Rhythmic Regulation of Photoreceptor and RPE Genes Important for Vision and Genetically Associated With Severe Retinal Diseases

Patrick Vancura,1 Erika Csicsely,1 Annalisa Leiser,1 P. Michael Iuvone,2 and Rainer Spessert1

1Institute of Functional and Clinical Anatomy, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany
2Department of Ophthalmology, Emory University School of Medicine, Atlanta, Georgia, United States

Correspondence: Rainer Spessert, Department of Functional and Clinical Anatomy, University Medical Center of the Johannes Gutenberg University Mainz, Saarstrasse 19-21, Mainz 55099, Germany; spessert@uni-mainz.de.
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PURPOSE. The aim of the present study was to identify candidate genes for mediating daily adjustment of vision.

METHODS. Genes important for vision and genetically associated with severe retinal diseases were tested for 24-hour rhythms in transcript levels in neuronal retina, microdissected photoreceptors, photoreceptor-related pinealocytes, and retinal pigment epithelium-choroid (RPE-choroid) complex by using quantitative PCR.

RESULTS. Photoreceptors of wildtype mice display circadian clock-dependent regulation of visual arrestins (Arr1, Arr4) and the visual cycle gene Rdh12, whereas cells of the RPE-choroid exhibit light-dependent regulation of the visual cycle key genes Lrat, Rpe65, and Rdb5. Clock-driven rhythmicity of Arr1, Arr4, and Rdb12 was observed also in rat pinealocytes, to persist in a mouse model of diabetic retinopathy (db/db) and, in the case of Arr1, to be abolished in retinase of mice deficient for dopamine D4 receptors. Therefore, the expression rhythms appear to be evolutionary conserved, to be unaffected in diabetic retinopathy, and, for Arr1, to require dopamine signaling via dopamine D4 receptors.

CONCLUSIONS. The data of the present study suggest that daily adjustment of retinal function combines clock-dependent regulation of genes responsible for phototransduction termination (Arr1, Arr4) and detoxification (Rdh12) in photoreceptors with light-dependent regulation of genes responsible for retinoid recycling (Lrat, Rpe65, and Rdb5) in RPE. Furthermore, they indicate circadian and light-dependent regulation of genes genetically associated with severe retinal diseases.

Keywords: circadian regulation, visual cycle, retina, visual arrestin

The mammalian retina has the ability to adjust its function to the marked daily changes in the environmental lighting conditions.1,2 This involves the adjustment of photoreception and visual processing3 that manifests in circadian changes in the retinal electrical responses to light, which can be measured using the ERG.4 Daily regulation of the retina might also be important to comply with daily changes in the occurrence of toxic light (elevated during the day) and oxidative stress (elevated at night). Accordingly, circadian output has been seen to affect photoreceptor viability and to promote ganglion cell survival during aging conditions.5

Daily adaptation of retina and photoreceptors is partly driven by the retina’s own circadian clock system,5,6 where circadian clocks are localized in various types of retinal neurons, including photoreceptors.7–9 The circadian regulation of visual function involves the neurohormones melatonin acting on MT1 and MT2 receptors,10,11 and dopamine acting on D4 receptors12–15 as biochemical transducers of night and day, respectively.

Vision in all vertebrates is initiated by the absorption of photons by the photoreceptive pigments rhodopsin (in rods) and opsins (in cones) located in the photoreceptor outer segments.16 In general, photons induce the conversion of the photopigment’s covalently bound 11-cis retinal to all-trans-retinal, inducing a structural switch that activates the photopigment. The reactions responsible for inactivation of photoexcited photopigment include phosphorylation of the photopigment by rhodopsin kinase followed by the binding of arrestin1 (Arr1) in rods and probably arrestin4 (Arr4, also referred to as cone arrestin) in cones.17–21

The maintenance of vision requires the regeneration of 11-cis retinal.22–25 To meet this requirement, all-trans-retinal is reisomerized to 11-cis retinal, a process that is performed by a multistep enzyme pathway called the visual (retinoid) cycle. The first catalytic step of the visual cycle, namely the reduction of all-trans-retinal to all-trans-retinol takes place in photoreceptors and is performed by five isozymes of the enzyme retinoid dehydrogenase/reductase (RDH)24 that are Rdb8 (prRdb), Rdb11, Rdb13, Rdb14, and Drbr5 (rretSdr1). Detoxification of retinaldehydes that exceed the reductive capacity of the outer segment compartment of the visual cycle is conducted by another isoform of Rdb, Rdb12, and a retinase-specific ABC transporter (Abca4).25,26

