

The retinal clock in mammals: role in health and disease

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Abstract: The mammalian retina contains an extraordinary diversity of cell types that are highly organized into precise circuits to perceive and process visual information in a dynamic manner and transmit it to the brain. Above this builds up another level of complex dynamic, orchestrated by a circadian clock located within the retina, which allows retinal physiology, and hence visual function, to adapt to daily changes in light intensity. The mammalian retina is a remarkable model of circadian clock because it harbors photoreception, self-sustained oscillator function, and physiological outputs within the same tissue. However, the location of the retinal clock in mammals has been a matter of long debate. Current data have shown that clock properties are widely distributed among retinal cells and that the retina is composed of a network of circadian clocks located within distinct cellular layers. Nevertheless, the identity of the major pacemaker, if any, still warrants identification. In addition, the retina coordinates rhythmic behavior by providing visual input to the master hypothalamic circadian clock in the suprachiasmatic nuclei (SCN). This light entrainment of the SCN to the light/dark cycle involves a network of retinal photoreceptor cells: rods, cones, and intrinsically photosensitive retinal ganglion cells (ipRGCs). Although it was considered that these photoreceptors synchronized both retinal and SCN clocks, new data challenge this view, suggesting that none of these photoreceptors is involved in photic entrainment of the retinal clock. Because circadian organization is a ubiquitous feature of the retina and controls fundamental processes, the coherence from cell to tissue is critical for circadian functions, and disruption of retinal clock organization or its response to light can potentially have a major impact on retinal pathophysiology and vision.

Keywords: retina, clock gene, circadian, ipRGC, photoreceptor, light

Introduction

From algae to mammals, nearly all organisms have adapted their lifestyle to the environmental 24 h light/dark (LD) cycle. Daily rhythms in behavior and physiology are programmed by a group of biological clocks widely present in a variety of tissues and cells, including the retina, and organized in a hierarchical manner. The central clock in the suprachiasmatic nucleus (SCN) lies at the top of the network and coordinates the phases of many peripheral clocks throughout the body. The temporal phase of the SCN is adjusted daily by light, which is captured by the retina, in order to synchronize organism-wide behavioral and physiological rhythms with the LD cycle. One of the first rhythms described in mammalian retina was the finding that rod outer segment disk shedding cycles in relationship with lighting conditions.¹ Furthermore, experiments performed in amphibians and birds demonstrated that the retina contains an endogenous circadian clock, able to oscillate in constant condition in a culture dish.^{2,3}

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However, it was not until 1996 that cultured hamster retina was reported to maintain autonomous circadian rhythms for melatonin secretion, providing evidence that an endogenous clock is also present in the mammalian retina,^{4,5} regardless of the absence of the SCN.^{6,7} Progress toward understanding the mechanisms of circadian rhythm generation and light entrainment of the retinal clock has been hampered by technical limitations. The successful development of bioluminescence recording using luciferase reporter coupled to clock genes fundamentally revolutionized studies on circadian oscillators.^{8,9} This technique provides the advantage of detecting and quantifying circadian rhythms in retinal living cells or explants, as well as revealing retinal circadian oscillations in vitro in long-term studies in mammals.¹⁰ Many reports have now clearly demonstrated that several molecular, cellular, and physiological retinal rhythms are under the control of a retinal circadian clock or, more likely, a network of clocks localized in multiple types of neurons and glial Müller cells (Figure 1).¹¹

Molecular organization and light entrainment of the retinal clock

Molecular basis of retinal circadian rhythms

The endogenous functioning of circadian clocks, including the retina, involves an integrated network of clock genes interacting in transcriptional–translational feedback loops.¹² Complexes of BMAL1 and CLOCK proteins bind to E-box enhancer elements in the promoters of *Period 1–3* (*Per1–3*), *Cryptochrome 1–2* (*Cry1–2*) clock genes, and “clock-controlled genes” and thereafter drive their expression. PER and CRY proteins form heterodimers that translocate back into the nucleus to inhibit CLOCK/BMAL1 transcriptional activity and therefore their own expression, forming the core negative feedback loop (Figure 1). Fine-tuning and reinforcement of this loop are ensured by another loop involving activation by CLOCK/BMAL1 of the *Ror* and *RevErb* nuclear hormone receptor–encoding genes. By competitive binding

