An In Vivo Doxycycline-Controlled Expression System for Functional Studies of the Retina

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PURPOSE. Transgenic mice were developed that express tetracycline-controlled transactivator 1 (tTA1) specifically in photoreceptor cells. In these mice the transcription of the gene of interest can be easily inactivated in the retina in a short time frame.

METHODS. A construct was prepared containing tTA1 under control of the murine rhodopsin regulatory region. This construct was used for the generation of transgenic mice. In situ hybridization was performed to study the distribution of the transactivator in the retina. The activity of the transactivator was analyzed by mating the lines with a luciferase reporter transgenic mouse. tTA1 activity and doxycycline’s ability to block it were analyzed by luciferase assay. The effects of tTA1 on the retina were assessed by histology and electrophysiology.

RESULTS. Two transgenic lines were developed that specifically express tTA1 in photoreceptor cells. The time course of transgene expression replicated transcription of endogenous rhodopsin. tTA1 was not toxic to the retina. Transactivator activity was blocked readily by doxycycline.

CONCLUSIONS. An expression system for photoreceptor cells was generated to drive transcription in a cell-specific and time-controllable manner. This system is suitable for the study of factors involved in retinal biology and of mutant forms of genes involved in retinal diseases. (Invest Ophthalmol Vis Sci. 2003;44:755–760) DOI:10.1167/iovs.02-0340

Functional studies of the retina would benefit from systems that allow the control of gene expression at a temporal and spatial level. For instance, the ability to block expression of either a protein or a mutant allele in a fast and complete way at a specific time point would be very useful in determining whether elimination of this factor changes either retinal physiology or a degenerative phenotype. A transgenic system in which these topics can be studied would also be beneficial to the development of therapeutic approaches for retinal degeneration. In the past several years, the tetracycline-controlled transactivator (tTA)–mediated transcription-activation system has been shown to be one of the best controllable systems for in vivo studies. In fact, it makes use of an effective, specific, and nontoxic transactivator that can act in a variety of cell types1 and transgenic animals.2–4 In the absence of tetracycline, tTA activates transcription by binding to an array of tet operator sequences (tetO). In the presence of tetracycline, transcription is blocked, because tTA cannot bind to its target. This system, called tet-off, differs in two aspects from the tet-on system (rtTA) developed for the retina.5 First, the tTA inactivates the transgene much faster, whereas the tet-on system quickly activates the transgene. Second, the dose-response of doxycycline (an analogue of tetracycline) on tTA shows an effective range at concentrations between 0.1 and 10 ng/mL, whereas on rtTA it is between 100 and 3000 ng/mL.5,6 Furthermore, in the absence of antibiotic, the tet-off system regulates transgene expression according to the tissue-specific and developmental regulation of the promoter used. In addition, the transgene can be inactivated easily and at will with low concentrations of doxycycline. Finally, considering that an overexpression of wild-type VP16 fusion protein may not be well tolerated by the cells,7 we used a VP16-derived minimal transactivator domain.8 This 12-amino-acid VP16 minimal domain eliminates potential targets for interaction with other transcription factors, as well as potential epitopes that elicit a cell immune response. Furthermore, we can achieve twice the transactivator activity of the original tTA by using the modification tTA1.8

A cell-specific expression of tTA1 in rod photoreceptor cells can be obtained with the characterized rhodopsin promoter.9 We generated transgenic mice in which a transgene can be specifically expressed in rod photoreceptor cells and can be easily inactivated at a defined time. In this article we present the characterization of two different transgenic mouse lines that express tTA1 in the photoreceptor cell layer, with temporal and spatial profiles similar to those of the endogenous rhodopsin gene.9 We assessed the transactivator’s activity and the response to doxycycline by mating the two transgenic lines with reporter mice (L7) carrying the luciferase gene downstream to tetO.2 The results demonstrate that the two lines have different expression patterns of tTA1 in the photoreceptor layer. However, both lines responded quickly to doxycycline treatment. Therefore, our system can be used to express genes involved in retinal biology and pathology and is helpful in the study of pathogenetic events leading to retinal degeneration.

