The retinal clock in mammals: role in health and disease

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
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, regardless of the absence of the SCN. 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. 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. 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).

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. 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](https://www.dovepress.com/)

**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. (Adapted from Servier Medical Art [homepage on the Internet]. Available from: http://www.servier.com/Powerpoint-image-bank. Accessed January 1, 2017. (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), Rorα/β, and RevErbα/β. PER and CRY proteins in turn inhibit CLOCK/BMAL1 transcriptional activity. By competitive binding to retina-related orphan receptors response elements (ROR-E) sites, the REVERB 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 Per1Brdn1/Per2Brdn1 mouse, exhibits delayed photoreceptor differentiation and alterations in short-wavelength cone distribution.16 Cry1–/Cry2– mice show a loss of ERG circadian rhythm, suggesting that functional Bmal1 and Cry1-Cry2 genes are required for the rhythmic regulation of light 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 RevErbα using small interfering RNA induces retinal abnormalities, such as pan-retinal spotting and decreased response to light.19 By contrast, Ait-Hmied et al20 reported a hypersensitivity to light in RevErbα+/– mice, comitant 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 Per2−/− 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 PER2Luc, 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 PER2Luc rhythm is still entrained to LD cycle in
Red/rd1/Opn4<sup>-/-</sup> mice, lacking rods, cones, and melanopsin. 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. Neuropsin is expressed in many vertebrate species and is localized in a subset of mouse RGCs, but it is unclear whether it is expressed in ganglion cells distinct from ipRGCs. However, Hughes et al. 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. 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. However, this methodology led to significant dissimilarities among the results observed in different species or even within the same species among different strains. 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. 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. 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 others failed to detect them. 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, although data obtained in the mouse support the opposite conclusion. 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. Conversely, several studies support the idea that the generation of rhythms takes place mainly in the inner retina.

The strongest evidence is provided by the study of Per<sup>2Luc</sup> mice. When analyzed on an rd background, on which photoreceptors degenerate quite early, PER2<sup>Luc</sup> activity was shown to be rhythmic and generated in the inner retina specifically. Responsible cell types have not been identified yet, although DAergic amacrine cells, which have been shown to express the whole set of clock genes, 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, 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. 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. 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 Per<sup>2Luc</sup> mice, we established that these 3 layers can act as functional, self-sustained circadian pacemakers. However, explants of
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). 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. 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. 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, and the D2-like DA receptors located on photoreceptors. 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, although they otherwise display robust PER2Luc oscillations. 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 PER2Luc retinas was barely affected. Only treatment with gamma-aminobutyric acid (GABA) decreased the amplitude, indicating that it has a negative impact on the synchronization. We observed that, upon dividing retinas from the same strain of mice into distinct cellular layers or combinations thereof,
the period of PER2
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
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 con-
tant conditions and, hence, to be regulated by a circadian
clock: this is the case for melatonin and DA synthesis,\textsuperscript{75}
phagocytosis of photoreceptor outer segments,\textsuperscript{1} amplitude
of the photopic ERG,\textsuperscript{35,76} contrast sensitivity,\textsuperscript{77} and rod–cone
coupling.\textsuperscript{78} Since the demonstration of the existence of an
autonomous clock in the mammalian retina,\textsuperscript{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 con-
text, 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.\textsuperscript{79} One
groundbreaking study conducted on whole eyes sampled
throughout the 24 h cycle nicely showed that, in LD con-
ditions, the knockout of Bmal1 induces an alteration of
rhythmic expression in 90% of the genes, with total loss of
rhythmicity in 60% of them.\textsuperscript{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.\textsuperscript{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 photorecep-
tor outer segment phagocytosis, and ZT9.\textsuperscript{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.\textsuperscript{81} Forty genes displaying differential day/night expres-
sion 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, Rorh, Pax4, and Pgc1a),
confirming that the clock controls many distinct processes
in photoreceptors.\textsuperscript{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 blot-
ting 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.\textsuperscript{2,3,83} In mammals, the site for
melatonin synthesis has not been located strictly to the PRL
because melatonin synthesis is retained upon photoreceptor
degeneration\textsuperscript{84} and also because the enzymes involved in its
synthesis are detected in the GCL.\textsuperscript{85} However, its regulation
in the PRL has been the best characterized. Expression of sev-
eral enzymes involved in the biochemical cascade generating
melatonin was shown to be rhythmic in the rat retina: TPH,
HIOMT, and AA-NAT,\textsuperscript{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.\textsuperscript{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 1
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)