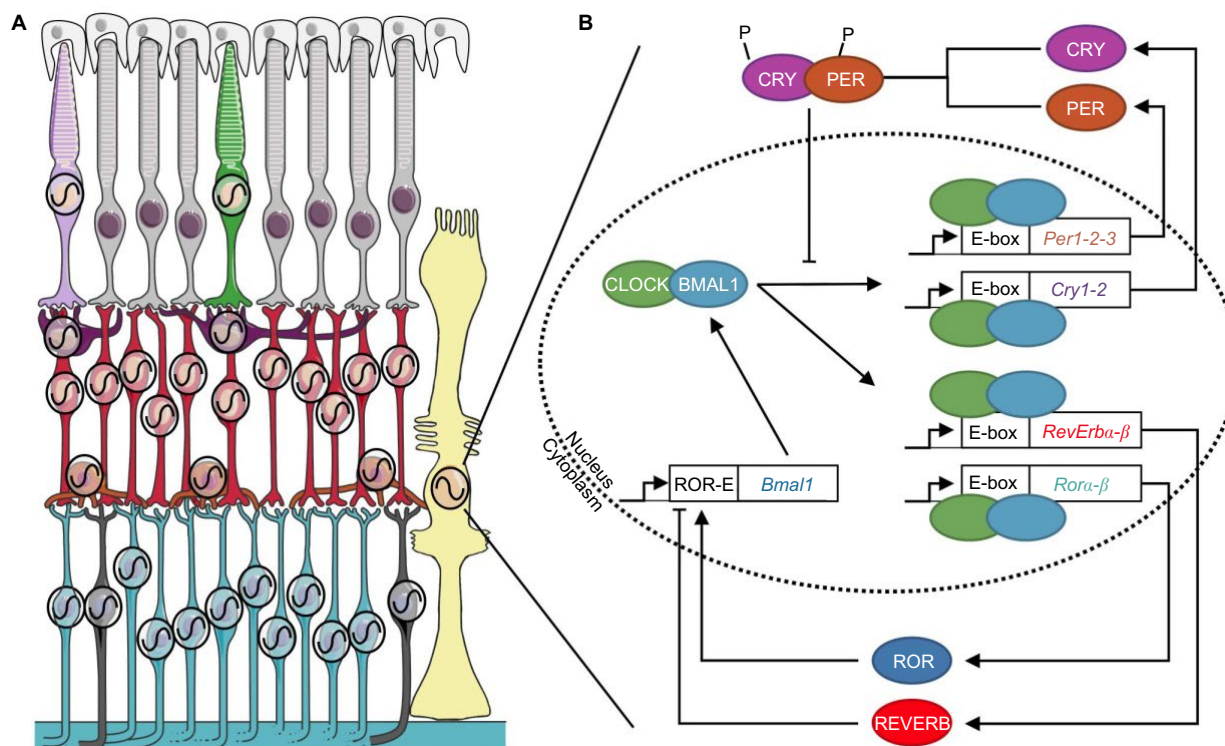


Figure 1 The circadian clock system in the mammalian retina.

Notes: (A) Clock machinery has been described in all retinal neurons, besides rods, as well as in glial Müller cells (black oscillation). Light purple: SW cone; green: MW cone; gray: rod; purple: horizontal cell; red: bipolar cell; orange: amacrine cell; blue: ganglion cell; black: ipRGCs; yellow: Müller cell; white: retinal pigmented epithelium.¹²⁴ (Adapted from Servier Medical Art [homepage on the Internet]. Available from: <http://www.servier.com/Powerpoint-image-bank>. Accessed January 1, 2017.x (<https://creativecommons.org/licenses/by/3.0/>). (B) The endogenous molecular mechanism generating circadian rhythms is based on transcriptional–translational feedback loops involving clock genes. The CLOCK/BMAL1 heterodimer binds to an enhancer box (E-box) and drives the transcription of the clock genes *Period* (*Per1–2–3*), *Cryptochrome* (*Cry1–2*), *Rora-β*, and *RevErbα-β*. PER and CRY proteins in turn inhibit CLOCK/BMAL1 transcriptional activity. By competitive binding to retinoid-related orphan receptors response elements (ROR-E) sites, the REVBER and ROR proteins repress or activate *Bmal1* transcription, respectively.

Abbreviations: MW, middle-wavelength sensitive; SW, short-sensitive.

to ROR-E sites, the REVERB and ROR proteins, respectively, repress or activate *Bmal1* gene transcription. Importantly, additional posttranslational events, such as proteasomal degradation, phosphorylation, acetylation, ubiquitination, and sumoylation, are also key components in the generation and timing of circadian rhythms. The culmination of activities of these transcription factors regulates circadian biology, from molecular mechanisms to overall behavior, thus constituting a time code for the cell and the entire organism.

Impact of clock gene deletion on retinal functions

Mutant and transgenic rodent models have provided a useful tool to evaluate a specific clock gene's function in circadian rhythms. However, the majority of clock genes have functionally redundant paralogs that are able to perform effectively as the primary gene. Thus, deleting a specific clock gene does not necessarily disrupt the clock mechanism within a cell but may change circadian parameters at the level of the tissue or the organism. *Per2*-knockout mice exhibit retinal vascular damage, reduction in retinal thickness, and a decrease in the electroretinogram (ERG) amplitude, similar to those found in type 2 diabetes.^{13–15} A double-clock gene mutant, the *Per1^{Brdm1}/Per2^{Brdm1}* mouse, exhibits delayed photoreceptor differentiation and alterations in short-wavelength cone distribution.¹⁶ *Cry1^{-/-}/Cry2^{-/-}* and *Bmal1* retina-specific knockout mice show a loss of ERG circadian rhythm, suggesting that functional *Bmal1* and *Cry1-Cry2* genes are required for rhythmic retinal processing of visual stimuli, independently from the central clock.^{17,18} In addition, *Bmal1^{-/-}* mice present abnormal circadian gene expression profiles in LD cycles, suggesting that *Bmal1* is involved in the light regulation of gene expression. Silencing of *RevErba* using small interfering RNA induces retinal abnormalities, such as pan-retinal spotting and decreased response to light.¹⁹ By contrast, Ait-Hmyed et al²⁰ reported a hypersensitivity to light in *RevErba^{-/-}* mice, concomitant with an increased number of melanopsin-containing ganglion cells, suggesting that this clock gene modulates light sensitivity and visual processing. Only one study has extensively explored the rhythmic phenotype of single clock gene knockout in retina. Using several clock-gene-deficient mice, Ruan et al²¹ reported that the retinal clock is more vulnerable to clock gene disruption than the SCN. The authors showed that *Per1*, *Cry1*, and *Clock* are each necessary for the expression of molecular circadian rhythms by the retina. By contrast, knocking out *Per2*, *Per3*, or *Cry2* individually does not blunt the rhythm but modulates its period or amplitude.²¹ For example, retinal explants from