MATERIAL AND METHODS

Animals

The use of animals in this work was in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Constructs

The 314-bp fragment from the EcoR1 site to the ATG of the murine rhodopsin gene9 was amplified from mouse genomic DNA using as
primers oligo forward, 5'-GAGTTGGAGGAGCATTG-3’, and oligo reverse, 5'-GCTGTTAGACATGCGGTGCCTAGG-3’. The PCR product was cloned in the pUHD21-1 vector upstream to the ITA1 sequence, using the Xbal site. We then cloned, at the EcoRI site, the 4.0 kb fragment (from KpnI to EcoRI) of the rhodopsin promoter region (a kind gift from Muna Naash). This construct was named RhoITAI.

**Production of the Transgenic Mice**

Transgenic founder mice were generated by pronuclear injection, by standard techniques. The transgene was excised as a 5.6 kb ClaI fragment, purified from agarose gel with kit (Qiagen Quick Gel Extraction; Basel, Switzerland) and microinjected into pronuclei of one-cell embryos from superovulating CD-1 mice (Charles River, Sulzfeld, Germany). Transgenic animals were identified by PCR and Southern blot analysis of genomic DNA prepared from tail samples. For PCR analysis ITA1-specific primers were used: tTA forward (tTAF), 5'-ATGAGGTTGGAATCGAAGG-3’; and tTA reverse (tTAR), 5'-GGCTACAATCTCAGTATGG-3’. For Southern blot analysis, 12 μg genomic DNA was digested with the NsiI restriction enzyme, cutting once within the transgene; fractionated on 0.8% agarose gel; and transferred onto a nylon membrane (Hybond-N; Amersham Pharmacia Biotech, Zurich, Switzerland). Blots were probed with the ITA1 fragment labeled using a random prime labeling system (Rediprime II; Amersham Pharmacia Biotech).

**Reverse Transcription—Polymerase Chain Reaction**

RT-PCR was performed as previously described. Total RNA was purified from retinas harvested from postnatal day (P)6, P7, P8, P9, and P15 and adult transgenic mice. tTA and tTAr primers were used for PCR analysis.

**Luciferase Assay**

ITA1 transgenic mice (RhotTA1-L5 and RhotTA1-L32) were bred with luciferase reporter L7 mice. Animals carrying both the transactivator (ITA1) and reporter (L7) genes were killed at different ages after birth. Eyes were enucleated and homogenized in 300 μL of 1X passive lysis buffer (Promega, Mannheim, Germany). A portion (20 μL) of the homogenate was used to assay luciferase activity (Luciferase Reporter Assay System; Promega) in a luminometer (AutoLumat; EG&G Berthold, Natick, MA). Luciferase activity was normalized to protein concentration and expressed as relative light units per microgram of total protein (RLU/μg). Transgenic mice were killed for assay at the following time points: P6 (RhotTA1-L5, n = 6; RhotTA1-L32, n = 9), P15 (RhotTA1-L5, n = 6; RhotTA1-L32, n = 4), and P20 (RhotTA1-L5, n = 13; and RhotTA1-L32, n = 19). Four other groups of P15 animals were exposed for either 1 or 10 days to doxycycline (Sigma, Milan, Italy) dissolved in their drinking water (2 mg/mL) containing 5% sucrose.

**In Situ Hybridization**

Mice eyes were harvested at P13 and P20, fixed in 4% paraformaldehyde, and embedded in paraffin. Sections (7 μm) were dewaxed and treated with 0.2% HCl for 15 minutes. Samples were incubated for 15 minutes in 20 μg/mL proteinase K, then washed with 0.4% glycine in PBS and postfixed with 4% paraformaldehyde. Acetylation with 0.2 M triethanolamine-HCl (pH 8.0) containing 0.25% acetic anhydride was performed twice for 5 minutes at RT. Slides were extensively washed in water and air dried. Sections were hybridized overnight at 65°C with 1 μg/mL digoxigenin-labeled riboprobes in 50% formamide, 1× Denhardt’s solution, 3× SSC, 10% dextran sulfate, 500 μg/mL tRNA, and 500 μg/mL salmon sperm DNA.

The 450-bp ITA PCR fragment was used as a template for transcription, either with T3 RNA polymerase after linearization with XbaI (antisense probe) or with T7 RNA polymerase after digestion with XbaI (sense control probe). The luciferase antisense probe was obtained by digestion with PstI and transcription with T3 RNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany), and the sense control probe was synthesized with T7 RNA polymerase (Roche) after digestion with XbaI.