The remaining steps of the visual cycle are performed in cells of the adjacent retinal pigment epithelium (RPE) that are (1) the esterification of all-trans-retinol to all-trans-retinyl esters
by Lrat, (2) the hydrolyzation and isomerization of all trans-retinyl esters to 11-cis retinol by Rpe65, and (3) the oxidation of 11-cis retinol to 11-cis retinal by the Rdb isoforms Rdb5, Rdb10, and Rdb11. Additional key players of the visual cycle are Rbp1 (also referred to as Crabp2) and Rbp1 (also referred to as Crabp), which catalyze the transport of 11-cis retinal and retinol within the RPE compartment.26 Due to the compartmentalization of the visual cycle into different cell types, retinoid intermediates have to translocate across the interphotoreceptor matrix, a process mediated by the interphotoreceptor retinoid-binding protein (encoded by the gene Rbp3), which is synthesized and secreted by photoreceptors.

Daily adjustment of vision involves 24-hour changes in the expression of Arr1 and Kcnv2, a channel essential for vision.27–30 The data included in the present study suggest that it also involves rhythmic regulation of cone Arr1 and key genes of the visual cycle in photoreceptors and RPE.

**Materials and Methods**

**Animals**

Adult male and female mice (see below) and rats (Sprague Dawley) with intact photoreceptors not carrying rd mutations were used in this study. With the exception of the mouse model for diabetic retinopathy (C57BL/6j db/db, C57BL/6j db/db, the mice used were melatonin-proficient (C3H/f þ/C0 þ) and melatonin-deficient for dopamine D4 receptors (db/db), the mice used were melatonin-proficient (C3H/f þ/C0 þ) and melatonin-deficient for dopamine D4 receptors (db/db), the mice used were melatonin-proficient (C3H/f þ/C0 þ) and melatonin-deficient for dopamine D4 receptors (db/db), the mice used were melatonin-proficient (C3H/f þ/C0 þ) and melatonin-deficient for dopamine D4 receptors (db/db), the mice used were melatonin-proficient (C3H/f þ/C0 þ) and melatonin-deficient for dopamine D4 receptors (db/db), the mice used were melatonin-proficient (C3H/f þ/C0 þ) and melatonin-deficient for dopamine D4 receptors (db/db), the mice used were melatonin-proficient (C3H/f þ/C0 þ) and melatonin-deficient for dopamine D4 receptors (db/db), the mice used were melatonin-proficient (C3H/f þ/C0 þ) and melatonin-deficient for dopamine D4 receptors 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<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Accession Number</th>
<th>Primer Sequence 5’ to 3’</th>
<th>PCR Product, bp</th>
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| m18S   | NR_003278.3              | Forward: CAACACGGGAAACCTCAC  
|        |                          | Reverse: TGGCTTCACCAACAACTAAGAC | 110 |
| mAbca4 | NM_007378.1              | Forward: TCGAGAAGGGTGCCACTG  
|        |                          | Reverse: GTCGAGCATATGGCGTGTAAG | 118 |
| mArr1  | NM_009118.2              | Forward: TCGTGAGGCCACTCTACTG  
|        |                          | Reverse: ATYCAAGAGGGGCGATCGAC | 116 |
| mArr4  | NM_133205.3              | Forward: ACCAATCTGGCCCTATACG  
|        |                          | Reverse: GCTCCACACCAACACTAC | 148 |
| mArrb1 | NM_177231.2              | Forward: CTGATAACCAGACCTTCTACG  
|        |                          | Reverse: GTTGGAGAACCTCGAAGAC | 103 |
| mArrb2 | NM_001271358.1           | Forward: GGGAGGGAAACAGTGAAAC  
|        |                          | Reverse: GATTTGGAGGCAGAAGTG | 111 |
| mDbp   | NM_016974.3              | Forward: GGAGGTGCTAATGACCTTTG  
|        |                          | Reverse: GGACTTTCCTTGCCTTCTC | 146 |
| mDhrs3 | NM_011303.6              | Forward: CTCGCCCTTCATGGAAGAC  
|        |                          | Reverse: CATGCCCTGGAACATTCGC | 106 |
| mE4bp4 | NM_017573.3              | Forward: CGTACCTACCTCTACCTAC  
|        |                          | Reverse: AAAGACTTGGCCACACTAC | 144 |
| mGapdh | BC082592.1               | Forward: CATCAGTAGCGTCGAAAC | 144 |
| mLrat  | NM_023624.4              | Forward: CCATACAGCCCTACTCTGGAAC  
|        |                          | Reverse: AAAGACGGGGAGCAGAC | 146 |