Per3^{-/-} mice show a period-shortening effect, whereas the period of the SCN clock is not altered. This differential pattern of circadian rhythm alterations between the retina and the SCN was proposed to relate to the fact that cellular coupling among clock cells/layers is not uniform throughout the retina,²² whereas oscillators are strongly coupled in the SCN.²³

Entrainment of the retinal clock by light

An especially important finding was that “blind” transgenic mice lacking functional rods and cones (*rd/rd cl*) exhibit near-normal photoentrainment of circadian rhythms.^{24,25} This subsequently led to the discovery of a novel opsin, melanopsin (OPN4), expressed in a subset of intrinsically photosensitive retinal ganglion cells (ipRGCs), with a peak of sensitivity at ~480 nm.^{26–28} It is now clear that ipRGCs combine their direct intrinsic responses with signals derived from rods and cones to regulate diverse non-image-forming responses,²⁹ with each photoreceptor encoding distinct parameters of the light.^{30–34} However, we currently lack a comprehensive understanding of how light regulates the retinal clock in mammals and of the role of the different photoreceptors.

Nevertheless, several lines of evidence suggest that ipRGCs influence retinal network and physiology. Melanopsin was shown to be critical for diurnal and circadian regulation of the mouse photopic and scotopic ERG, suggesting influence of melanopsin up to the photoreceptor level.^{35–37} In agreement with this, we reported that circadian rhythms of clock gene expression are lost in the photoreceptor layer (PRL) of *Opn4^{-/-}* mice.³⁸ In addition, synaptic contact of ipRGCs with other retinal neurons has been reported in rodents, monkeys, and humans.^{39–42} Specifically, ipRGCs provide excitatory sustained light responses to dopaminergic (DAergic) amacrine cells, which are influential modulators of retinal circuitry.⁴³ In the absence of melanopsin, this sustained response is lost,⁴⁰ and blue light fails to activate the DAergic system and to induce *Per1-Per2* mRNA expression in the PRL.³⁸ The function of these retrograde connections is currently unknown, but they represent a potential mechanism for transmitting irradiance information to the outer retina.

In mammals, the first rhythm shown in vitro to be synchronized with LD cycles was melatonin secretion in the mouse retina.⁵ Ten years later, Ruan et al¹⁰ clearly established that a pulse of white light administered at circadian time (CT)13 or CT19, respectively, delays or advances the phase of *PER2^{Luc}*, principally via the DA D1 receptor. Recent data suggest that the photoreceptors regulating light response of retinal rhythms differ from those used by the SCN.^{44,45} *PER2^{Luc}* rhythm is still entrained to LD cycle in

rd1/rd1;Opn4^{-/-} mice, lacking rods, cones, and melanopsin.⁴⁴ The authors propose that an ultraviolet (UV)-sensitive opsin, neuropsin (OPN5), is the sole opsin required to phase-shift the retinal clock in response to light.⁴⁵ Neuropsin is expressed in many vertebrate species and is localized in a subset of mouse RGCs,⁴⁵⁻⁴⁸ but it is unclear whether it is expressed in ganglion cells distinct from ipRGCs. However, Hughes et al⁴⁹ suggested a limited role for neuropsin in driving light response in the mouse retina because UV light stimulation failed to induce both c-FOS and excitatory responses in mice lacking the essential components of the phototransduction signaling pathways present in rods, cones, and ipRGCs.⁴⁹ All these studies raise important questions about the relationship between neuropsin and melanopsin and their respective roles in the light response of the retinal clock.

Cellular and layer organization of the retinal clock

At the cellular level

In early studies, analysis of the expression pattern of core clock genes in the whole retina was the classic approach used to assess the functioning of the retinal clock.⁵⁰ However, this methodology led to significant dissimilarities among the results observed in different species or even within the same species among different strains.⁵⁰ Moreover, the cellular diversity of mammalian retina renders difficult the analysis of the circadian clock at the whole-tissue level because differences in phasing and period between cells/layers may result in low-amplitude rhythmicity or masking of coherent clock gene expression.^{22,38,51-55} It is therefore essential to determine which cell types possess clock properties to understand rhythm generation in the retina. Based on the molecular and physiological data from amphibians and birds, the initial prevailing model for circadian organization in vertebrate retinas proposed that photoreceptors contain self-sustained circadian clocks.⁵⁶⁻⁵⁸ By contrast, the cellular location of the retinal clock in mammals has been a matter of long debate. In early research, retinal degenerative animal models such as the *rd* mouse and the Royal College of Surgeons rat, both characterized by progressive degeneration of photoreceptors, showed arrhythmic melatonin release, whereas the daily rhythms of DA were retained,^{5,59} suggesting that photoreceptors are necessary to maintain rhythmic melatonin production, but their integrity was not required for DA signaling. With the discovery of clock genes, photoreceptors have been the site of the most intensive controversy because initial reports claimed that expression of *Clock*, *Bmal1*, *Per1-2*, or *Cry2* takes place in the outer nuclear layer (ONL) of rodents,⁶⁰⁻⁶²

