Published in final edited form as:

Bioessays. 2008 July ; 30(7): 624–633.

The Circadian Clock System in the Mammalian Retina

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Abstract

Daily rhythms are a ubiquitous feature of living systems. Generally, these rhythms are not just passive consequences of cyclic fluctuations in the environment, but instead originate within the organism. In mammals, including humans, the master pacemaker controlling 24-hour rhythms is localized in the suprachiasmatic nuclei of the hypothalamus. This circadian clock is responsible for the temporal organization of a wide variety of functions, ranging from sleep and food intake, to physiological measures such as body temperature, heart rate and hormone release. The retinal circadian clock was the first extra-SCN circadian oscillator to be discovered in mammals and several studies have now demonstrated that many of the physiological, cellular, and molecular rhythms that are present within the retina are under the control of a retinal circadian clock, or more likely a network of hierarchically organized circadian clocks that are present within this tissue.

Introduction

The daily alternation of light and darkness is the most reliable timing cue on our planet and, therefore, it is not surprising that most organisms have evolved precise timing mechanisms (circadian clocks) that allow them to anticipate and adapt to dramatic changes in light intensity during the 24-hour day. These circadian clocks control a wide variety of functions within the organisms. In mammals, the master circadian pacemaker is localized in the suprachiasmatic nuclei (SCN) of the hypothalamus. The SCN circadian clock is responsible for the temporal organization of several functions, ranging from sleep and food intake, to body temperature, heart rate and hormone release (1). We now know that although the master pacemaker in the SCN regulates the timing of most circadian rhythms in mammals, either directly or indirectly, many organs/tissues of the body can generate circadian rhythms independently of the SCN *in vitro*, and are thus called peripheral circadian oscillators (2). Among these peripheral circadian oscillators, the mammalian retina represents a unique case since it appears to be rather independent from the SCN and may influence the circadian rhythms generated by the SCN.

The aim of this review is to describe the retinal circadian clock system, its organization, and its contribution to the general circadian organization of mammals.

The molecular circadian clock

The current model of the mammalian circadian clock includes two interlocking transcription-translation feedback loops comprised of several so-called “clock” genes and their protein

products, which ultimately regulate the transcription of “clock-controlled” genes (Figure 1). These feedback loops consist of positive and negative components. The positive components include the basic helix-loop-helix-PAS domain transcription factors, CLOCK and BMAL1. These transcription factors heterodimerize, translocate from the cytosol to the nucleus, and bind to circadian E-box promoter elements that enhance the transcription of genes encoding the negative components PERIOD 1 & 2 and CRYPTOCHROME 1 & 2. The CRYPTOCHROME and PERIOD proteins feedback inhibit the transcription of the *Cryptochrome* and *Period* genes by blocking CLOCK/BMAL1-mediated trans-activation. The second feedback loop involves the trans-activation of the *Rev-Erba* and *Rora* genes by CLOCK/BMAL1. The protein products of these genes compete for binding to RRE elements in the *Bmal1* promoter, driving a daily rhythm of *Bmal1* transcription and closing the second feedback loop (3). Rhythmic expression of these clock gene products produces circadian clock outputs by regulating transcription of clock-controlled genes (CCGs). At least some of these CCGs, including *aanat*, the gene encoding the penultimate enzyme in the melatonin biosynthetic pathway, contain circadian E boxes, which have a core nucleotide sequence of CACGTG and are activated rhythmically by CLOCK/BMAL1 (4). Post-translational regulation, including phosphorylation, acetylation, ubiquitination, sumoylation and proteasomal degradation are also important in the regulatory mechanisms generating the circadian oscillation (5,6).

Regulation of retinal melatonin synthesis by circadian clocks

Studies in the early eighties demonstrated that a circadian clock was present in the eye of the frog *Xenopus laevis* (7). A few years later, circadian clocks in *Xenopus* and chick retinæ were localized to the photoreceptor cells (8–10). These clocks drive rhythms of melatonin production and expression of red cone opsin mRNA. Experimental evidence collected in the early 1990s suggested that the mammalian retina, like the retinæ of non-mammalian vertebrates, contains an autonomous circadian clock (11–12) that controls circadian rhythms in the eye (13). Additional studies demonstrated that at least one of these rhythms, outer segment disc shedding of the rod photoreceptors, persists in animals with SCN lesions or transected optic nerves, indicating that this rhythm was driven by an extra-SCN circadian clock (12).

The direct demonstration that the mammalian retina contained an independent circadian clock was obtained about ten years ago when it was reported that melatonin release followed a circadian pattern in cultured mammalian retina (14). This circadian rhythm of melatonin release free-ran in constant darkness, was entrained by light *in vitro* (14) and was temperature-compensated (15). Such results directly demonstrate that the retina is a *bona fide* circadian pacemaker since it satisfies the three fundamental properties of a circadian rhythm (i.e., free-running, entrainment, and temperature compensation). Moreover, the observation that a mutation of casein kinase 1 epsilon, which binds to and phosphorylates clock proteins (16), has a similar effect on circadian timing in the retina and the SCN, indicated that the mechanism generating the circadian oscillation in the retina is similar, if not identical, to the one in the SCN (14). The photic and circadian regulation of retinal melatonin depends primarily on arylalkylamine *N*-acetyltransferase (*aanat*) transcription, post-translational modification, and degradation (17–19). Therefore, several studies have focused on the regulation of this enzyme to investigate the mechanisms by which the retinal circadian clock regulates melatonin synthesis. In the mammalian retina, *aanat* mRNA has been localized to the photoreceptor layer (20–22) and, to a much lesser extent, the inner nuclear layer and the ganglion cell layer (21, 22). Retinal *aanat* mRNA levels and activity show marked day-night and circadian variations in many species (21,23–25). In the rat retina, *aanat* mRNA rhythmicity persists in animals in which the SCN of the hypothalamus have been lesioned (26) and after disruption of the dopaminergic and GABAergic neurons in the inner retina (27).

The promoter region of the rat *aanat* gene contains an E-box (28) and the transcription of this gene in photoreceptors is directly regulated by the CLOCK/BMAL1 complex (28,29). However, *aanat* transcription and the stability of the enzyme protein are also subject to photic control via the activation of the cAMP pathway that follows depolarization of the photoreceptors in darkness (30). Interestingly, the activation of this pathway is directly gated by the circadian clock since darkness can increase melatonin production only in the late day and the night, and has no effect in the morning (Figure 2) (25). This gating occurs because the cAMP signaling cascade is under control of the circadian clock (25). The circadian clock regulates the cAMP signaling cascade by directly controlling the transcription and, presumably, the protein level and activity, of adenylyl cyclase type 1 (AC1), a Ca^{2+} /calmodulin stimulated enzyme (25). AC1 expression and activity, and cAMP levels are also circadian in chicken retina (31,32), suggesting that this pathway is conserved.