| mNrl   | NM_008736.3              | Forward: GTGGGAAGAGGGTGACTACG  
|        |                          | Reverse: GAACGTGGAGGCGTTGTAAC | 92 |
| mPer1  | NM_011065.5              | Forward: CCGAGAGGTTGGTTACTGAG  
|        |                          | Reverse: GCGAGGTCTTCTCTTGAGCATG | 120 |
| mPer2  | NM_011060.6              | Forward: AGTGGCAGATGTCGAAACCC  
|        |                          | Reverse: TGAAGACTGTCACACCATAGAA | 100 |
| mPer3  | NM_011067.2              | Forward: CGCCGCCCTACAGTGAAAG  
|        |                          | Reverse: GCCCCACGCTGTTAATCCT | 145 |
| mRbp1  | NM_011254.5              | Forward: GGACTTCAGCGGGTACTG  
|        |                          | Reverse: GATCATGTGGTCGCCATC | 143 |
| mRbp3  | NM_015745.2              | Forward: ATGGCTACGCTCTTCTTTG  
|        |                          | Reverse: ATGGCTACGCTCTTCTTTG | 104 |
| mRdb5  | NM_134006.4              | Forward: TGAGGGCTGTGGCTTCTTC  
|        |                          | Reverse: GTAGGGCTGTGGCTTCTTC | 137 |
| mRdb8  | NM_001030290.1           | Forward: TGCGCTACCTCGGCGAGTCG  
|        |                          | Reverse: GTGGTCTGTCTGCGGAGTG | 110 |
| mRdb10 | NM_133832.3              | Forward: GTGCTCTGTTGTTGTCTTC  
|        |                          | Reverse: TCGAGCTACGCTGATCTC | 132 |
| mRdb11 | NM_021557.5              | Forward: AAGCGTACGAGGGAACAG  
|        |                          | Reverse: CGCCGCCATGTTGATGAG | 125 |
| mRdb12 | NM_030017.4              | Forward: CTTCTCTACCCCTCTTCTC  
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| mRdb13 | NM_175372.4              | Forward: AGACAGTGGGACACCAAAG  
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| mRdb14 | NM_025697.2              | Forward: CAATGAGAGGTGGCCAGAC  
|        |                          | Reverse: GCCACCTAGTCTCATAG | 114 |
| mRlbp1 | NM_020599.2              | Forward: GCTACAGAGGGCTCTTCTC  
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| mTb    | NM_009377.1              | Forward: GTGGTCTGTCTGCGGAGTG  
|        |                          | Reverse: GATGGGTCAAACTCTACAG | 136 |
| r18S   | NR_046237.1              | Forward: GTTGGTGGAGCGATTTGTC  
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| rArr1  | NM_013025.2              | Forward: GGCCCTCAGGAACATATC  
|        |                          | Reverse: GCACCTCAGTCCAGCTTAC | 148 |
| rArr4  | NM_001190993.1           | Forward: ACCAATCTGGGCTCTACTG  
|        |                          | Reverse: GCTCCACACCAACATCAC | 107 |
| rE4bp4 | NM_053727.2              | Forward: TCGGGAACATGGGCAATC  
|        |                          | Reverse: TCTTCTACGCGGGAACACC | 89 |
| rGapdh | NM_017008.4              | Forward: TGACTCTACCCAGGGCAAG  
|        |                          | Reverse: CTGGGAAGATGGTGAGGTT | 139 |
| rRdb12 | NM_001108037.1           | Forward: GGAATCCAGGTGGAAATAG  
|        |                          | Reverse: AGTGGCAGAGTGTTACAG | 139 |
Statistical Analysis

All data are expressed as the mean ± standard error of the mean (SEM) of four qPCR experiments from four independent tissue samples. Transcript levels were calculated relative to average expression of each dataset throughout 24 hours to plot temporal expression. Cosinor analysis was used to evaluate variations among the groups in the 24-hour profile and to fit sine-wave curves to the circadian data to mathematically estimate the time of peaking gene expression (acrophase) and to assess the amplitude. The model can be expressed according to the following equation:

\[ f(t) = A + B \cos \left( \frac{2\pi}{T} (t + C) \right) \]

where \( f(t) \) indicates relative expression levels of target genes, \( t \) specifying the time of sampling (hours after light-on), \( A \) representing the mean value of the cosine curve (mesor; midline estimating statistic of rhythm), \( B \) indicating the amplitude of the curve (half of the sinusoid), and \( C \) indicating the acrophase (point of time, when the function \( f(t) \) is maximum). \( T \) gives the time of the period, which was fixed at 24 hours for this experimental setting. Significance of daily regulation was defined by showing a \( P < 0.05 \).