whereas others failed to detect them.^{17,52,63} More recently, by using ONL isolated by laser microdissection, vibratome sectioning, or following kainic acid treatment, several studies demonstrated clock property in rat photoreceptors under an LD cycle, but these rhythms were severely damped in constant darkness, indicating that the photoreceptor clock is not robust,⁶⁴⁻⁶⁶ although data obtained in the mouse support the opposite conclusion.³⁸ This latter analysis was the first to compare the kinetics of clock gene expression in ONL and inner retina (ganglion cell layer [GCL] + inner nuclear layer [INL]) and concluded that both retinal compartments show clock gene oscillations, interestingly without any obvious phase relationship (Figure 2). However, only ONL displayed the complete set of oscillating clock genes, suggesting that the main clock is located within the photoreceptors.³⁸ Conversely, several studies support the idea that the generation of rhythms takes place mainly in the inner retina.^{17,52,63,67} The strongest evidence is provided by the study of *Per2^{Luc}* mice. When analyzed on an *rd* background, on which photoreceptors degenerate quite early, *PER2^{Luc}* activity was shown to be rhythmic⁵² and generated in the inner retina specifically.¹⁰ Responsible cell types have not been identified yet, although DAergic amacrine cells, which have been shown to express the whole set of clock genes,⁶⁸ are a good candidate. Although their contribution to clock gene oscillations in the retina has been poorly evaluated, ganglion cells have also been reported to harbor clock gene transcripts or products,^{52,61-63,69} sometimes with sparse distribution. The most extensive ex vivo analysis of the distribution of clock gene expression in the retina was conducted by immunohistochemistry in the mouse. Surprisingly, the authors came to the conclusion that, although clock proteins are present in most cell types besides rods, rhythmic expression is detected uniquely in cones.⁵³ Müller cells, the main glial cell type of the retina, have been demonstrated to also express sustained rhythms in primary culture of rodent or human cells transduced by luciferase-expressing viruses.⁷⁰ Taken together and in spite of some discrepancy, these data suggest that clock genes are expressed throughout the retina.

At the level of layers

Studying the specific clock properties and robustness of a retinal subregion by ex vivo analyses is limited by the existence of potential input received from neighboring cells. By using an original, vibratome-based procedure to isolate the retinal cellular layers (PRL, INL, and GCL) from *Per2^{Luc}* mice, we established that these 3 layers can act as functional, self-sustained circadian pacemakers.²² However, explants of

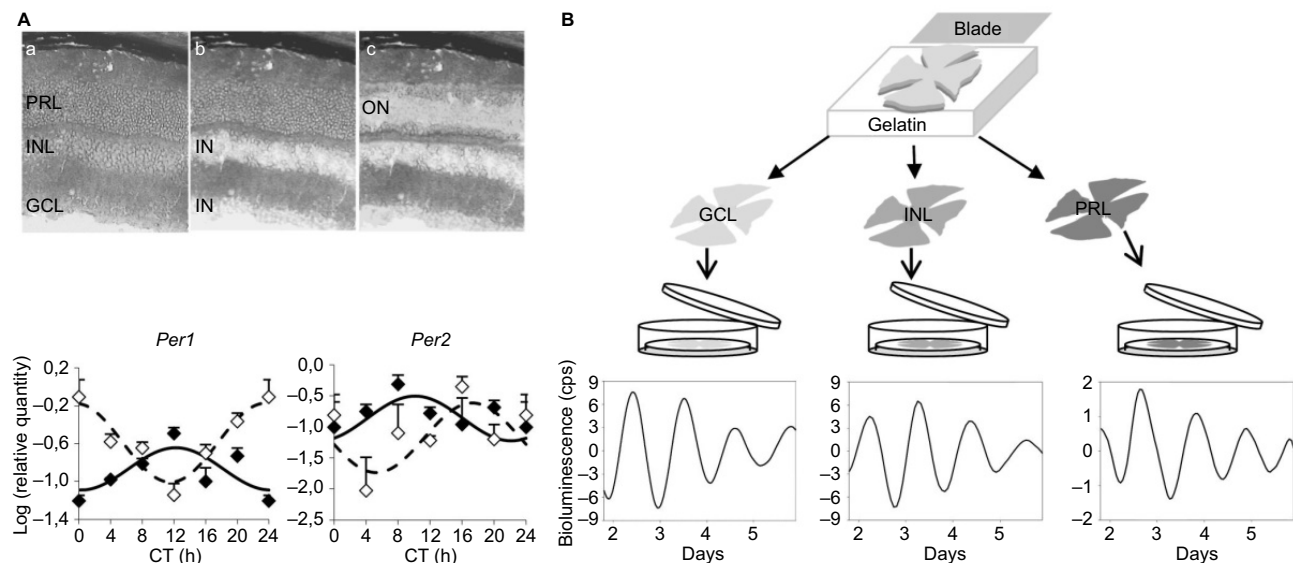


Figure 2 The retinal clock contains a network of clocks.

Notes: (A) Top panel: photomicrographs of a retinal section of wild-type mouse (a) before and after laser microdissection of (b) the IN of the retina, including the INL + GCL, and (c) the ON, which includes the PRL. Bottom panel: circadian expression of *Per1* and *Per2* clock genes in the inner (solid line) and the outer (dashed line) retinal layers of the wild-type mouse. Note that both compartments, the IN and the ON, show clock gene oscillations, interestingly without any obvious phase relationship. *Cell Mol Life Sci*. The absence of melanopsin alters retinal clock function and dopamine regulation by light, 70(18), 2013, 3435–3447, Dkhissi-Benyahya O, Coutanson C, Knoblauch K, et al. With permission of Springer.³⁸ (B) Top panel: Vibratome-sectioning method for isolation of the PRL, the INL, and the GCL from freshly mounted retinas. Bottom panel: Representative bioluminescence recordings (detrended data following 24 h running average subtraction) of a GCL (left panel), an INL (middle panel), and a PRL (right panel) sample from *PER2^{Luc}* mice during the first week of culture. Data from Jaeger et al and Sandu et al.^{22,66}

Abbreviations: cps, counts per second; CT, circadian time; GCL, ganglion cell layer; IN, inner region; INL, inner nuclear layer; ON, outer region; PRL, photoreceptor layer.