A series of recent studies also investigated the effects of different photoperiods on the expression of *aanat* mRNA. The results obtained indicated that the circadian regulation of *aanat* mRNA is affected by the photoperiodic history (33,34) thus suggesting that the pacemaker driving *aanat* transcription in the photoreceptors can store photoperiodic information in a manner similar to that reported for SCN. Another similarity between the SCN clock and the pacemaker driving *aanat* transcription in the retina has been reported with the respect to the effect of restricted feeding in the rat retina (35). Restricted feeding shifted the phase of the rhythms of *Period 2* (*Per2*) and *albumin D-site binding protein* (*Dbp*) transcripts in the retina. However, the peak of the *aanat* mRNA rhythm did not change with restricted feeding, although an additional minor peak was produced at mid-day. Thus, unlike most peripheral oscillators, the pacemaker regulating the major peak of the retinal *aanat* mRNA rhythm oscillated independently of the imposed feeding cycle, as does the SCN clock.

These observations indicate that the regulation of *aanat* mRNA in the retina represents an ideal model to investigate the functioning of circadian clocks and how the external environment interacts with circadian oscillators to regulate a physiologically relevant circadian output (i.e., melatonin).

Circadian organization of the retinal clock network

Melatonin and dopamine (DA) play opposing roles in the regulation of retinal adaptive physiology (36–38). DA functions as a humoral signal for light, producing light-adaptive physiology. Melatonin, on the other hand, has dark-adaptive effects. In many species, the synthesis and release of both melatonin and DA are under circadian control, with melatonin released at night and DA during the daytime (Figure 3) (37,38). Melatonin inhibits the release of DA through an action on melatonin receptors (39,40), and DA inhibits the synthesis and release of melatonin from photoreceptor cells by acting on D_2 -like dopamine receptors (41–43). Thus, the melatonin secreting photoreceptors and DA secreting neurons form an intercellular feedback loop functioning to regulate circadian retinal physiology.

Studies in *Xenopus* photoreceptors have demonstrated that DA, acting on photoreceptor cells, plays an important role in the entrainment of retinal circadian rhythms. Light and DA both can phase shift the photoreceptor clock (44). However, the pathway used by DA involves suppression of cAMP levels, whereas light uses a cAMP-independent pathway (45). *Per2* induction in *Xenopus* photoreceptors is directly responsive to light and DA, suggesting that PERIOD 2 acts as the molecular link between the light/dark cycle or DA receptor activation and the circadian clock (46,47).

Recent studies have also suggested that DA is the key player in the control of retinal circadian rhythmicity in mammals. Retinal DA neurons express the core circadian clock genes (48–51). These findings support the hypothesis that dopaminergic amacrine cells contain an

autonomous circadian clock that drives DA release and metabolism. However, the ability to generate circadian rhythms of DA metabolism appears to depend on the melatonin feedback loop. Retinal DA content and metabolism are circadian in mice that synthesize melatonin, but not in mice that are genetically incapable of synthesizing the neurohormone (52,53). In the latter strains of mice, DA release is strictly light driven; and daily injections of melatonin induce circadian rhythms of DA in retinæ of mice that are unable to synthesize melatonin (53). Thus, the circadian rhythm of DA and DA metabolites in the mouse retina depends upon the presence of the circadian rhythm of melatonin. Likewise, circadian rhythms of DA release and metabolism in retinæ of fish, birds and lizards appear to require melatonin (54–56). Whether melatonin drives the rhythms of DA metabolism or entrains the oscillators in DA neurons is an unresolved issue.

In rat retina, disruption of DA signaling (and destruction of most of the inner retina) does not abolish the circadian rhythm of *aanat* mRNA in the photoreceptor cells (27), and the isolated photoreceptor layer can rhythmically synthesize melatonin *in vitro* (57). These data provide compelling experimental evidence that the circadian rhythm in *aanat* mRNA is not driven by DA, but rather by a circadian clock present in the photoreceptors.

However, the phase of the circadian rhythm in *aanat* mRNA is advanced in rats in which the inner retina has been removed (27). Thus, although the rat photoreceptors contain the circadian pacemaker driving melatonin synthesis, some interaction must exist between the photoreceptor and the inner retinal (dopaminergic ?) clocks; this relationship may be important to set the phase of the clock in the photoreceptors (Figure 3). A dopamine clock also appears to be important for regulating rhythms in the inner retina, as disruption of DA signaling in the rat retina abolishes the circadian rhythms in the transcript and protein levels of melanopsin (58–59), the photopigment of the intrinsically-photosensitive ganglion cells that transmit light-information from the retina to the SCN (60).

In mice, DA modulates diurnal rhythms of cAMP- and CaM kinase-dependent protein phosphorylation in photoreceptor cells, decreasing phosphorylation state during the day (61). The photoreceptor-specific protein phosducin, which is phosphorylated at distinct sites by cAMP- and Ca²⁺/calmodulin-dependent kinases, undergoes a robust diurnal rhythm of phosphorylation at both sites (61). Blockade of DA receptors or genetic deletion of D4 receptors reduces the amplitude of the diurnal rhythm of phosducin phosphorylation at both the cAMP- and the Ca²⁺/calmodulin-dependent kinase phosphorylation sites, indicating that the rhythm is modulated by DA. Interestingly, the rhythm of phosducin phosphorylation persists in constant darkness in melatonin-synthesizing C3H/f^{+/+} mice but not in melatonin-deficient C57Bl/6J mice. This circadian rhythm of phosducin phosphorylation state in C3H/f^{+/+} mice is eliminated by DA receptor blockade. These results provide compelling evidence that DA modulates rhythms of photoreceptor metabolism in mice, and that the DA-melatonin feedback loop, first established in non-mammalian vertebrate retina (36), plays an important role in the circadian organization of the mouse retina.

Circadian clock localization within the retina

The retina is a heterogeneous tissue with many different types of specialized cells, several of which have been shown to express clock genes. It is clear that the circadian clocks driving melatonin rhythms in *Xenopus* and chickens are localized in photoreceptor cells (7,31). In mammals, we know that the circadian pacemaker driving melatonin synthesis is localized within the neural retina, but the cell type(s) that contain(s) the circadian oscillators are still unclear.