After hybridization, slides were washed in 50% formamide and 2× SSC at 65°C and then equilibrated in NTE (0.5 M NaCl, 10 mM Tris-HCl [pH 8], and 5 mM EDTA), and treated with 20 μg/mL RNase A for 30 minutes at 57°C in NTE. After washing with 50% formamide, 1× SSC at 65°C and then with 2× SSC at RT, sections were blocked for 1 hour at RT with 1% blocking reagent (Roche) in MAB-T solution (100 mM maleic acid, 150 mM NaCl, 0.1% Tween-20 [pH 7.5]). Anti-digoxigenin-AP conjugate antibody (1:2000; Roche) in 1% blocking solution was incubated overnight at 4°C. After extensive washes with TBS (100 mM Tris-HCl [pH 7.5], and 150 mM NaCl) and NTM (100 mM NaCl, 100 mM Tris-HCl [pH 9.5], and 50 mM MgCl2), sections were exposed to the nitroblue tetrazolium/5-bromo-4-chloro-3-indoyl phosphate (NBT/BCIP) substrate for alkaline phosphate (Sigma). Reaction was observed with a microscope and blocked with 4% paraformaldehyde for 20 minutes. Slides were coverslipped with 70% glycerol in PBS and photographed using a microscope with Nomarski optics (Axioskop; Carl Zeiss, Oberkochen, Germany).

**Histologic Analysis**

Eyes enucleated from adult transgenic mice were fixed overnight in Carnoy’s solution at 4°C, embedded using a kit (JB-4 Plus Embedding Kit; Polyscience, Inc., Eppelheim, Germany) and sectioned parallel to the sagittal plane. Sections (1 μm) were stained with 50% methylene blue and 50% azure II.

**Electroretinograms**

Animals were dark adapted for 12 hours and anesthetized with 100 mg/kg body weight ketamine and 5 mg xylazine. Pupils were dilated with 1 drop of a mixture of 1.7% tropicamide and 3.3% of phenylephrine. The ground electrode was a subcutaneous needle in the tail, the reference electrode was placed subcutaneously between the eyes, and
the active electrodes were gold wires placed on the cornea below the pupil with a drop of methylhydroxypropyl cellulose (Methocel; Dow Chemical Co., Zürich, Switzerland). Measurements were performed on 6-month-old animals, with five mice in each group.

Recordings were made simultaneously in both eyes with a data acquisition system (Espion Console; Diagnosys LLC, Littleton, MA). The mouse was placed on a specially designed operating table that also contained the electrode mounts (High-Throughput Mouse-ERG; STZ for Biomedical Optics and Functiontesting, Tübingen, Germany) which could be introduced into a Ganzfeld LED stimulator (Espion Color-Burst; Diagnosys LLC). All electroretinographic (ERG) responses were obtained within 30 minutes after injection of anesthesia.\(^{13}\)

Pulses of 10 ms were delivered at a frequency of 0.48 Hz. Results were obtained at 11 steps with illumination levels as indicated by the manufacturer’s setting at 0.5 \(\times 10^{-3}\), 12.5 \(\times 10^{-3}\), 25 \(\times 10^{-3}\), 125 \(\times 10^{-3}\), 500 \(\times 10^{-3}\), 125, 5, 12.5, 50, 125, and 500 cd/sec per meter after 5 to 20 averaging.

A statistical analysis program (Matlab; the MathWorks, Natick, MA) was used to search for maxima and minima in predefined time windows to determine amplitudes, which were checked manually. Left and right eyes of all mice within one group were averaged for dark- and light-adapted ERGs separately. The Naka-Rushton fits for the amplitude of the b-wave\(^{14-17}\) were iteratively performed by computer (Delphi software; Borland Software Corp., Scotts Valley, CA) to determine the values of \(n\) and \(k\) for each mouse, until a least-square fit over all luminance-levels reached a minimum. \(V_{\text{max}}\) is the maximum of the b-wave amplitude, \(k\) is the intensity at which the b-wave amplitude reaches half saturation, and \(n\) is a dimensionless constant related to the slope of the intensity-response function. The significance of differences between groups in all three parameters of Naka-Rushton fit and the a-wave amplitude were evaluated by Kruskal-Wallis followed by the Scheffe’s F post hoc test.

