circadian clock. FEBS Letters. 2011;**585**:1485-1494

[56] Kobayashi Y, Ishikawa T, Hirayama J, Daiyasu H, Kanai S, Toh H, et al. Molecular analysis of zebrafish photolyase/cryptochrome family: Two types of cryptochromes present in zebrafish. Genes to Cells. 2000;**5**:725-738

[57] Dekens MP, Whitmore D. Autonomous onset of the circadian clock in the zebrafish embryo. The EMBO Journal. 2008;**27**:2757-2765

[58] Kaneko M, Cahill GM. Light-dependent development of circadian gene expression in transgenic zebrafish. PLoS Biology. 2005;**3**:e34

[59] Cahill GM. Clock mechanisms in zebrafish. Cell and Tissue Research. 2002;**309**:27-34

[60] Kazimi N, Cahill GM. Development of a circadian melatonin rhythm in embryonic zebrafish. Brain Research. Developmental Brain Research. 1999;**117**:47-52

[61] Thomas GM, Huganir RL. MAPK cascade signalling and synaptic plasticity. Nature Reviews. Neuroscience. 2004;**5**:173-183

[62] Pagano C, Siauciunaite R, Idda ML, Ruggiero G, Ceinos RM, Pagano M, et al. Evolution shapes the responsiveness of the D-box enhancer element to light and reactive oxygen species in vertebrates. Scientific Reports. 2018;**8**:13180

[63] Mracek P, Santoriello C, Idda ML, Pagano C, Ben-Moshe Z, Gothilf Y, et al. Regulation of per and cry genes reveals a central role for the D-box enhancer in light-dependent gene expression. PLoS One. 2012;**7**:e51278

[64] Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT. Hydrogen peroxide and nitric oxide as signalling molecules in plants. Journal of Experimental Botany. 2002b;**53**:1237-1247

[65] Neill S, Desikan R, Hancock J. Hydrogen peroxide signalling. Current Opinion in Plant Biology. 2002a;**5**:388-395

[66] Osaki T, Uchida Y, Hirayama J, Nishina H. Diphenyleneiodonium

chloride, an inhibitor of reduced nicotinamide adenine dinucleotide phosphate oxidase, suppresses light-dependent induction of clock and DNA repair genes in zebrafish. Biological & Pharmaceutical Bulletin. 2011;**34**:1343-1347

[67] Hockberger PE, Skimina TA, Centonze VE, Lavin C, Chu S, Dadras S, et al. Activation of flavin-containing oxidases underlies light-induced production of H_2O_2 in mammalian cells. Proceedings of the National Academy of Sciences of the United States of America. 1999;**96**:6255-6260

[68] Todo T. Functional diversity of the DNA photolyase/blue light receptor family. Mutation Research. 1999;**434**:89-97

[69] Cashmore AR, Jarillo JA, Wu YJ, Liu D. Cryptochromes: Blue light receptors for plants and animals. Science. 1999;**284**:760-765