The first attempt to localize the circadian clock within the retina used a mouse model (*rd* mouse) (62), in which rod photoreceptors degenerate within a few weeks of birth (63). In these mice,

retinal melatonin synthesis becomes arrhythmic once rod photoreceptors have degenerated (62). This observation suggested that mouse rods contain the circadian pacemaker that drives retinal melatonin synthesis, but that rods are not the only retinal cell type capable of melatonin synthesis (62). Additional studies using another animal model of retinal degeneration, the Royal College of Surgeons (RCS) rat, indicated a rather different picture. In RCS rats, DA and DA metabolite levels *in vivo* are still under circadian control after the rod photoreceptors have degenerated (64), initially leading to the conclusion that melatonin is not required for generating DA rhythms. However, further studies indicated that *aanat* mRNA expression in the retina of these rats shows remarkable plasticity with respect to cellular localization and regulation. In retinas of intact, wild type rats, *aanat* mRNA appears to be rhythmically expressed exclusively in photoreceptors, with a low level of constitutive expression in the inner nuclear layer (65). After degeneration of photoreceptors in the RCS rats, *aanat* expression becomes up-regulated and rhythmic in the inner nuclear layer and melatonin continues to be rhythmically produced (65). This reorganization of melatonin synthetic capability following photoreceptor degeneration may account for the persistence of circadian rhythms of DA metabolism in the RCS rat. A further experiment also indicated that intraocular injection of kainic acid - which destroys most of the inner retinal neurons but spares photoreceptor cells - abolished the circadian rhythms of *aanat* mRNA in the RCS rats with no photoreceptors, but not in the congenic, wild type controls. These data demonstrated that at least two circadian clock systems are present in the retina of the RCS rats. Nevertheless, it is not clear if the inner retinal circadian clock is present in the normal retina or is the result of changes in retinal organization that follow photoreceptor degeneration.

The mechanism responsible for generating the circadian oscillation requires the presence within a cell of certain clock genes (1,3). Therefore, a simple, but indirect, method to identify clock cells is to perform *in situ* hybridization to identify which cells express the mRNA for the clock genes. In *Xenopus* and chicken retinas, clock genes are indeed expressed in the photoreceptors cells (46,66–68) and disruption of the CLOCK protein in *Xenopus* photoreceptors abolished the circadian rhythms of melatonin synthesis (69).

In rat retina, *Period1* (*Per1*) mRNA expression is low in photoreceptors and more abundant in the inner retinal neurons (70). A similar pattern has been observed with *Bmal1* and *Clock* mRNAs (71). A recently published report, in which laser capture microdissection combined with RT-PCR was employed, supports these early findings by demonstrating that low levels of most of the clock genes are present in rat photoreceptors (57).

In the mouse retina, the situation is much more complicated and the results obtained are rather contradictory. Initially, it was reported that *Per1*, *Clock*, and *Bmal1* mRNAs were present and co-localize in photoreceptors and the inner retinal neurons (72). Additional studies reported that photoreceptors do (73,74) or do not express (48,75) *Per1* transcripts. More recently Ruan et al. (50), using single cell RT-PCR, detected expression of circadian clock gene transcripts in a small percentage of photoreceptors. However, co-expression of the six core clock genes (*Per1*, *Per2*, *Cry1*, *Cry2*, *Clock*, *Bmal1*) within the same photoreceptor cells was not detected. In contrast, inner retinal neurons, and in particular DA neurons, co-express these clock genes (48–51).

A few studies have also reported the pattern of expression of clock genes in the retina, but the results reported do not provide much insight into the functioning of retinal circadian clocks (76–78). As mentioned earlier, the retina is a heterogeneous tissue with several types of cells. *In situ* hybridization studies have shown that clock genes may be expressed in different cell types and at different levels (48,74) and, possibly, with different phases (57). Therefore, it is unlikely that measuring mRNA levels from the whole retina will provide any useful indication about the functional characteristics of retinal circadian clocks.

A recent paper reported a circadian rhythm of *Per2* promoter-driven luciferase expression in the retinae of mice with almost no photoreceptors (50). The circadian rhythms in bioluminescence in these cultured retinae persisted for several days before damping out, after which forskolin stimulation restored the circadian rhythm. Interestingly, the authors also reported that intact retinae did not express a circadian rhythm in *Per2*-luciferase bioluminescence. This result is quite interesting since in the mouse (as well as in the rat) *Per2* transcripts are not readily detectable in photoreceptors cells (57,70,75) and, therefore, it is unclear why the intact retina should behave in a different manner from the retina lacking photoreceptors.

Another recent report indicated that isolated photoreceptor layers (PRLs) obtained from *Per1*-luciferase (*Per1*-luc) rats express a robust circadian rhythm in bioluminescence, thereby demonstrating that rat photoreceptors contain a circadian pacemaker (57). In contrast, intact rat retinae showed low amplitude rhythms or no rhythm at all in *Per1*-luc bioluminescence (57). The absence of rhythmicity in *Per1*-luc bioluminescence in intact retina can be explained by the fact that *Per1* is expressed in more than one retinal layer and therefore it is likely that the *Per1*-luc bioluminescence observed in the intact retinal preparation represents the expression of multiple clocks that may be out of phase with one another, canceling out each other's rhythms of photon emission (50). This interpretation is supported by observations with *Per1*-luc rat retina in which the phase of the PRL rhythms was significantly different from the phase in the few intact retinae in which a rhythm was observed (57) and by the results obtained in kainic acid treated retina (27).

The absence of the *Per1*-luc circadian rhythm in the intact retina is not due to the lack of viability of these cultures, since forskolin stimulation induced circadian oscillations in arrhythmic retinae or increased the amplitude of the rhythms in retinae that showed low amplitude rhythms (Tosini, unpublished results). This observation suggests that the lack of circadian rhythmicity in intact retinae is probably a consequence of the desynchronization among the different circadian oscillators present in the retina once it is explanted and placed in culture under constant conditions.

Finally, it should also be pointed out that the presence of clock genes does not necessarily imply the presence of an autonomous functioning clock. For example, the rhythmic expression of *Per1* mRNA in the rodent pituitary gland requires heterologous sensitization of the adenosine A2b receptor by melatonin, acting on the MT1 receptor (79). In the absence of nocturnal melatonin stimulation, *Per1* transcript expression is arrhythmic. Similarly, rhythm generation in retinal cells may require neurohumoral signaling. A recent study comparing melatonin-producing and melatonin-deficient mice suggested that melatonin may affect clock function by post-translational regulation of PER1 and CRY1 protein levels and CREB phosphorylation (74). However, as the melatonin-producing retinas lacked photoreceptors, it is difficult to determine if the effects observed are causally related to the presence or absence of melatonin or to adaptive changes following photoreceptor loss.

Retinal cell viability and circadian clock functions

Early studies described the diurnal dependence of retinal light damage (80,81). More recent reports demonstrate that a single, relatively short, intense light exposure causes circadian time-dependent loss of photoreceptor cells (82,83). The retina was most sensitive to light damage in the middle of the subjective night and photoreceptor loss was almost completely prevented when light treatment began during the day. These results suggest that intrinsic factor(s) expressed in the retina in a circadian manner influence the extent of light-induced photoreceptor cell death. Also, induction of *c-fos*, which is thought to be involved in light-induced retinal damage (84), exhibits circadian dependency in the rat retina (85,86).