RESULTS

Visual Arrestins and Rdh12 are Under Circadian Regulation in Neuronal Retina and Photoreceptors

To investigate whether the neural retina (devoid of the RPE-choroid) and photoreceptors of mice display daily regulation of genes important for visual processing, 24-hour profiling of the mRNA levels of visual arrestins and enzymes of the photoreceptor compartment of the visual cycle was performed. Among the genes tested, \( \text{Arr}1, \text{Arr}4, \) and \( \text{Rdh}12 \) were seen to display significant daily rhythms in neural retina and photoreceptors of mouse (Fig. 1, blue and red lines; for statistical analysis, see Table 2). Peak expression occurred for \( \text{Arr}1 \) in retina at Zeitgeber time (ZT) 8.2 and in photoreceptors at ZT7.7, for \( \text{Arr}4 \) in retina at ZT12.5 and in photoreceptors at ZT11.4, and for \( \text{Rdh}12 \) in retina at ZT9.4 and in photoreceptors at ZT7.8. No daily periodicity was observed for the visual cycle genes \( \text{Rdh}8, \text{Rdh}11, \text{Rdh}13, \text{Rdh}14, \text{Dhrs}3, \text{Abca}4, \) and \( \text{Rbp}3 \).

The daily rhythmicity of \( \text{Arr}1, \text{Arr}4, \) and \( \text{Rdh}12 \) may be driven by a true circadian clock or light/dark transitions. To test circadian regulation, 24-hour profiling of the genes was performed in mice kept for one cycle under constant darkness (Fig. 1, black lines; for statistical analysis, see Table 2). Consistent with the concept that daily rhythmicity of the genes is promoted by a true circadian clock, daily changes of the genes under investigation persisted in the absence of light/dark transitions.

To test the validity of the experimental system used, the clock-controlled gene \( \text{E4bp}4 \) was recorded in the same transcriptomes as those used for analyzing the other genes. Consistent with the validity of the results obtained, \( \text{E4bp}4 \) transcript amount was observed to be rhythmic in retina and photoreceptors, under LD 12:12 and DD (Fig. 1; for statistical analysis, see Table 2).
Daily Regulation of Visual Genes

Table 2. Statistical Analysis of Transcriptional Profiling Illustrated in Figures 1 to 4

<table>
<thead>
<tr>
<th>Source of Transcriptomes and Lighting Conditions</th>
<th>$P$ Value</th>
<th>Acrophase, Amplitude, $%$</th>
<th>Figure</th>
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<tr>
<td>Mouse retina (C3H/ft)</td>
<td>$&lt;0.05$</td>
<td>8.2, 31.0</td>
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<td>Mouse photoreceptors (C3H/ft)</td>
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<td>D11 (KO) mouse retina</td>
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<td>3.2, 45.0</td>
<td>8</td>
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<td>KO, knock out; WT, wild type.</td>
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Visual Arrestins and Rdh12 Are Also Rhythmic in Rat Pineal Gland

Mammalian photoreceptors and pinealocytes phylogenetically and ontogenetically descend from a common ancestor cell type. To investigate whether rhythmicity of the genes is phylogenetically conserved, the 24-hour profiling of the genes was compared in retina and pinealocytes. Because the pineal gland is much larger in rat than in mice, rat tissue was used to obtain sufficient mRNA levels for this purpose. Arr1, Arr4, and Rdh12 were rhythmically expressed in rat pinealocytes with similar profiles as those observed in rat retina (Fig. 2, blue and red lines; for statistical analysis, see Table 2).

Consistent with the validity of the results obtained, the clock-controlled gene E4bp4 was observed to be rhythmic in the same transcriptomes as those used for profiling the other genes (Fig. 2, blue and red lines; for statistical analysis, see Table 2).