PRLs showed the lowest amplitude and robustness, which correlated with the absence of rhythms in PRLs isolated similarly from *Per1*-luciferase rats, in which GCL and INL showed clear rhythms (Figure 2).²² These results confirm that the retina is a network of circadian oscillators located in the different layers and in which the INL and GCL provide major contribution. In conclusion, expression of clock genes has been detected in most retinal neurons as well as in glial cells.⁷⁰ The identity of the major pacemaker, if any, still warrants identification but it is likely that, as in the SCN, the global network plays a role in the sustainability of the retinal clock.

Intercellular/layer synchronization in the retina

Irrespective of the exact cellular location of the retinal clock in mammals, data from early studies indicate that the retinal oscillators are interconnected, notably through the DA/melatonin feedback loop.⁵⁰ In the current view essentially derived from amphibian and avian models, the secretion of DA by a subset of amacrine cells in the light period and the synthesis of melatonin in PRLs during the night signal to the retina that it is day and night, respectively. Both molecules display reciprocal negative effects, involving, respectively, the melatonin receptors present in the INL, amacrine cells in particular,^{71,72} and the D2-like DA recep-

tors located on photoreceptors.^{73,74} Taken together, these cells and neuromodulators constitute an intercellular feedback loop that regulates retinal rhythmic physiology (melatonin and DA displaying overt largely distributed receptors) and sustains the rhythmic activity of the clocks that have not been localized yet. In this respect, the case of most mouse lines is odd because they do not synthesize melatonin and are unable to maintain rhythmic DA release in constant dark conditions,⁵⁹ although they otherwise display robust *PER2^{Luc}* oscillations.^{10,22} This result indicates that DAergic cells, at least in melatonin-deficient mice, are not the site of rhythm generation and that photoreceptors in melatonin-proficient mice, contain a clock releasing melatonin as a synchronizing signal. The localization of the clock contained in the inner retina still awaits identification, but it has been suggested that it does not rely on any synchronizing signal from the remainder of the retina or within the clock population itself. Indeed, in the presence of agonists or antagonists of most common neurotransmitters, as well as upon blocking of neural transmission, the cycling bioluminescence from *PER2^{Luc}* retinas was barely affected.^{10,22} Only treatment with gamma-aminobutyric acid (GABA) decreased the amplitude, indicating that it has a negative impact on the synchronization.^{10,22} We observed that, upon dividing retinas from the same strain of mice into distinct cellular layers or combinations thereof,

the period of *PER2^{Luc}* oscillations was strongly correlated with the degree of dissociation of the tissue. Moreover, communication between the INL and GCL appeared to rely on their synaptic connection because both layers sampled individually and cultured together exhibited periods similar to those of the independent layers and significantly higher than those of INL + GCL sampled together. In addition, the blocking of gap junctions, similar to dissociating the retinas, increased the period of the retinal clock, although this effect was only partial. These data support the idea that the retinal clock is rather composed of several oscillators, which are located in the distinct layers and strongly connected not only by gap junctions but also by other (notably synaptic) signals that remain to be identified.

Functions controlled by the retina clock

Many functions have been described to be rhythmic in the retina exposed to the LD cycle and are thought to optimize adaptation of vision to the daily changes in light intensity. Many of them have been shown to remain rhythmic in constant conditions and, hence, to be regulated by a circadian clock: this is the case for melatonin and DA synthesis,⁷⁵ phagocytosis of photoreceptor outer segments,¹ amplitude of the photopic ERG,^{35,76} contrast sensitivity,⁷⁷ and rod–cone coupling.⁷⁸ Since the demonstration of the existence of an autonomous clock in the mammalian retina,⁴ it has been essentially assumed that the rhythmic retinal functions are under the control of the retina clock seen as a whole. The ongoing paradigm about how circadian clocks control their outputs is based on the idea that clock transcription factors transfer their rhythmicity to their target genes. In this context, generation of tissue-specific cycling gene expression programs can result from either indirect transcriptional activation by transcription factors that are targets of the clock or direct effects from the combination of clock factors and tissue-specific regulators. By using high-throughput transcriptome analyses, it has been demonstrated that ~43% of protein-encoding genes in mammals are rhythmic.⁷⁹ One groundbreaking study conducted on whole eyes sampled throughout the 24 h cycle nicely showed that, in LD conditions, the knockout of *Bmal1* induces an alteration of rhythmic expression in 90% of the genes, with total loss of rhythmicity in 60% of them.¹⁷ This study hence places the circadian clock in the center of rhythmic functions in the retina, *Bmal1* being required even to mediate the effects of the LD cycle on gene expression. However, analysis of the genes identified as rhythmic in this study did not reveal

clear enrichment in specific functions, most major pathways, including photoreceptor signaling and synaptic transmission, being found in the gene list.¹⁷ A similar study was reported more recently, with whole eye globes sampled at 2 zeitgeber times (ZT): ZT1.5, coinciding with the peak of photoreceptor outer segment phagocytosis, and ZT9.⁸⁰ The authors describe rhythmic expression of genes related to both the phosphoinositide metabolism and regulation of phagocytosis, with maxima correlating with the peak of phagocytosis in the retina. Concomitantly, another study was conducted on rat photoreceptors isolated by laser microdissection at ZT6 and ZT18.⁸¹ Forty genes displaying differential day/night expression in PRLs and confirmed in whole retina samples were reported, notably linked to transcriptional regulation, cell cycle and cell death, cell response to environmental signals, and retina-specific functions (*Grk1*, *Rorb*, *Pax4*, and *Pgc1a*), confirming that the clock controls many distinct processes in photoreceptors.⁸¹

More globally, insight into how the clock controls retinal function has been mainly obtained as a result of targeted analysis of gene expression, in most cases by Northern blotting and/or real-time quantitative polymerase chain reaction (qPCR), which has led to a wide list of cycling genes, either in LD or in LD and constant dark (DD) conditions (Table 1). Not surprisingly, the genes identified as rhythmic mainly refer to visual function, indicating that, even if there is a bias in the analysis, photoreceptors display rhythmic activity regulated at the gene expression level. They also indicate that part of this regulation involves cycling transcription factors and signaling proteins that are not retina specific.