Melatonin may contribute to the circadian rhythm of light damage sensitivity. Administration of exogenous melatonin increases light-induced photoreceptor degeneration (87,88) and intraocular injection of luzindole, a melatonin receptor antagonist, decreases light damage (89). In contrast, melatonin may have protective effects on other retinal cell types. Melatonin protects retinal pigment epithelial cells from oxidative stress and ischemia-induced cell death (90,91). Similarly, melatonin protects serotonin-accumulating and GABA-containing retinal neurons, presumably amacrine cells, from kainate- or ischemia-induced neurotoxicity (92). Moreover, detoxification has been proposed as an evolutionarily conserved role of melatonin-synthesis enzyme arylalkylamine N-acetyltransferase (93).

Recent studies have somewhat questioned the role for clocks in retinal cell viability since *Bmal1* knock-out and double *Cryptochrome1/Cryptochrome2* knock-out mice do not show any significant alteration of retinal structure or photoreceptor morphology (75,94). However, it is important to note that these studies were performed in young animals and, therefore, it is possible that negative effects due to the loss of the circadian clock may become evident at a later point in the life cycle or if the photoreceptors are exposed damaging light or other stressors.

Several investigations have also reported that intraocular pressure (IOP) shows a circadian rhythm in many mammalian species, including humans (95). In all the species tested, IOP was found to be low during the day and high during the night. The IOP rhythm is not observed in *Cryptochrome1/Cryptochrome2* KO mice, in which IOP is constantly maintained at the nocturnal (high) levels (96). Since elevated IOP is believed to be a major contributing factor to the development and progression of open-angle glaucoma (97), it is plausible that alteration in the retinal circadian clock system may lead to an increased risk for the development of glaucoma.

Influence of the retinal clock on retinal physiology and visual processing

Early studies in humans and in rats demonstrated circadian rhythms of electroretinogram (ERG) responses. Tassi et al. (98) reported that human visual sensitivity is increased in the early morning. Although these data provided compelling experimental evidence for a circadian control of the ERG, they did not provide a definitive demonstration that the rhythmic changes in the ERG were driven by a retinal circadian clock. Such a demonstration was recently achieved in an elegant study by Weitz and colleagues (75). In their study, wild-type mice displayed a clear circadian rhythm in the amplitude of the b-wave of the photopic ERG. This circadian regulation of the ERG is completely abolished in mice with a retina-specific genetic deletion of *Bmal1*. These data demonstrate that a functional circadian clock within the retina is required for the circadian regulation of retinal visual processing *in vivo*. Finally, it is noteworthy that loss of the ganglion cell photopigment melanopsin abolishes circadian control in some parameters of the cone ERG (99), thus revealing an important role for inner retinal photoreceptors in optimizing classical visual pathways according to time of day.

Potential influences of retinal circadian clocks on the central circadian system

Several studies have established that the retina is the only tissue capable of visual and non-visual photoreception in mammals (100). Therefore, the retina plays a vital role in circadian organization of the whole organism, as it is the only source of photic input to the SCN and, hence, to the rest of the body. It has been proposed that the retina may also contribute to overall circadian organization since changes in the period of the circadian rhythm in locomotor activity have been observed after photoreceptor degeneration or bilateral enucleation (101–103). However, the free-running period in *rd/rd* mice and in mice lacking all of the known retinal photopigments (i.e., rhodopsin, cone opsins, and melanopsin) is not different from that in wild-

type mice (104,105). Overall, these data indicate that the effects of retinal circadian clock system on the free-running period may be difficult to predict. It is not yet clear if the changes observed are the result of interactions between the two clock systems or to other unknown factors that may be altered by photoreceptor degeneration or removal of the eyes.

A number of other studies have suggested that retinal factors, possibly clocks, may contribute to central circadian organization. For example, enucleation abolishes the circadian rhythm of MAP kinase phosphorylation in neurons in the core region of the SCN (106). Targeted disruption of the dopamine D2 receptor gene dampens the circadian rhythm of *Per1* mRNA in retina (73) and impairs light-induced masking behavior (suppression of locomotor activity at night) (107). The impaired masking may result from loss of D2 receptors in the retina, as the adult SCN does not express those receptors (108). Genetic deletion of the DA and cAMP-regulated phosphoprotein-32 (DARPP-32) attenuated light-evoked behavioral phase delay and light-mediated *Per2* induction in the SCN (109). Finding that DARPP-32 is expressed in the retina but not in the SCN or intergeniculate leaflet, the authors concluded that DARPP-32 is involved in the retinal pathways that transmit photic information that resets the SCN clock. However, the possibility that the effects of D2 receptors and DARPP-32 on central circadian function are localized outside the retina (i.e., elsewhere in the central nervous system) cannot be excluded until retina-specific disruption of DA function is tested.

Conclusions

The presence of an independent circadian clock system within the mammalian retina has been established by a series of *in vivo* and *in vitro* studies and using a wide variety of techniques. The experimental evidence indicates that the retinal circadian clock network is hierarchically organized. The circadian clock that generates melatonin rhythms in rat retina is located in photoreceptor cells and persists following destruction of the inner retina with kainic acid and in cultured PRLs and, therefore, appears to be relatively independent of inner retinal clocks. In contrast, the circadian rhythm of DA metabolism and release is dependent on circadian rhythms in melatonin release. The circadian rhythms in DA play important roles in conveying the circadian signal to other retinal cells and may contribute to the stability and precision of the retinal circadian system. The retinal circadian clock system controls many retinal functions from gene transcription to photoreceptor viability to visual processing, and some experimental data also support the notion that the interaction between the retinal and the SCN clock may be important for the circadian organization of the entire organism.

Finally, important differences may exist between the retinal clock systems of the mouse and the rat. Although some of these disparities may be attributable to species-specific differences, we speculate that some of the apparent dissimilarities are due to the use of strains of mice that are unable to synthesize melatonin (62,110) and, therefore, lack a pivotal component of the retinal circadian system. This notion is reinforced by the observation that the circadian organization of the rat retina is very similar to that of the birds, lizards, *Xenopus* and fish (44, 45,54–56,111), indicating a rather evolutionarily conserved organizational plan for the circadian clock system within the vertebrates.