Expression of Arr1 Is Arrhythmic in Dopamine D4 Receptor–Deficient Mice

In order to evaluate the contribution of dopamine to circadian regulation, 24-hour profiling of the genes was performed in mice deficient for the D4 receptor (Fig. 3, blue versus red lines; for statistical analysis, see Table 2). Daily regulation of Arr1 was not observed in retina of D4-deficient mice, but Arr4 and Rdh12 were rhythmically expressed. This suggests that circadian regulation of Arr1, but not that of Arr4 and Rdh12, requires dopamine signaling via D4 receptors.

As expected for a clock-controlled gene, E4bp4 rhythmicity was evident in the same transcriptomes as those used for analysis of the other genes. Interestingly, E4bp4 rhythmicity mitigates in mice deficient for D4 receptors (Fig. 3, blue versus red lines; for statistical analysis, see Table 2). Because E4bp4 transcription is known to be directed by clock gene products, abolished E4bp4 rhythmicity may mirror an influence of dopamine signaling on the retinal clock function.

Circadian Regulation of Visual Arrestins and Rdh12 Expression Persists in Diabetic Retinopathy

In diabetic retinopathy, visual function is impaired. To investigate whether disturbed circadian control of Arr1, Arr4, and Rdh12 plays a role in this context, the db/db mouse, a worldwide applied model of type II diabetes and diabetic retinopathy, was used. Irrespective of the melatonin deficiency of the db/db mouse (C57BL/6j background), the nondiabetic phenotype (db/) was seen to display daily rhythms in Arr1, Arr4, and Rdh12 mRNA levels (Fig. 4, blue lines; for statistical analysis, see Table 2). This suggests that circadian regulation does not require melatonin signaling. Moreover, rhythmicity of the genes persisted in diabetic (db/db) mice (Fig. 4, blue versus red lines; for statistical analysis, see Table 2). Therefore, circadian regulation of Arr1, Arr4, and Rdh12 appears not to be affected in diabetic retinopathy.

Consistent with the validity of the experimental system used, E4bp4 was observed to be rhythmic in the nondiabetic phenotype (db/). The daily profile in the E4bp4 transcript was statistically arrhythmic in diabetic (db/db) mice, although it resembled that in nondiabetic (db/) mice (Fig. 4, blue versus red lines; for statistical analysis, see Table 2).

Visual Cycle Genes Are Under Daily Regulation in the RPE-Choroid

Because the different steps of the visual retinoid cycle are diversified on photoreceptors and the adjacent RPE, and the
visual cycle gene \textit{Rdh12} was seen to be rhythmic in mouse photoreceptors, possible daily fluctuations in the expression of genes of the RPE part of the visual cycle were investigated in preparations of the RPE-choroid obtained from mice. Among the genes tested, \textit{Lrat}, \textit{Rpe65}, and \textit{Rdh5} were seen to undergo daily rhythms with peaks at the early daytime (\textit{Lrat}, ZT4.2; \textit{Rpe65}, ZT1.3; \textit{Rdh5}, ZT1.9) (Fig. 5, blue lines; for statistical analysis, see Table 3). No daily periodicity was evident for the visual cycle genes \textit{Rbp1}, \textit{Rdh10}, \textit{Rdh11}, and \textit{Rlbp1}.

To check clock-dependent regulation of \textit{Lrat}, \textit{Rpe65}, and \textit{Rdh5}, 24-hour profiling of the genes was performed in mice kept for one cycle under DD (Fig. 5, black lines; for statistical analysis, see Table 3). Consistent with the concept that daily rhythmicity of \textit{Lrat}, \textit{Rpe65}, and \textit{Rdh5} expression does not depend on a true circadian clock but requires LD transitions, daily periodicity of the transcripts vanished under DD. Consistent with the presence of a true circadian clock in RPE-choroid and the validity of the experimental system used, the clock-controlled gene \textit{E4bp4} was seen to display periodicity not only under LD 12:12 but also under DD (Fig. 5, blue versus black lines; for statistical analysis, see Table 3).