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Lighting condition in which temporal variation in gene expression over ~24 h was demonstrated in whole retina samples, and related references</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2aR</td>
<td>LD: Li et al&lt;sup&gt;125&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
| Aanat       | LD: Roseboom et al<sup>124</sup>; Schneider et al<sup>95</sup>  
LD and DD: Sakamoto et al<sup>84</sup>; Sakamoto et al<sup>127</sup>; Bai et al<sup>128</sup>; Bobu et al<sup>129</sup> |
| Acadm       | LD and DD: Vancura et al<sup>130</sup>                                                                                           |
| Adcy1       | LD: Kwang et al<sup>77</sup>  
DD: Jackson et al<sup>131</sup>  
LD and DD: Fukuwara et al<sup>89</sup> |
| Adra1b      | LD and DD: Kunst et al<sup>81</sup>                                                                                                                                 |
| Arr3        | LD and DD: Bobu et al<sup>129</sup>                                                                                                                                 |
| Asns        | LD: Kunst et al<sup>81</sup>  
DD: Jackson et al<sup>131</sup>  
LD and DD: Fukuhara et al<sup>89</sup> |
| Atf3        | LD: Kunst et al<sup>81</sup>  
DD: Jackson et al<sup>131</sup>  
LD and DD: Fukuhara et al<sup>89</sup> |
| Cdkn1b      | LD: Kunst et al<sup>81</sup>  
DD: Jackson et al<sup>131</sup>  
LD and DD: Fukuwara et al<sup>89</sup> |
| Cerk        | LD and DD: Kunst et al<sup>81</sup>                                                                                                                                 |
| Cx36        | LD and DD: Katti et al<sup>132</sup>                                                                                                                                 |
| Cox1        | LD and DD: de Zavalia et al<sup>133</sup>                                                                                         |
| Cpt1a       | LD and DD: Vancura et al<sup>130</sup>                                                                                           |
| Ctnnal1     | LD: Kunst et al<sup>81</sup>  
DD: Jackson et al<sup>131</sup>  
LD and DD: Fukuwara et al<sup>89</sup> |
| Dbp         | DD: Sakamoto et al<sup>84</sup>; Storch et al<sup>17</sup>; Lahouaoui et al<sup>85</sup>                                       |
| Dicer       | LD: Yan et al<sup>134</sup>  
DD: Jackson et al<sup>131</sup>  
LD and DD: Fukuwara et al<sup>89</sup> |
| Drd4        | LD: Li et al<sup>132</sup>; Klitten et al<sup>135</sup>  
DD: Storch et al<sup>17</sup>  
LD and DD: Bai et al<sup>128</sup>; Jackson et al<sup>131</sup> |
| Dyrk2       | LD: Kunst et al<sup>81</sup>  
DD: Jackson et al<sup>131</sup>  
LD and DD: Fukuwara et al<sup>89</sup> |
| Egr1        | LD: Humphries and Carter<sup>136</sup>; Man et al<sup>137</sup>  
LD and DD: Bai et al<sup>128</sup> |
| Egr2        | LD: Bai et al<sup>128</sup>  
DD: Jackson et al<sup>131</sup>  
LD and DD: Fukuwara et al<sup>89</sup> |
| Esrb        | LD: Kunst et al<sup>81</sup>  
DD: Jackson et al<sup>131</sup>  
LD and DD: Fukuwara et al<sup>89</sup> |
| Fos         | LD: Humphries and Carter<sup>136</sup>; Yoshida et al<sup>137</sup>; Kamphuis et al<sup>140</sup>                 |
| Gapdh       | LD: Kamphuis et al<sup>140</sup>                                                                                                   |
| Gnat1       | LD: Brann and Cohen<sup>131</sup>                                                                                                 |
| Grk1        | LD: Kunst et al<sup>81</sup>  
DD: Jackson et al<sup>131</sup>  
LD and DD: Fukuwara et al<sup>89</sup> |
| Gsk3b       | LD: Vancura et al<sup>130</sup>                                                                                                   |
| Gys1        | LD: Vancura et al<sup>130</sup>                                                                                                   |
| Hiont       | LD: Gauer and Kraft<sup>42</sup>                                                                                                   |
| c-Jun       | LD: Kamphuis et al<sup>140</sup>                                                                                                   |
| Kcnb1       | LD: Holter et al<sup>133</sup>                                                                                                     |
| Kcnv2       | LD: Holter et al<sup>143</sup>                                                                                                     |
| Mertk       | LD: Law et al<sup>144</sup>                                                                                                       |
| Ncst        | LD and DD: Wang et al<sup>145</sup>                                                                                               |
| Nr2c1       | LD: Kunst et al<sup>138</sup>                                                                                                     |
| Nr2e3       | LD: Molemen et al<sup>93</sup>  
LD and DD: Kunst et al<sup>146</sup>                                                                                                   |
| Nr4a1       | LD and DD: Kunst et al<sup>146</sup>                                                                                               |
| Nupr1       | LD: Kunst et al<sup>138</sup>                                                                                                     |
| Opn1mw      | LD: Sakamoto et al<sup>147</sup>                                                                                                   |
| Opn1sw      | LD and DD: Bobu et al<sup>129</sup>                                                                                               |
| Opn2        | LD: Sakamoto et al<sup>147</sup>                                                                                                   |
| Opn4        | LD and DD: Sakamoto et al<sup>150</sup>; Hannibal et al<sup>151</sup>                                                          |