Acknowledgements

Supported by NIH grants NS 43459 to G.T. and EY004864 and EY 014764 to P.M.I

Abbreviations

AC1

Adenylyl cyclase type 1

aanat	Arylalkylamine N-acetyltransferase
CCG	Clock- Controlled Gene
Cry1	Cryptochrome 1
Cry2	Cryptochrome 2
DA	Dopamine
ERG	Electroretinogram
IOP	Intraocular pressure
Per1	Period 1
Per 2	Period 2
PRL	Photoreceptor layer
SCN	Suprachiasmatic nuclei

References

1. Herzog ED. Neurons and networks in daily rhythms. *Nat Rev Neurosci* 2007;8:790–802. [PubMed: 17882255]
2. Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, et al. Resetting central and peripheral circadian oscillators in transgenic rats. *Science* 2000;288:682–685. [PubMed: 10784453]
3. Ko CH, Takahashi JC. Molecular components of the mammalian circadian clock. *Hum Mol Genet* 2006;2:R271–277. [PubMed: 16987893]
4. Munoz E, Baler R. The circadian E-box: when perfect is not good enough. *Chronobiol Int* 2003;20:371–88. [PubMed: 12868535]
5. Gatfield D, Schibler U. Proteasomes keep the circadian clock ticking. *Science* 2007;316:1135–1136. [PubMed: 17495136]
6. Sehgal A. Ac-ing the Clock. *Neuron* 2008;57:8–10. [PubMed: 18184560]
7. Besharse JC, Iuvone PM. Circadian clock in *Xenopus* eye controlling retinal serotonin N-acetyltransferase. *Nature* 1983;305:133–135. [PubMed: 6888555]
8. Cahill GM, Besharse JC. Circadian clock functions localized in *Xenopus* retinal photoreceptors. *Neuron* 1993;10:573–577. [PubMed: 8476609]
9. Pierce ME, Sheshberadaran H, Zhang Z, Fox LE, Applebury ML, et al. Circadian regulation of iodopsin gene expression in embryonic photoreceptors in retinal cell culture. *Neuron* 10:579–584. [PubMed: 8476610]
10. Thomas KB, Tigges M, Iuvone PM. Melatonin synthesis and circadian tryptophan hydroxylase activity in chicken retina following destruction of serotonin immunoreactive amacrine and bipolar cells by kainic acid. *Brain Res.* 1993

11. La Vail MM. Rod outer segment disk shedding in the rat retina: relationship to cyclic lighting. *Science* 1976;194:1071–1073. [PubMed: 982063]
12. Terman JS, Remé CE, Wirz-Justice A. Rod outer segment disk shedding in rats with lesions of the suprachiasmatic nucleus. *Brain Res* 1993;605:256–264. [PubMed: 8481775]
13. Reme CE, Wirz-Justice A, Terman M. The visual input stage of the mammalian circadian pacemaking system: I. Is there a clock in the mammalian eye? *J Biol Rhythms* 1991;6:5–29. 601, 303–307. [PubMed: 1773080]
14. Tosini G, Menaker M. Circadian rhythms in cultured mammalian retina. *Science* 1996;272:419–421. [PubMed: 8602533]
15. Tosini G, Menaker M. Temperature Compensation of retinal circadian oscillators in wild-type and *tau* mutant hamsters. *Neuroreport* 1998;9:1001–1005. [PubMed: 9601657]
16. Lowrey PL, Shimomura K, Antoch MP, Yamazaki S, Zemenides PD, et al. Positional syntenic cloning and functional characterization of the mammalian circadian mutation *tau*. *Science* 2000;288:483–492. [PubMed: 10775102]
17. Klein DC, Coon SL, Roseboom PH, Weller JL, Bernard M, et al. The melatonin rhythm-generating enzyme: molecular regulation of serotonin N-acetyltransferase in pineal gland. *Recent Progress in Hormone Research* 1997;52:307–358. [PubMed: 9238858]
18. Iuvone PM, Brown AD, Haque R, Weller J, Zawilska JB, et al. Retinal melatonin production: role of proteasomal proteolysis in the circadian and photic control of arylalkylamine N-acetyltransferase. *Invest Ophthalmol Vis Sci* 2002;43:564–572. [PubMed: 11818405]
19. Tosini G, Chaurasia SS, Iuvone PM. Regulation of AANAT in the retina. *Chronobiology International* 2006;23:381–391. [PubMed: 16687311]
20. Niki T, Hamada T, Ohtomi M, Sakamoto K, Suzuki S, et al. The localization of the site of arylalkylamine N-acetyltransferase circadian expression in the photoreceptor cells of mammalian retina. *Biochem Biophys Res Comm* 1998;248:115–120. [PubMed: 9675096]
21. Coon SL, Del Olmo E, Young WS, Klein DC. Melatonin synthesis enzymes in *Macaca mulatta*: focus on arylalkylamine N-acetyltransferase (EC 2.3.1.87). *J Clin Endocrinol Metab* 2002;87:4699–4706. [PubMed: 12364461]
22. Liu C, Fukuhara C, Wessel JH, Iuvone PM, Tosini G. Localization of *Aa-nat* mRNA in the rat retina by fluorescence in situ hybridization and laser capture microdissection. *Cell Tissue Res* 2004;315:197–201. [PubMed: 14618388]
23. Sakamoto K, Ishida N. Circadian expression of serotonin N- acetyltransferase mRNA in the rat retina. *Neurosci Lett* 1998;245:113–116. [PubMed: 9605498]
24. Sakamoto K, Ishida N. Molecular cloning of serotonin N-acetyltransferase gene from the mouse and its daily expression in the retina. *Neurosci Lett* 1998;250:181–184. [PubMed: 9708862]
25. Fukuhara C, Liu C, Ivanova TN, Chan GC-K, Storm DR, et al. Gating of the cAMP signaling cascade and melatonin synthesis by the circadian clock in mammalian retina. *J Neurosci* 2004;24:1803–1811. [PubMed: 14985420]
26. Sakamoto K, Oishi K, Shiraishi M, Hamano S, Otsuka H, et al. Two circadian oscillatory mechanisms in the mammalian retina. *Neuroreport* 2000;18:3995–3997. [PubMed: 11192616]
27. Sakamoto K, Liu C, Kasamatsu M, Iuvone PM, Tosini G. Intraocular injection of kainic acid does not abolish the circadian rhythm of *Aanat* mRNA in the rat photoreceptors. *Mol Vision* 2006;12:117–124.
28. Chen W, Baler R. The rat arylalkylamine N- acetyltransferase E- box: different use in a master vs. slave oscillator *Mol Brain Res* 2000;81:43–50.
29. Tosini G, Fukuhara C. The mammalian retina as a clock. *Cell Tissue Res* 2002;309:119–126. [PubMed: 12111542]
30. Iuvone, PM. Cell biology and metabolic activity of photoreceptor cells: light-evoked and circadian regulation. In: Djamgoz, MBA.; Archer, S.; Vallerga, S., editors. *Neurobiology and clinical aspects of the outer retina*. London: Chapman and Hall; 1995. p. 25-55.
31. Ivanova TN, Iuvone PM. Circadian rhythm and photic control of cAMP level in chick retinal cell cultures: a mechanism for coupling the circadian oscillator to the melatonin-synthesizing enzyme, arylalkylamine N-acetyltransferase, in photoreceptor cells. *Brain Res* 2003;991:96–103. [PubMed: 14575881]