Regarding clock-controlled genes, the most investigated ones relate to the regulation of melatonin synthesis, described as a characteristic output from the retinal clock in most vertebrate species examined.^{2,82,83} In mammals, the site for melatonin synthesis has not been located strictly to the PRL because melatonin synthesis is retained upon photoreceptor degeneration⁸⁴ and also because the enzymes involved in its synthesis are detected in the GCL.⁸⁵ However, its regulation in the PRL has been the best characterized. Expression of several enzymes involved in the biochemical cascade generating melatonin was shown to be rhythmic in the rat retina: TPH, HIOMT, and AA-NAT,⁸⁶ which catalyzes the penultimate, rate-limiting step. The promoter of the *Aanat* gene contains an E-box sequence, which was shown to be stimulated by BMAL1/CLOCK.⁸⁷ This gene is likely regulated as well through its well-conserved CRE site, which mediates cyclic adenosine monophosphate (cAMP) activation potentially during the night, following photoreceptor depolarization.

Table I Genes whose expression (mRNA or protein) was reported to vary over the 24 h cycle in light/dark (LD), constant dark (DD), or both conditions (LD and DD)

Gene symbol	Lighting condition in which temporal variation in gene expression over ~24 h was demonstrated in whole retina samples, and related references
<i>A2aR</i>	LD: Li et al ¹²⁵
<i>Aanat</i>	LD: Roseboom et al ¹²⁶ ; Schneider et al ⁶⁵
	LD and DD: Sakamoto et al ⁸⁴ ; Sakamoto et al ¹²⁷ ; Bai et al ¹²⁸ ; Bobu et al ¹²⁹
<i>Acadm</i>	LD and DD: Vancura et al ¹³⁰
<i>Adcy1</i>	LD: Hwang et al ⁷⁷
	DD: Jackson et al ¹³¹
	LD and DD: Fukuhara et al ⁸⁸
<i>Adra1b</i>	LD and DD: Kunst et al ⁸¹
<i>Arr3</i>	LD and DD: Bobu et al ¹²⁹
<i>Asns</i>	LD: Kunst et al ⁸¹
<i>Atf3</i>	LD: Kunst et al ⁸¹
<i>Cdkn1b</i>	LD: Kunst et al ⁸¹
<i>Cerk</i>	LD and DD: Kunst et al ⁸¹
<i>Cx36</i>	LD and DD: Katti et al ¹³²
<i>Cox1</i>	LD and DD: de Zavalía et al ¹³³
<i>Cpt1a</i>	LD and DD: Vancura et al ¹³⁰
<i>Ctnnal1</i>	LD: Kunst et al ⁸¹
<i>Dbp</i>	DD: Sakamoto et al ⁷ ; Storch et al ¹⁷ ; Lahouaoui et al ⁵⁵
<i>Dicer</i>	LD: Yan et al ¹³⁴
<i>Drd4</i>	LD: Li et al ¹²⁵ ; Klitten et al ¹³⁵
	DD: Storch et al ¹⁷
	LD and DD: Bai et al ¹²⁸ ; Jackson et al ¹³¹
<i>Dyrk2</i>	LD: Kunst et al ⁸¹
<i>Egr1</i>	LD: Humphries and Carter ¹³⁶ ; Man et al ¹³⁷
	LD and DD: Bai et al ¹²⁸
<i>Egr2</i>	LD: Bai et al ¹²⁸
<i>Esrrb</i>	LD: Kunst et al ¹³⁸
<i>Fos</i>	LD: Humphries and Carter ¹³⁶ ; Yoshida et al ¹³⁹ ; Kamphuis et al ¹⁴⁰
<i>Gapdh</i>	LD: Kamphuis et al ¹⁴⁰
<i>Gnat1</i>	LD: Brann and Cohen ¹⁴¹
<i>Grk1</i>	LD: Kunst et al ⁸¹
<i>Gsk3b</i>	LD: Vancura et al ¹³⁰
<i>Gys1</i>	LD: Vancura et al ¹³⁰
<i>Hiomt</i>	LD: Gauer and Kraft ¹⁴²
<i>c-Jun</i>	LD: Kamphuis et al ¹⁴⁰
<i>Kcnb1</i>	LD: Holter et al ¹⁴³
<i>Kcnv2</i>	LD: Holter et al ¹⁴³
<i>Mertk</i>	LD: Law et al ¹⁴⁴
<i>Noct</i>	LD and DD: Wang et al ¹⁴⁵
<i>Nr2c1</i>	LD: Kunst et al ¹³⁸
<i>Nr2e3</i>	LD: Mollema et al ¹⁹
	LD and DD: Kunst et al ¹⁴⁶
<i>Nr4a1</i>	LD and DD: Kunst et al ¹⁴⁶
<i>Nupr1</i>	LD: Kunst et al ¹³⁸
<i>Opn1mw</i>	LD: Sakamoto et al ¹⁴⁷
	LD and DD: Bobu et al ¹²⁹
<i>Opn1sw</i>	LD: Sakamoto et al ¹⁴⁷
	DD: von Schantz et al ¹⁴⁸
	LD and DD: Bobu et al ¹²⁹
<i>Opn2</i>	LD: Sakamoto et al ¹⁴⁷
	DD: von Schantz et al ¹⁴⁸
	LD and DD: Bobu et al ¹²⁹
<i>Opn4</i>	LD: Bobu et al ¹²⁹ ; Mathes et al ¹⁴⁹
	LD and DD: Sakamoto et al ¹⁵⁰ ; Hannibal et al ¹⁵¹