**RESULTS**

**Generation of Rhodopsin Promoter tTA1 Transgenic Lines**

We cloned 4.4 kb of the murine opsin regulatory region\(^9\) upstream to \(\text{ITA1}\) substituting the ATG of rhodopsin with the \(\text{tTA1}\) start codon in the \(\text{pUHD21-1}\) vector\(^8\) (Fig. 1A). This construct was used to generate transgenic mice. Founders were screened by PCR and Southern blot analysis. One of the founders transmitted the transgene to the progeny. Comparison of the Southern blot patterns identified two different lines in which the transgene integrated in different genomic sites and in different copy numbers (Fig. 1B, arrow).

To investigate the temporal pattern of \(\text{tTA1}\) expression in the retina, we collected retinas from the two transgenic lines at different ages after birth. We performed RT-PCR analysis, using \(\text{tTA1}\)-specific primers. As shown in Figure 1C, expression of \(\text{tTA1}\) starts at 8 days after birth in both lines. Expression increases in the following days, and it is maintained until adulthood. It should be noted that expression in our transgenic

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**Translated Table:**

<table>
<thead>
<tr>
<th>RhotTA1-L5</th>
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<tr>
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<td>P13</td>
<td>A</td>
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<td>P20</td>
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**Figure 2.** In situ hybridization analysis of double-transgenic retinas from RhotTA1-L5 and RhotTA1-L32 mice mated with L7 mice. Adjacent sections of P13 retinas (A–D) were hybridized either with the \(\text{tTA}\) probe (A, C) or the luciferase probe (B, D). Retinas from P20 mice (E–H) were analyzed, either with the \(\text{tTA}\) probe (E, G) or the luciferase probe (F, H). In all the samples, expression was restricted to the photoreceptor cell layer. P15 mice were fed with doxycycline (2 mg/mL). Luciferase expression was inhibited in both of the lines after 1 or 10 days of treatment with the antibiotic (J, L, N, P), whereas \(\text{tTA1}\) expression was maintained as expected (I, K, M, O). os, outer segment; onl, outer nuclear layer; opl, outer plexiform layer.
lines replicates the temporal expression pattern of the endogenous rhodopsin gene\textsuperscript{18} and of other rhodopsin promoter transgenic lines.\textsuperscript{9,19}

Specificity of tTA1 Expression in Transgenic Mice

We analyzed expression of tTA1 by in situ hybridization in sections from retinas harvested from the two transgenic mice. Expression of the transactivator was found only in the outer nuclear layer of the transgenic retinas (Figs. 2A, 2E, 2C, 2G). At 13 days after birth, tTA1 transcripts were detectable in both lines (Figs. 2A, 2C), and, at P20, expression was evident and well distributed in the photoreceptor cells (Figs. 2E, 2G). When we analyzed contiguous sections from the temporal to the nasal side of the eye, we did not notice differences in distribution of the transcripts.

Expression of tTA1 did not interfere with retinal development and did not affect retinal morphology. In fact, histologic analysis of the retinal tissue in adult transgenic animals (6 to 7 months) confirmed that tTA1 did not cause morphologic anomalies in photoreceptor cells (Figs. 3A, 3B). Furthermore, we analyzed the retinas at the electrophysiological level. Table 1 shows a summary of the parameter estimates for the Naka-Rushton fits of the b-wave amplitudes in wild-type and transgenic mice. In the dark-adapted ERGs (Fig. 3C), analysis revealed no significant difference between wild-type and transgenic mice in any of the tested parameters for either the a- or b-wave. In the light-adapted ERG (Fig. 3D), the parameter $V_{max}$ was not significantly different between the groups, except for RhotTA1-L5 compared with the wild type ($P = 0.0064$). No significant differences were observed between any of the groups in the parameter $k$ and $n$ of the Naka-Rushton fits. Finally, analysis of the a-wave amplitudes showed no statistical difference between the three groups (Table 2).

