Functional Characterization of Mouse RDH11 as a Retinol Dehydrogenase Involved in Dark Adaptation in Vivo*

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From the Departments of ‡Molecular Genetics, **Internal Medicine, ‡‡Pathology and Molecular Biology, and ¶¶Ophthalmology, University of Texas Southwestern Medical Center, Dallas, Texas 75390, the Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104, the Retina Foundation of the Southwest, Dallas, Texas 75231, and §§Regeneron Pharmaceuticals, Incorporated, Tarrytown, New York 10591

We previously cloned mouse RDH11 (mRDH11) as a gene regulated by the transcription factor sterol regulatory element-binding proteins and showed that it is a retinol dehydrogenase expressed in non-ocular tissues such as the liver and testis and in the retina (Kasus-Jacobi, A., Ou, J., Bashmakov, Y. K., Shelton, J. M., Richardson, J. A., Goldstein, J. L., and Brown, M. S. (2003) J. Biol. Chem. 278, 32380–32389). It was proposed to function in the recycling of the visual chromophore 11-cis-retinal after photoisomerization by a bleaching light, a pathway referred to as the visual cycle. In this work, we describe our studies on the ocular function of mRDH11. We created a knockout mouse by replacing the mrdh11 coding sequence with the lacZ reporter gene for expression profiling. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) staining demonstrated active transcription of this gene in photoreceptor cells. We show by immunoblot analysis that mRDH11 is associated with retinal membranes purified from a non OUTER segment fraction of the retina. No obvious retinal defect was found during development and aging of RDH11-deficient mice. The functional consequences of mRDH11 disruption were investigated by electrotretinography. Dark adaptation was delayed by a factor of 2.5–3 compared with wild-type mice. However, the kinetics of 11-cis-retinal recycling during dark adaptation was not affected, suggesting that mRDH11 is not involved in the visual cycle. We propose that mRDH11 disruption affects retinoid metabolism in photoreceptor inner segments and delays the kinetics of dark adaptation through modulation of calcium homeostasis.

Short-chain dehydrogenases/reductases form a large family of functionally heterogeneous enzymes. The vast majority of them are NAD(P)(H)-dependent oxidoreductases and display specificity toward a wide spectrum of substrates ranging from steroids, retinoids, prostaglandins, and carbohydrates to xenobiotics. In humans, ~60 members of this family have been identified in the genome (1). Some of them have been associated with important functions and lead to various diseases if mutated (2). The function of several members of this family is still unknown.

Mouse RDH11 (mRDH11)1 is an enzyme of the short-chain dehydrogenase/reductase family that has been cloned as a gene regulated by the transcription factor sterol regulatory element-binding proteins and was previously named SCALD for short-chain aldehyde reductase (3). mRDH11 protein is 85% identical to its human ortholog, a protein that has been given three different names: PSDR1 (prostate short-chain dehydrogenase/reductase-1) (4), RalR1 (retinal reductase-1) (5), and RDH11 (6). For convenience, we will refer to the human protein as hRDH11 and to the mouse protein as mRDH11. The function of this protein is unknown.

Both human and mouse enzyme activities have been characterized in vitro. They are able to reduce both all-trans- and cis-retinaldehydes with low Km values ranging from 0.1 to 1 μM (3, 5). The reverse reaction, oxidation of all-trans-retinol, is not catalyzed by mRDH11 (3). hRDH11 is able to catalyze this reaction, however, at a lower catalytic efficiency compared with all-trans-retinal reduction (5, 7). In addition to retinaldehydes, mRDH11 catalyzes the reduction of short-chain aldehydes such as nonanal with Km = 30 μM (3). Such activity has not been studied for the human protein. Both enzymes specifically use NADP(H) as coenzyme.

Expression of mRDH11 has been examined in various mouse tissues; it was found abundantly in the liver and testis (3). As revealed by immunofluorescence, the protein is also expressed in four layers of the mouse retina, including photoreceptor inner segments (3). Northern blot analysis showed that hRDH11 is expressed in a wide variety of human tissues, including the liver, testis, and prostate (4). By immunofluorescence using a monoclonal antibody generated against hRDH11, a signal was detected in monkey and bovine eye, mostly in the retinal pigment epithelium (RPE) (6). Only a faint signal was detected in photoreceptor inner segments (6). Expression of mRDH11 in photoreceptor cells and its high affinity for retinoid substrates suggest that this enzyme is involved in retinoid metabolism in the eye.