(Continued)

Table 1 (Continued)

Gene symbol	Lighting condition in which temporal variation in gene expression over ~24 h was demonstrated in whole retina samples, and related references
<i>Otx2</i>	LD: Kunst et al ¹³⁸
<i>Pax4</i>	LD: Kunst et al ⁸¹
<i>Pde8a</i>	LD and DD: Kunst et al ⁸¹
<i>Pde10a</i>	LD and DD: Wolloscheck et al ¹⁵²
<i>Pla2g1b</i>	LD: Kunst et al ⁸¹
<i>Ppargc1a</i>	LD: Kunst et al ⁸¹
<i>Rcvrn</i>	LD: Kamphuis et al ¹⁴⁰ ; Wiechmann and Sinicola ¹⁵³
<i>Rhobtb1</i>	LD: Kunst et al ⁸¹
<i>Sirt1, 2, 5-7</i>	LD: Ban et al ¹⁵⁴
<i>Th</i>	LD: Sakamoto et al ¹⁵⁰
<i>Tph1</i>	LD: Kamphuis et al ¹⁴⁰ ; Liang et al ¹⁵⁵
<i>Tph2</i>	LD: Liang et al ¹⁵⁵
<i>Usp2a, 2b</i>	LD: Scoma et al ¹⁵⁶

This CRE-mediated control probably works also in constant conditions because cAMP is rhythmic in constant darkness.⁸⁸ Indeed, the BMAL1/CLOCK dimer directly stimulates transcription of *Ac1* gene (encoding adenylate cyclase 1), potentially restricting its expression to the end of the day and night, thus gating cAMP increase and *Aanat* activation to the same temporal window.⁸⁸

The potential role of the retinal clock in eye disease

A myriad of circadian rhythms has been described in the mammalian eye. Most of them, if not all, are regulated by the retinal clock.⁵⁰ Furthermore, the mammalian retinal clock controls cell survival and growth processes in the eye, including the susceptibility of photoreceptors to degeneration from light damage or during aging.^{89,90} The widespread control of signaling, metabolism, and gene expression exerted by the retinal circadian clock suggests that its integrity is critical for maintaining normal rhythmic responses and retina homeostasis. Thus, this organ represents a major target for impairments induced by circadian misalignment or retinal diseases.

Several ocular pathologies, such as diabetes, glaucoma, or age-related macular degeneration, are associated with degeneration of retinal cells, including photoreceptors, which receive and integrate light signals from the environment. These pathologies represent the major causes of blindness that not only affect vision but also lead to a decrease or loss of the photic input to the circadian system, impairing proper synchronization to the environmental 24 h cycle and thus adding to vision loss another strong handicap.

Dysregulation of clock gene expression has been identified as a key factor in the pathogenesis of several diseases or

retinal alterations. *Bmal1*^{-/-} mice display ocular abnormalities, including corneal neovascularization, keratinization, and progressive inflammation,⁹¹ associated with reduced life span, as well as premature aging symptoms, such as cataracts, also reported in *Clock*-deficient mice.⁹² *Clock* mutant and *Bmal1* conditional knockout mice develop type 1 diabetes,⁹³ whereas *Per2*-knockout mice exhibit retinal phenotype similar to that found in type 2 diabetes.¹³⁻¹⁵ Conversely, diabetes is also associated with circadian disruption of clock gene expression. In rodent models of type 1^{94,96} or type 2⁹⁵ diabetes, the expression of several clock genes, including *Clock*, *Bmal1*, *Per1-Per2*, and *Cry1-2*, was downregulated in the retina, whereas no alterations were observed in the SCN. We reported similar observations using a mouse model of type 1 diabetes induced by streptozotocin injection.⁵⁵ In this model, the amplitude of *Per1* and *Cry1* gene expression is upregulated, whereas *Per3* loses its circadian rhythmicity in the retina. In addition to the dysregulation of the retinal clock organization, we reported disturbances in one of its main outputs, the DAergic system, with 56% reduction in the total number of tyrosine hydroxylase (TH)-positive cells in diabetic mice,⁵⁵ in accordance with previous studies performed both in mouse and rat models of diabetes.⁹⁷⁻¹⁰¹ The diabetes-associated visual dysfunction reported in rodent models^{100,102,103} and in diabetic patients who exhibit defects in color perception and contrast sensitivity can be treated by DA precursors or DA agonists.¹⁰⁰ However, the underlying mechanisms are still unclear. The decline in DA content during diabetes may be related to a reduced number of retinal DAergic neurons, low levels of DA synthesis, or an impairment of DA release from the cells. In addition, a deficit in light induction of *Th*, *c-Fos*, and *Per1* in response to 480 nm light has been reported during the development