\begin{table}[h]
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\begin{tabular}{lcccc}
\hline
Genotype & \multicolumn{1}{c}{Dark-Adapted ERG} & & & \\
 & $V_{max}$ & $k$ & $n$ & \\
\hline
Wild type ($n = 5$) & 219.10 $\pm$ 75.97 & 1.97 $\pm$ 2.22 & 0.69 $\pm$ 0.14 & \\
RhotTA1-L5 ($n = 5$) & 160.06 $\pm$ 87.20 & 0.45 $\pm$ 0.41 & 0.53 $\pm$ 0.17 & \\
RhotTA1-L32 ($n = 5$) & 219.51 $\pm$ 54.61 & 1.24 $\pm$ 1.43 & 0.59 $\pm$ 0.17 & \\
\hline
\hline
Genotype & \multicolumn{1}{c}{Light-Adapted ERG} & & & \\
 & $V_{max}$ & $k$ & $n$ & \\
\hline
Wild type ($n = 5$) & 216.84 $\pm$ 49.03 & 5.97 $\pm$ 2.29 & 0.88 $\pm$ 0.26 & \\
RhotTA1-L5 ($n = 5$) & 160.06 $\pm$ 87.20 & 0.45 $\pm$ 0.41 & 0.53 $\pm$ 0.17 & \\
RhotTA1-L32 ($n = 5$) & 219.51 $\pm$ 54.61 & 1.24 $\pm$ 1.43 & 0.59 $\pm$ 0.17 & \\
\hline
\end{tabular}
\caption{Summary of Parameter Estimates for b-Wave Analysis by the Naka-Rushton Fits}
\end{table}

Data are expressed as the mean $\pm$ standard deviation.

** $P < 0.01$; all other comparisons were statistically non significant.

\textsuperscript{2} We then analyzed luciferase expression in the transgenic line bearing the luciferase reporter gene under control of tetO. To investigate whether tTA1 activates in vivo transcription in the retina, we collected eyes from double-transgenic mice (RhotTA1/L7). The distribution of luciferase transcripts overlapped the tTA1 mRNA expression pattern (Figs. 2B, 2D, 2F, 2H). To quantify the transactivator activity in the two lines, we collected eyes from RhotTA1/L7 mice and assayed luciferase activity in protein extracts. As shown in Figure 4A luciferase activity in RhotTA1-L5 rose above background at P8 (tTA1 single transgenic mice show 0.24 RLU/µg) and quickly increased in the following days to reach a plateau at P13. In RhotTA1-L32, the kinetics of induction of luciferase expression appears to be slower, in fact at P8 luciferase activity is lower than in RhotTA1-L5. Expression increases at P13 and reaches its high-

\begin{figure}[h]
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\caption{Histologic analysis of transgenic adult eyes. Seven-month-old RhotTA1-L5 (A) and 6.5-month-old RhotTA1-L32 (B) retinas were sectioned (1 µm) and stained with methylene blue and azure II. Dark-adapted (C) light-adapted (D) ERG parameter $V_{max}$ measured on wild-type (□), RhotTA1-L5 (△), and RhotTA1-L32 (○) mice.
}
\end{figure}
suggest that expression of tTA1 is driven specifically in the outer layer of the retina.

We also performed luciferase activity assays in other tissues (tongue, liver, heart, lung, kidney, spleen, and brain) harvested from the double-transgenic lines. We did not measure significant activity in liver, kidney, and spleen. In tongue, brain, lung, and heart we detected low levels of luciferase activity, as previously reported for L7 single-transgenic mice.2 The data suggest that expression of tTA1 is driven specifically in the outer layer of the retina.

Doxycycline's Effect on tTA1 Activity

The major advantage of the tTA1 system is that transcription can be blocked by the use of doxycycline. We therefore studied whether doxycycline treatment of RhotTA1/L7 mice blocks reporter gene expression. We added 2 mg/mL doxycycline in the drinking water of RhotTA1/L7 mice. We found that the response to the antibiotic was very rapid. In fact, 1 day of exposure to doxycycline was sufficient to block expression completely in RhotTA1-L5 mice (0.35 ± 0.07 RLU/μg; Fig. 4A). Inactivation of tTA1 was maintained for a long time (10 days) by continued administration of the antibiotic (Fig. 4A). Luciferase activity after doxycycline treatment was comparable to the activity from L7 single-transgenic mice (0.5 RLU/μg). In RhotTA1-L32 mice, suppression was not complete after 24 hours (Fig. 4B). However, a longer exposure to doxycycline brought expression of luciferase to basal levels (0.66 ± 0.1 RLU/μg; Fig. 4B).

We also analyzed the effect of doxycycline at the histologic level, by using in situ hybridization. One day after the addition of doxycycline to the drinking water, luciferase mRNA was not detectable, whereas, as expected, expression of ITA1 was not affected (Figs. 2I–P).