(Continued)
This CRE-mediated control probably works also in constant conditions because cAMP is rhythmic in constant darkness. 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.

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. 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. 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, associated with reduced life span, as well as premature aging symptoms, such as cataracts, also reported in Clock-deficient mice. Clock mutant and Bmal1 conditional knockout mice develop type 1 diabetes, whereas Per2-knockout mice exhibit retinal phenotype similar to that found in type 2 diabetes. Conversely, diabetes is also associated with circadian disruption of clock gene expression. In rodent models of type 1 or type 2 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. 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, in accordance with previous studies performed both in mouse and rat models of diabetes. The diabetes-associated visual dysfunction reported in rodent models and in diabetic patients who exhibit defects in color perception and contrast sensitivity can be treated by DA precursors or DA agonists. 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 of diabetes.

### Table 1 (Continued)

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<tr>
<td>Pax4</td>
<td>LD: Kunst et al81</td>
</tr>
<tr>
<td>PdeB8a</td>
<td>LD and DD: Kunst et al81</td>
</tr>
<tr>
<td>Pde10a</td>
<td>LD and DD: Wolloscheck et al152</td>
</tr>
<tr>
<td>Pia2g1b</td>
<td>LD: Kunst et al81</td>
</tr>
<tr>
<td>Ppargc1a</td>
<td>LD: Kunst et al81</td>
</tr>
<tr>
<td>Rcrn</td>
<td>LD: Kamphuis et al80; Wiechmann and Sinacola153</td>
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<tr>
<td>Rhobb1</td>
<td>LD: Kunst et al80</td>
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<td>Sir1, 2, 5-7</td>
<td>LD: Ban et al154</td>
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<td>Th</td>
<td>LD: Sakamoto et al155</td>
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<td>Tph1</td>
<td>LD: Kamphuis et al80; Liang et al155</td>
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<td>Tph2</td>
<td>LD: Liang et al155</td>
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<td>Usp2a, 2b</td>
<td>LD: Scoma et al156</td>
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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 suited 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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