**DISCUSSION**

In the present study, the genes \textit{Arr1}, \textit{Arr4}, \textit{Lrat}, \textit{Rdh5}, \textit{Rpe65}, and \textit{Rdh12} were observed to display daily rhythms in either photoreceptors (\textit{Arr1}, \textit{Arr4}, and \textit{Rdh12}) or RPE-choroid (\textit{Lrat}, \textit{Rpe65}, and \textit{Rdh5}). Since they encode indispensable components of either the phototransduction pathway (\textit{Arr1}, \textit{Arr4}), or the visual retinoid cycle (\textit{Rdh12}, \textit{Lrat}, \textit{Rpe65}, and \textit{Rdh5}), two processes essential to vision, daily regulation appears to contribute to daily adjustment of vision to comply with 24-hour changes in lighting conditions. Remarkably, rhythmic regulation of the photoreceptor genes (\textit{Arr1}, \textit{Arr4}, and \textit{Rdh12}) was seen to be driven by a circadian clock, whereas that of the RPE genes (\textit{Lrat}, \textit{Rpe65}, and \textit{Rdh5}) was observed to depend on LD transitions. This suggests that daily

| Table 3. Statistical Analysis of Transcriptional Profiling Illustrated in Figure 5 |
|-----------------|-----|----------|-----------|----------|
| \textbf{Gene}   | \textbf{LD 12:12} | \textbf{DD} |
|                 | \textbf{P Value} | \textbf{Acrophase, h} | \textbf{Amplitude, %} | \textbf{P Value} | \textbf{Acrophase, h} | \textbf{Amplitude, %} | \textbf{See Figure} |
| \textit{Lrat}   | <0.05 | 4.2 | 38.1 | >0.05 | - | - | 5 |
| \textit{Rdh5}  | <0.05 | 1.9 | 42.8 | >0.05 | - | - | 5 |
| \textit{Rpe65} | <0.05 | 1.3 | 41.7 | >0.05 | - | - | 5 |
| \textit{E4bp4} | <0.05 | 21.1 | 35.7 | <0.05 | 21.1 | 67.1 | 5 |

FIGURE 2. Daily rhythmicity of \textit{Arr1}, \textit{Arr4}, and \textit{Rdh12} in rat pineal gland and rat retina. Transcript levels of the genes were monitored in comparison to the clock-controlled gene \textit{E4bp4} under LD 12:12 in pineal gland (blue lines) and retina (red lines) of rats by using qPCR. The mRNA levels are plotted as a function of ZT and the lines represent the periodic sinusoidal functions determined by cosinor analysis (\textit{solid line for } P < 0.05 \textit{ in cosinor analysis). Data represent a percentage of the average value of the transcript amount during the 24-hour period. Statistical analysis of transcriptional profiling is provided in Table 2. The value of ZT0 is plotted twice at both ZT0 and ZT24. The \textit{solid bars} indicate the dark period. Each value represents mean ± SEM (\textit{n} = 4; each \textit{n} represents two retinas and a pineal gland of one animal).
adjustment of visual processing combines clock-driven gene regulation in photoreceptors with light-driven gene regulation in RPE. Circadian regulation of photoreceptor genes should derive from clocks located in photoreceptors, inner retinal neurons, and RPE-choroid but not from the master clock within the suprachiasmatic nucleus (SCN). 2

**Arr1** is abundant in rod and cone photoreceptors. 18–21 Due to the limitation of the present study that photoreceptor transcript preparations derive from both rods and cones, **Arr1** rhythms could reflect average rod and cone values and may not necessarily be valid for each type of photoreceptor. However, in the rod-dominant mouse retina, the observed 24-hour changes in **Arr1** expression may mainly derive from rods. The phenotype of **Arr1** is evolutionarily conserved and its function is not redundant with that of **Arr4**.20,21 Accordingly, both types of visual arrestin appear to play complementary roles in the daily adjustment of retinal function.

In rod and cone photoreceptors, protein formation occurs in the cell body and the inner segment. Therefore, increased transcription of the visual arrestins during the day may contribute to the accumulation of arrestin protein in these cell compartments at night. In response to light, both arrestins are translocated to the outer segment,41–45 where they influence phototransduction. 46 Hence, circadian regulation of **Arr1** and **Arr4** may allow rods and cones to prepare an arrestin reservoir at night, ready for translocation to the outer segment in response to light.

**Lrat**, **Rpe65**, and **Rdh5** encode the key enzymes of the RPE compartment of the visual cycle. 22 Therefore, concurrent upregulation of the genes during the daytime (this study) may result in a daytime peak in the capacity of the RPE to perform chromophore regeneration. This suggests that the 24-hour rhythms of **Lrat**, **Rpe65**, and **Rdh5** complies with the requirement of the RPE to increase chromophore regeneration during light exposure/daytime.