Finally, we studied whether we could induce tTA1 activity to resume. We withdrew the antibiotic from the drinking water after 3 days of treatment and tested luciferase activity at different time points. We found that, in 3-week-old mice, luciferase activity started to be detectable 4 days after we stopped the treatment (in RhotTA1-L5 mice it reached 51 ± 39 RLU/μg and in RhotTA1-L32 3628 ± 7730 RLU/μg). These data are in accordance with those of Robertson et al.20

DISCUSSION

We have generated an in vivo system to control gene expression in the retina. Classic transgenic approaches are not versatile enough, because of the persistence of transgene expression. To overcome this limitation, different inducible and repressible systems were developed to allow the control of transgene expression in a temporal and spatial manner. The use of such a system specific for the retina will be of great value in more clearly understanding the physiological changes occurring in retinal diseases and in developing therapeutic approaches. In fact, these studies will benefit from an in vivo model in which the effects of molecules can be tested by blocking their expression at will. For this purpose, we chose to express tTA1 in the retina. This transactivator is an improved version of the original tTA. It prevents the toxic effects of the VP16 transactivator domain and shows twice the transactivator activity. Furthermore, this system is very sensitive to doxycycline, even at low concentrations (0.1-10 ng/mL).6 This is very important, because doxycycline is usually administered through the drinking water, and the local concentration in the tissues can be very low.

In this report, we present data on two different transgenic lines expressing tTA1 in rod photoreceptors. To assess the system we used three techniques: histology, in situ hybridization, and enzymatic assay. Histology of the retina from transgenic mice showed that expression of tTA1 did not cause morphologic changes in the photoreceptor cells. Furthermore, no statistically significant difference was found between wild-type and transgenic mice in the dark-adapted or, in other words, rod-dominated ERG, as indicated by the parameters $V_{max}$, k, and n of the Naka-Rushton fits of the b-wave amplitudes. The a-wave analysis demonstrated that photoreceptor function was unaltered in the transgenic mice. A reduced $V_{max}$, observed solely in the light-adapted or cone-dominated state in RhotTA1-L5 mice, without any impairment of sensitivity (as shown by the parameters n and k of the Naka-Rushton fits) and without any reduction in a-wave amplitude could point to a limited alteration of the inner retinal layer or the synaptic transmission from the cone system to it. Considering the fact that rhodopsin is only expressed in rod photoreceptor cells, the difference in $V_{max}$ can probably be attributed to the fact that CD1 mice are outbred or to the small sample size, which could not overcome interindividual variability. That the rod system of the transgenic mice was not altered in any of the electrophysiologically tested parameters is in good accordance with histologic findings.
The cell specificity of transcription of the transgene was analyzed by in situ hybridization. Both lines showed specific expression of tTA1 in the photoreceptor nuclear layer. However, transcript distribution in the retina did not show a homogeneous pattern, and we detected photoreceptors highly expressing tTA1 among cells with lower level of expression. Heterogeneous expression of transgenes is a common phenomenon in mice and has been reported in many transgenic lines.\textsuperscript{20–22} This effect was more evident in one of the two lines (RhotTA1-L32), whereas expression was more homogeneous in RhotTA1-L5. However, when we analyzed the distribution of expression in the entire retina we found that tTA1 activated transcription in the entire adult organ and transcription of tTA1 started at the same time as that of endogenous rhodopsin.\textsuperscript{18}

Finally, tTA1 transcriptional activity was studied by breeding the transgenic lines with the L7 reporter line. We analyzed the ability of tTA1 to activate transcription in vivo in the retina by measuring the amount of luciferase activity. Based on an enzymatic luciferase assay, we defined good transactivating activity in both lines, demonstrating that the system works well in the retina. Then, by feeding the mice with doxycycline, we showed that a 1-day treatment is effective in completely inactivating the transgene in RhotTA1-L5. RhotTA1-L32 needs a longer exposure to the antibiotic to block the activity of tTA1. These data demonstrate that RhotTA1 transgenic mice are a versatile system to control expression in the photoreceptor cells. The rapid response to low doses of antibiotic makes these mice an effective tool for functional studies in retinal biology. Furthermore, this animal model represents an important component in the development of therapies that may be applied to human diseases in the future.

Acknowledgments

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References