32. Chaurasia SS, Haque R, Pozdeyev N, Jackson CR, Iuvone PM. Temporal coupling of cyclic AMP and Ca/calmodulin-stimulated adenylyl cyclase to the circadian clock in chick retinal photoreceptor cells. *J Neurochem* 2006;99:1142–1150. [PubMed: 16981891]
33. Engel L, Vollrath L, Spessert R. Arylalkylamine N-acetyltransferase gene expression in retina and pineal gland of rats under various photoperiods. *Biochem Biophysical Res Comm* 2004;318:983–986.
34. Rohleder C, Langer C, Maus C, Spiwoks-Becker I, Emser A, et al. Influence of photoperiodic history on clock genes and the circadian pacemaker in the rat retina. *Eur J Neurosci* 2006;1:105–111. [PubMed: 16420420]
35. Oishi K, Sakamoto K, Ishida N. Bimodal circadian expression of serotonin N-acetyltransferase mRNA in the retina of rats under restricted feeding. *Neurosci Lett* 2003;351:21–24. [PubMed: 14550904]
36. Iuvone PM, Besharse JC. Dopamine receptor-mediated inhibition of serotonin N-acetyltransferase in retina. *Brain Res* 1986;369:168–176. [PubMed: 3697740]
37. Green CB, Besharse JC. Retinal circadian clocks and control of retinal physiology. *J Biol Rhythms* 2004;19:91–102. [PubMed: 15038849]
38. Iuvone PM, Tosini G, Pozdeyev N, Haque R, Klein DC, et al. Circadian Clocks, Clock-Controlled Genes and Melatonin Biosynthesis in the Retina. *Prog Retin Eye Res* 2005;24:433–456. [PubMed: 15845344]
39. Dubocovich ML, Massana MI, Stanca I, Sauri DM. Melatonin receptor antagonists that differentiates between the human Mel1a and Mel1b recombinant subtypes are used to assess the pharmacological profile of the rabbit retina ML1 presynaptic heteroreceptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 1998;355:365–375.
40. Boatright JH, Rubim NM, Iuvone PM. Regulation of endogenous dopamine release in amphibian retina by melatonin: the role of GABA. *Vis Neurosci* 1994;11:1013–1018. [PubMed: 7947394]
41. Zawilska JB, Iuvone PM. Melatonin synthesis in chicken retina: effect of kainic acid-induced lesions on the diurnal rhythm and D2-dopamine receptor-mediated regulation of serotonin N-acetyltransferase activity. *Neurosci Lett* 1992;135:71–74. [PubMed: 1347416]
42. Nguyen-Legros J, Chanut E, Versaux-Botteri C, Simon A, Trouvin JH. Dopamine inhibits melatonin synthesis in photoreceptor cells through a D2-like receptor subtype in the rat retina: biochemical and histochemical evidence. *J Neurochem* 1996;67:2514–2520. [PubMed: 8931485]
43. Tosini G, Dirden JC. Dopamine Inhibits Melatonin release in the Mammalian Retina: in Vitro Evidence. *Neuroscience Lett* 2000;286:119–122.
44. Cahill GM, Besharse JC. Resetting the circadian clock in cultured *Xenopus* eyecups: regulation of retinal melatonin rhythms by light and D2 dopamine receptors. *J Neurosci* 1991;11:2959–2971. [PubMed: 1682423]
45. Hasegawa M, Cahill GM. A role for cyclic AMP in entrainment of the circadian oscillator in *Xenopus* retinal photoreceptors by dopamine but not by light. *J Neurochem* 1999;72:1812–1820. [PubMed: 10217257]
46. Steenhard BM, Besharse JC. Phase shifting the retinal circadian clock: xPer2 mRNA induction by light and dopamine. *J Neurosci* 2000;20:8572–8577. [PubMed: 11102460]
47. Besharse JC, Zhuang M, Freeman K, Fogerty J. Regulation of photoreceptor Per1 and Per2 by light, dopamine and a circadian clock. *Eur J Neurosci* 2004;20:167–174. [PubMed: 15245489]
48. Witkovsky P, Veseinberger E, LeSauter J, Yan L, Johnson M, et al. Cellular location and circadian rhythm of expression of the biological clock gene *Period 1* in the mouse retina. *J Neurosci* 2003;23:7670–7676. [PubMed: 12930806]
49. Gustincich S, Contini M, Gariboldi M, Puopolo M, Kadota K, et al. Gene discovery in genetically labeled single dopaminergic neurons of the retina. *Proc Natl Acad Sci U S A* 2004;101:5069–5074. [PubMed: 15047890]
50. Ruan G-X, Zhang D-Q, Zhou T, Yamazaki S, McMahon DG. Circadian organization of the mammalian retina. *Proc Natl Acad Sci U S A* 2006;103:9703–9708. [PubMed: 16766660]
51. Dorendos R, Contini M, Hirasawa H, Gustincich S, Raviola E. *Vis Neurosci* 2007;24:1–8. [PubMed: 17430604]
52. Nir I, Haque R, Iuvone PM. Diurnal metabolism of dopamine in the mouse retina. *Brain Res* 2000;870:118–125. [PubMed: 10869508]