**Rdh12** is localized to the inner segment of both rod and cone photoreceptors. 47 It does not play a significant role in visual cycle function but is necessary to protect photoreceptors from toxic retinaldehydes that exceed the reductive capacity of the outer segment compartment of the photoreceptor cells. 48–50 Therefore, upregulation of the gene at the earlier time of day may fulfill the demand to protect the photoreceptors from increasing concentrations of retinaldehydes released during light exposure/daytime. 51–53 Interestingly, the release of retinaldehydes during light exposure/daytime and thus the requirement for protection depends on **Rpe65** activity. 54,55 Therefore, upregulation of **Rdh12** during the
daytime may be necessary to compensate for an Rpe65-dependent increase in retinaldehyde release.

The genes observed to be under daily regulation in the present study are important for maintaining vision and for protecting photoreceptors from cytotoxic byproducts of the visual pathways. Accordingly, mutations of these genes have been genetically linked to various forms of severe retinal diseases. Not only is Arr1 genetically associated to Oguchi disease\textsuperscript{56,57} and retinitis pigmentosa,\textsuperscript{58} but also are Lrat, Rpe65, and Rdh12 to Leber’s congenital amaurosis.\textsuperscript{59} Mutations of Rdh5 are associated with fundus albipunctatus.\textsuperscript{60} Moreover, mice deficient for Arr1, Arr4, Rpe65, and Rdh12 suffer from dystrophy of rods (Arr1,\textsuperscript{61} Rpe65,\textsuperscript{62} and Rdh12\textsuperscript{52}) and/or cones (Arr1,\textsuperscript{19} Arr4,\textsuperscript{20,21} Rpe65,\textsuperscript{63} and Rdh12\textsuperscript{52}). These findings indicate that the abundance of each of the gene products is a prerequisite for retinal health. Thus, correct upregulation of the genes during early (Lrat, Rpe65, Rdh5, and Rdh12) or late (Arr1 and Arr4) daytime might be essential for retinal health.

Rhythmicity of Arr1, Arr4, and Rdh12 persisted in the db/db mouse, a mouse model of diabetic retinopathy. Therefore, daily regulation of these genes might also be unaffected in diabetic retinopathy of humans, one of the most common causes of blindness in Europe and United States.\textsuperscript{64} Accordingly, the pathogenesis of diabetic retinopathy appears not to derive from disturbed circadian regulation of visual arrestins or Rdh12.

Circadian regulation of Arr1 appears to be mediated by dopamine signaling via D\textsubscript{4} receptors. Therefore, dopamine-dependent control of photoreceptors\textsuperscript{12} appears to derive from the clock-driven release of dopamine from amacrine cells in the inner retina\textsuperscript{2} and/or from circadian expression of Drd4, the gene that encodes the dopamine D4 receptor.\textsuperscript{13} Therefore, circadian regulation of Arr1 in photoreceptors may be promoted by a molecular clock located within amacrine cells and photoreceptor cells.

Daily regulation of Arr1, Arr4, and Rdh12 was seen in the present study to also occur in rat pineal gland, a neuroendocrine transducer of the circadian system.\textsuperscript{27,65} In mammalian pineal gland, rhythmicity of gene expression is driven by the master clock in the SCN.\textsuperscript{66} This suggests that regulation of the visual genes is circadian in retina and pineal gland but depends on different clocks, viz. the intraretinal clock system and the master clock in the SCN. Mammalian photoreceptors and pinealocytes phylogenetically and ontogenetically descend from a common ancestor cell type even if pinealocytes have lost direct photoreception and endogenous clock function during evolution.\textsuperscript{67} Therefore, the circadian regulation of the visual genes under investigation appears to be evolutionary conserved.

In conclusion, the data of the present study suggest that genes important for phototransduction shutoff and retinoid renewal are not only important for maintaining vision, but also for mediating adjustment of vision to comply with 24-hour changes in lighting conditions. As a consequence, mutations of the respective genes might impair daily adjustment of the retina and this deficiency might contribute to the pathogenesis of the respective gene associated retinal disorders. Moreover,
circadian regulation of \textit{Rdh12} may adjust the detoxification capacity of photoreceptors to changing amounts of cytotoxic byproducts of visual pathways. Therefore, \textit{Rdh12} is a candidate gene for mediating the positive influence of the retinal clock on photoreceptor survival. 

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References