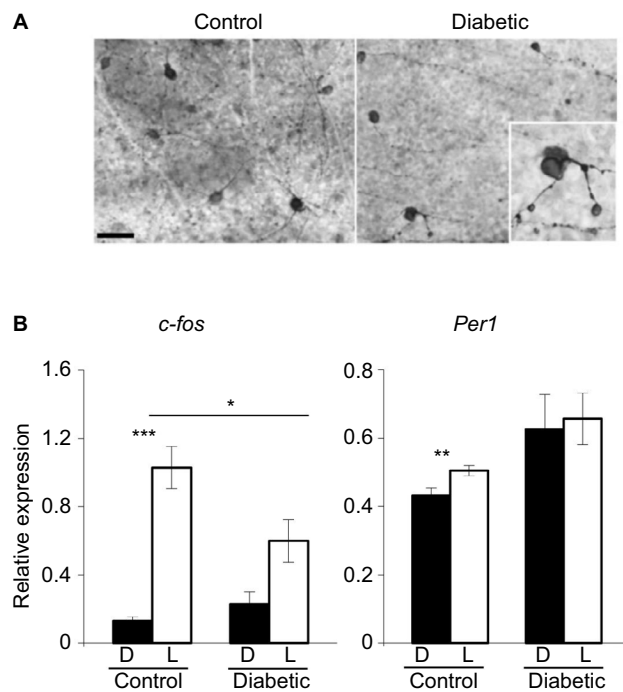


Figure 3 ipRGCs and light-induced gene expression are altered in a mouse model of diabetic retinopathy.

Notes: (A) Representative photomicrographs of flat-mounted retinas from control and diabetic mouse models immunostained by melanopsin antibody at 12 weeks postdiabetes. Morphological abnormalities of ipRGCs, such as soma swelling and increased varicosities in the dendrites, were observed in the peripheral retina of diabetic animals. Enlarged image of a melanopsin-positive cell (inset: magnification $\times 100$). Scale bar = 50 μ m. (B) Relative light-induced *c-Fos* and *Per1* mRNA levels in the retinas of control and diabetic mice at 12 weeks postdiabetes. Control and diabetic mice were exposed to a 480 nm monochromatic light pulse (1.17×10^{14} photons/cm²/s, 15 min duration) delivered at CT16. White bars represent animals that received the light pulse (L). The dark control mice (D, black bars) were handled the same way but did not receive any light stimulus. Data are presented as mean value \pm SEM. Asterisk indicates a statistically significant difference: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (modified from^{55,104}). Adapted from Lahouaoui H, Coutanson C, Cooper HM, Bennis M, Dkhissi-Benyahya O. Diabetic retinopathy alters light-induced clock gene expression and dopamine levels in the mouse retina. *Mol Vis.* 2016;22:959.⁵⁵ Adapted from Lahouaoui H, Coutanson C, Cooper HM, Bennis M, Dkhissi-Benyahya O. Clock genes and behavioral responses to light are altered in a mouse model of diabetic retinopathy. *PLoS One.* 2014;9(7):e101584.¹⁰⁴

Abbreviations: CT, circadian time; ipRGC, intrinsically photosensitive retinal ganglion cell; SEM, standard error of the mean.

of diabetic retinopathy, possibly through impaired ipRGCs (Figure 3).^{55,104} In patients with type 2 diabetes, ipRGC dysfunction has been proposed to be investigated for diagnostic purpose by using the postillumination pupil response (PIPR), a sustained constriction of the pupil after light offset.¹⁰⁵

The classic hallmark of glaucomatous optic neuropathies is the progressive loss of RGCs and their axons, with concomitant insidious defects in the visual field. As for RGCs, ipRGC abnormalities have been reported in rodent models of experimental glaucoma^{106–109} and in glaucomatous patients.^{110,111} We reported an ~50%–70% reduction of RGC axon terminals (including ipRGCs) in several visual and non-visual structures, notably in the SCN, and an alteration of the expression of opsin genes in the retina in an experimental rat

model of glaucoma,¹⁰⁶ as observed by others.¹¹² In addition, several nonvisual responses to light were affected, such as the pupillary light response, light-induced nocturnal pineal melatonin suppression, and locomotor activity rhythm.^{106,108} Dysfunction of ipRGCs in animal models of glaucoma have been corroborated by human studies because glaucoma patients with sleep disorders showed a reduction in the pupillary light response and suppression of nocturnal melatonin by light.^{113–118}

The potential role of ipRGCs has been described in other pathological processes. Concurrent regression of embryonic hyaloid vasculature and formation of the retinal vasculature occur postnatally in the mouse,¹¹⁹ and the light response pathway that regulates both processes involves melanopsin.¹²⁰ In neurodegenerative diseases such as Parkinson's and Alzheimer's, a contribution of retinal deficits to circadian abnormalities has been suggested because loss of ipRGCs has been found to be associated with β -amyloid deposits.¹²¹ Degeneration of cone and melanopsin pathways has also been reported in a mouse model of Huntington's disease.¹²² All these data suggest alteration of both retinal photoreception and photic signal transmission necessary to entrain the retinal and the central clocks in several degenerative diseases. Using impaired circadian photoreception as a marker or biological indicator of retinal or brain pathologies will represent a valuable research tool for diagnostic purposes. ERG anomalies are, for instance, associated with several psychiatric disorders.¹²³

Conclusion

The retina constitutes a fascinating clock model perfectly suitable both to understand the molecular mechanisms of circadian rhythm generation at the cellular and tissue levels and to characterize the synchronizing factors coordinating multiple oscillators at the tissue level. To elucidate these key unresolved questions, the development of in vitro culture of isolated cells or retinal explants from bioluminescent mice models, coupled with a spatial resolution of the signal, is crucial to monitor clock oscillations at the single-cell level in all retinal layers and over several days. Disruption of retinal clock organization or its entrainment by light may have major impacts on visual and nonvisual functions. In addition, given its highly rhythmic physiology, the retina potentially represents a major target for impairments induced by circadian misalignment. Thus, it will be important to assess the potential detrimental effects of repetitive clock phase shifts, such as those induced by shift work or repetitive jet lag, on retina function and health.

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Disclosure

The authors report no conflicts of interest in this work.

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