53. Doyle SE, Grace MS, McIvor W, Menaker M. Circadian rhythms of dopamine in mouse retina: the role of melatonin. *Vis Neurosci* 2002;19:593–601. [PubMed: 12507326]
54. Adachi A, Suzuki Y, Nogi T, Ebihara S. The relationship between ocular melatonin and dopamine rhythms in the pigeon: effects of melatonin inhibition on dopamine release. *Brain Res* 1999;815:435–440. [PubMed: 9878866]
55. Ribelayga C, Wang Y, Mangel SC. A circadian clock in the fish retina regulates dopamine release via activation of melatonin receptors. *J Physiol* 2004;554:467–482. [PubMed: 14565990]
56. Bartell PA, Miranda-Anaya M, McIvor W, Menaker M. Interactions between dopamine and melatonin organize circadian rhythmicity in the retina of the green iguana. *J Biol Rhythms* 2007;22:515–523. [PubMed: 18057326]
57. Tosini G, Davidson AJ, Fukuhara C, Kasamatsu M, Castanon-Cervantes O. Localization of a circadian clock in mammalian photoreceptors. *FASEB J* 2007;21:3866–3871. [PubMed: 17621597]
58. Sakamoto K, Liu C, Tosini G. Classical Photoreceptors regulate melanopsin mRNA levels in the rat retina. *J Neurosci* 2004;24:9693–9697. [PubMed: 15509757]
59. Sakamoto K, Liu C, Pozdeyev N, Iuvone PM, Tosini G. Dopamine regulates melanopsin expression in intrinsically photosensitive retinal ganglion cells. *Eur J Neurosci* 2005;22:3536–3544.
60. Foster RG, Hankins MW. Circadian vision. *Curr Biol* 2007;4:R746–751. [PubMed: 17803920]
61. Pozdeyev N, Tosini G, Li L, Ali F, Rozov S, Lee HL, Iuvone PM. Dopamine modulates diurnal and circadian rhythms of protein phosphorylation in photoreceptor cells of mouse retina. *Eur J Neurosci*. 2008(in press)
62. Tosini G, Menaker M. The clock in the mouse retina: melatonin synthesis and photoreceptor degeneration. *Brain Res* 1998;789:221–228. [PubMed: 9573370]
63. Carter-Dawson L, LaVail MM, Sidman RL. Differential effects of the rd mutation on rods and cones in the mouse retina. *Invest Ophthalmol Vis Sci* 1978;17:489–498.
64. Doyle SE, McIvor WE, Menaker M. Circadian rhythmicity in dopamine content of mammalian retina: role of the photoreceptors. *J Neurochem* 2002;83:211–219. [PubMed: 12358745]
65. Sakamoto K, Liu C, Tosini G. Circadian Rhythms in the retina of rats with photoreceptor degeneration. *J Neurochem* 2004;90:1019–1024. [PubMed: 15287909]
66. Zhu H, Green CB. Three cryptochromes are rhythmically expressed in *Xenopus laevis* retinal photoreceptors. *Mol Vis* 2001;7:210–215. [PubMed: 11533577]
67. Bailey MJ, Chong NW, Xiong J, Cassone VM. Chickens' Cry2: molecular analysis of an avian cryptochrome in retinal and pineal photoreceptors. *FEBS Lett* 2002;513:169–174. [PubMed: 11904144]
68. Haque R, Chaurasia SS, Wessel JH 3rd, Iuvone PM. Dual regulation of cryptochrome 1 mRNA expression in chicken retina by light and circadian oscillators. *Neuroreport* 2002;13:2247–2251. [PubMed: 12488805]
69. Hayasaka N, LaRue SI, Green CB. In vivo disruption of *Xenopus* CLOCK in the retinal photoreceptor cells abolishes circadian melatonin rhythmicity without affecting its production levels. *J Neurosci* 2002;22:1600–1607. [PubMed: 11880490]
70. Namihira M, Honma S, Abe H, Masubuchi S, Ikeda M, et al. Circadian pattern, light responsiveness and localization of rPer1 and rPer2 gene expression in the rat retina. *Neuroreport* 2001;12:471–475. [PubMed: 11234748]
71. Namihira M, Honma S, Abe H, Tanahashi Y, Ikeda M, et al. Circadian rhythms and light responsiveness of mammalian clock gene, Clock and Bmal1, transcripts in the rat retina. *Neurosci Lett* 1999;271:1–4. [PubMed: 10471199]
72. Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, et al. Role of the CLOCK protein in the mammalian circadian mechanism. *Science* 1998;280:1564–1569. [PubMed: 9616112]
73. Yujnovsky I, Hyrayama J, Doi M, Borrelli E, Sassoni-Corsi P. Signaling mediated by dopamine D2 receptor potentiates circadian regulation by CLOCK:BMAL1. *Proc Natl Acad Sci U S A* 2006;103:6386–6391. [PubMed: 16606840]
74. Dinet V, Ansari N, Torres-Farfan C, Korf H-W. Clock gene expression in the retina of melatonin-proficient (C3H) and melatonin-deficient (C57BL) mice. *J Pineal Res* 2007;42:83–91. [PubMed: 17198542]

75. Storch K-F, Paz C, Signorovitch J, Raviola E, Pawlyk B, et al. Intrinsic Circadian clock of the mammalian retina: Importance for retinal processing of visual information. *Cell* 2007;130:730–741. [PubMed: 17719549]
76. Tosini G, Sakamoto K, Kasamatsu M. Clock Gene Expression in the Rat Retina: Effects of Lighting Conditions and Photoreceptor Degeneration. *Brain Res* 2007;1159:134–140. [PubMed: 17560558]
77. Kamphuis W, Cailotto C, Dijk F, Bergen A, Buijs RM. Circadian expression of clock genes and clock-controlled genes in the rat retina. *Biochem Biophys Res Commun* 2005;330:18–26. [PubMed: 15781226]
78. Peirson SN, Butler JN, Duffield GE, Takher S, Sharma P, et al. Comparison of clock gene expression in SCN, retina, heart and liver of mice. *Biochem Biophysical Res Comm* 2006;351:800–807.
79. Von Gall C, Garabette ML, Kell CA, Frenzel S, Dehghani F, et al. Rhythmic gene expression in pituitary depends on heterologous sensitization by the neurohormone melatonin. *Nat Neurosci* 2002;5:234–238. [PubMed: 11836530]
80. Duncan TE, O'Steen WK. The diurnal susceptibility of rat retinal photoreceptors to light induced damage. *Exp Eye Res* 1985;41:497–507. [PubMed: 4085578]
81. White MP, Fisher LJ. Degree of light damage to the retina varies with time of day of bright light exposure. *Physiol Behav* 1987;39:607–613. [PubMed: 3588706]
82. Organisciak DT, Darrow RM, Barsalou L, Kutty RK, Wiggert B. Circadian-dependent retinal light damage in rats. *Invest Ophthalmol Vis Sci* 2000;41:3694–3701. [PubMed: 11053264]
83. Vaughan DK, Nemke JL, Fliesler SJ, Darrow RM, Organisciak DT. Evidence for a circadian rhythm of susceptibility to retinal light damage. *Photochem Photobiol* 2002;75:547–553. [PubMed: 12017483]
84. Wenzel A, Grimm C, Samardzija M, Reme CE. Molecular mechanisms of light-induced photoreceptor apoptosis and neuroprotection for retinal degeneration. *Prog Retin Eye Res* 2005;24:275–306. [PubMed: 15610977]
85. Yoshida K, Kawamura K, Imaki J. Differential expression of c-fos mRNA in rat retinal cells: regulation by light/dark cycle. *Neuron* 1993;10:1049–1054. [PubMed: 8318229]
86. Humphries A, Carter DA. Circadian dependency of nocturnal immediate-early protein induction in rat retina. *Biochem Biophys Res Commun* 2004;320:551–556. [PubMed: 15219864]
87. Bubenik GA, Purtil RA. The role of melatonin and dopamine in retinal physiology. *Can J Physiol Pharmacol* 1980;58:1457–1462. [PubMed: 7237242]
88. Wiechmann AF, O'Steen WK. Melatonin increases photoreceptor susceptibility to light-induced damage. *Invest Ophthalmol Vis Sci* 1992;33:1894–1902. [PubMed: 1582795]
89. Sugawara T, Sieving PA, Iuvone PM, Bush RA. The melatonin antagonist luzindole protects retinal photoreceptors from light damage in the rat. *Invest Ophthalmol Vis Sci* 1998;39:2458–2465. [PubMed: 9804154]
90. Osborne NN, Nash MS, Wood JP. Melatonin counteracts ischemia-induced apoptosis in human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 1998;39:2374–2383. [PubMed: 9804146]
91. Liang FQ, Green L, Wang C, Alssadi R, Godley BF. Melatonin protects human retinal pigment epithelial (RPE) cells against oxidative stress. *Exp Eye Res* 2004;78:1069–1075. [PubMed: 15109913]
92. Cazevielle C, Safa R, Osborne NN. Melatonin protects primary cultures of rat cortical neurones from NMDA excitotoxicity and hypoxia/reoxygenation. *Brain Res* 1997;768:120–124. [PubMed: 9369308]
93. Klein DC. The 2004 Aschoff/Pittendrigh lecture: Theory of the origin of the pineal gland--a tale of conflict and resolution. *J Biol Rhythms* 2004;19:264–279. [PubMed: 15245646]
94. Selby CP, Thompson C, Schmitz TM, Van Gelder RN, Sancar A. Functional redundancy of cryptochromes and classical photoreceptors for nonvisual ocular photoreception in mice. *Proc Natl Acad Sci U S A* 2000;97:14697–14702. [PubMed: 11114194]
95. Liu JH. Circadian rhythm of intraocular pressure. *J Glaucoma* 1998;7:141–147. [PubMed: 9559503]
96. Maeda A, Tsujiya S, Higashide T, Toida K, Todo T, et al. Circadian intraocular pressure rhythm is generated by clock genes. *Invest Ophthalmol Vis Sci* 2006;47:4050–4052. [PubMed: 16936122]
97. Sit AJ. Nocturnal Pressure: the sleeping giant. *Glaucoma* 2006:53–55.

98. Tassi P. Diurnal rhythmicity for visual sensitivity in humans? *Chronobiology International* 1997;14:35–48. [PubMed: 9042550]
99. Barnard AR, Hattar S, Hankins MW, Lucas RJ. Melanopsin regulates visual processing in the mouse retina. *Curr Biol* 2006;16:389–395. [PubMed: 16488873]
100. Yamazaki S, Goto M, Menaker M. No evidence for extraocular photoreceptors in the circadian system of the Syrian hamster. *J Biol Rhythms* 1999;14:197–201. [PubMed: 10452331]
101. Lupi D, Cooper HM, Froehlich A, Stanford L, McCall MA, et al. Transgenic ablation of rod photoreceptors alters the circadian phenotype of mice. *Neurosci* 1999;89:363–374.
102. Yamazaki S, Alones V, Menaker M. Interaction of the retina with suprachiasmatic pacemakers in the control of circadian behavior. *J Biol Rhythms* 2002;17:315–329. [PubMed: 12164248]
103. Tosini G, Aguzzi J, Bullock NM. Effect of photoreceptor degeneration on circadian photoreception and free-running period in the Royal College of Surgeons rat. *Brain Res* 2007;1148:76–82. [PubMed: 17382912]
104. Foster RG, Provencio I, Hudson D, Fiske S, De Grip W, Menaker M. Circadian photoreception in the retinally degenerate mouse (rd/rd). *J Comp Physiol [A]* 1991;169:39–50.
105. Panda S, Provencio I, Tu DC, Pires SS, Rollag MD, et al. Melanopsin is required for non-image-forming photic responses in blind mice. *Science* 2003;301:525–527. [PubMed: 12829787]
106. Lee HS, Nelms JL, Nguyen M, Silver R, Lehman MN. The eye is necessary for a circadian rhythm in the suprachiasmatic nucleus. *Nat Neurosci* 2003;6:111–112. [PubMed: 12536213]
107. Doi M, Cho S, Yujnovsky I, Hirayama J, Cermakian N, et al. Impaired light masking in dopamine D2 receptor-null mice. *Nat Neurosci* 2006;9:732–734. [PubMed: 16715079]
108. Weiner DM, Levey AI, Sunahara RK, Niznik HB, O'Dowd BF, et al. D1 and D2 dopamine receptor mRNA in rat brain. *Proc Natl Acad Sci U S A* 1991;88:1859–1863. [PubMed: 1825729]
109. Yan L, Bobula JM, Svenningsson P, Greengard P, Silver R. DARPP-32 involvement in the photic pathway of the circadian system. *J Neurosci* 2006;26:9434–9438. [PubMed: 16971527]
110. Goto M, Oshima I, Tomita T, Ebihara S. Melatonin content of the pineal gland in different mouse strains. *J Pineal Res* 1989;7:195–204. [PubMed: 2769571]
111. Bernard M, Iuvone PM, Cassone VM, Roseboom PH, Coon SL, et al. Avian melatonin synthesis: photic and circadian regulation of serotonin N-acetyltransferase mRNA in the chicken pineal gland and retina. *J Neurochem* 1997;68:213–224. [PubMed: 8978728]

Figure 1.

The feedback-loop generating the circadian oscillation is composed of two feedback loops. The positive feedback loop involves the transcription factors *Bmal1* and *Clock*, which form heterodimers and activate the transcription of *Period (Per)* and *Cryptochrome (Cry)* genes as well as clock controlled genes (CCGs). In the cytoplasm, PERIOD and CRYPTOCHROME proteins form heterodimers that enter the nucleus and interact with BMAL1/CLOCK, thus inhibiting the transcription (negative feedback). BMAL1 and CLOCK also stimulate the transcription of *Rev-Erbα* and *Rora*. REV-ERB α protein inhibits *Bmal1* transcription whereas RORA protein enhances *Bmal1* transcription.

Figure 2.

The circadian clock regulates the rhythmic expression of *aa-nat* mRNA through the BMAL1:CLOCK complex and its interaction with the E-box located on the *aa-nat* promoter. The circadian clock also provides for the rhythmic expression of AC1, temporally regulating the ability of Ca²⁺ to stimulate cAMP synthesis. Darkness depolarizes the photoreceptor plasma membrane, leading to Ca²⁺ influx and stimulation of cAMP synthesis late in the day and at night, when AC1 protein expression is high, but not during the early morning. Thus, AC1 expression provides a gate controlling the time of day when cAMP levels are maximally stimulated by darkness. The increase of cAMP level in darkness stimulates *aa-nat* transcription via PKA and the CRE present in the *Aa-nat* promoter, increasing the rate of AA-NAT protein synthesis.

Figure 3.

Melatonin and dopamine play opposing roles in the regulation of retinal adaptive physiology. Dopamine functions as a humoral signal for light, producing light-adaptive physiology, while melatonin has dark-adaptive effects. The synthesis and release of both melatonin and dopamine are under circadian control, with melatonin released at night and dopamine during the daytime. Melatonin inhibits the release of dopamine through an action on melatonin receptors and dopamine inhibits the synthesis and release of melatonin from photoreceptor cells by acting on dopamine receptors present on the dopaminergic neurons located in the inner retina. Thus, the melatonin synthesizing photoreceptors and dopamine secreting inner retinal neurons form a cellular feedback loop that regulates circadian retinal physiology.