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1 The abbreviations used are: mRDH11, mouse RDH11; hRDH11, human RDH11; RPE, retinal pigment epithelium; RDH, retinol dehydrogenase; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; HPLC, high pressure liquid chromatography; ROS, rod outer segment; RR, rest of the retina; ERG, electoretinogram.
In higher animals, retinoids have two distinct functions: regulation of gene expression for morphogenesis and differentiation of vitamin A-dependent tissues and light absorption in the retina. Vertebrate eye development requires vitamin A (all-trans-retinol) as demonstrated in gestational vitamin A deficiency studies, where ocular defects were one of the most common malformations observed (8). Vitamin A is also critically involved in vision as a source of 11-cis-retinal. Vertebrate vision begins with the absorption of light by visual pigments in photoreceptor cells. Visual pigments, or opsins, are seven membrane-spanning, G-protein-coupled receptors located in the outer segment disc membranes of rods and cones. The light-sensitive chromophore 11-cis-retinal is covalently attached to opsin proteins in the dark. Light stimulation results in isomerization of 11-cis-retinal to all-trans-retinal, which causes a change in the conformation of rhodopsin. The resulting photoactivated metarhodopsin II triggers the phototransduction cascade that leads to the generation of an electrical signal and ultimately to inhibition of neurotransmitter release at the synaptic terminal. After isomerization, all-trans-retinal is released from opsin and enzymatically converted to all-trans-retinol by a retinol dehydrogenase (RDE). This activity has been characterized; it is located in the membrane of outer segment discs and is NADPH-dependent (9–12). Candidate RDHs, photoreceptor RDH and RDH14, have been identified, but their functions have not been demonstrated in vivo (6, 13). All-trans-retinol is then transported to the RPE, where it is ultimately converted to 11-cis-retinol and oxidized to 11-cis-retinal by RDH5. This enzyme is located in the RPE and is NAD-dependent. It was suggested that there are additional not yet identified RDHs catalyzing this step, and hRDH11 was proposed to be one of them (6, 14). 11-cis-Retinal is returned to the outer segment discs for the regeneration of photosensitive rhodopsin. This biochemical pathway is referred to as the visual cycle (15). Recently, a number of RDHs that belong to the retinal short-chain dehydrogenase/reductase 1 family have been cloned (Table 1). They are proposed to function in the visual cycle, although, with the exception of RDH5 and RDH12, their roles have not been demonstrated in vivo. Mutations in RDH5 cause a mild retinal disease called fundus albipunctatus, which is characterized by delayed dark adaptation (16) due to a delay in regeneration of the 11-cis-retinal chromophore (17). Mutations in RDH12, the enzyme most closely related to hRDH11 with 71% identical residues, cause the severe early-onset retinal dystrophy Leber’s congenital amaurosis (18, 19). No mutations in hRDH11 have been associated with human retinal dysfunction.

In this work, we describe our studies on the function of mRDH11 in vivo. We disrupted the rdh11 gene in mice and showed a delay in dark adaptation in these mice, confirming the role of this enzyme in vision. However, our results show that this delay is not due to a defect in 11-cis-retinal regeneration in knockout mice. We propose that mRDH11 disruption affects retinoid metabolism in photoreceptor inner segments and delays the kinetics of dark adaptation through modulation of calcium homeostasis.

### EXPERIMENTAL PROCEDURES

**Materials**—We obtained mouse monoclonal anti-GPK1 (G-protein-associated rhodopsin kinase-1) IgG from Affinity BioReagents; X-gal from Gold Biotechnology, Inc.; and other chemicals, including all-trans-retinol, all-trans-retinal, and all-trans-retinyl palmitate, from Sigma. All-trans-retinal was used to produce all-trans-retinal oximes (syn and anti) as described (20). These retinoids and derivatives were used as standards for HPLC analysis.

**Generation of rdh11 Knockout Mice and Genotyping**—VelociGene, a high-throughput automated approach used to generate RDH11 knockout mice (along with 199 other knockouts), has been described (21). The resulting mice were genotyped by Southern blot analysis of BamHI-digested tail DNA using a DNA probe (0.7-kb fragment) starting 1.5 kb upstream of the first exon and by PCR using primers 5'-GGATGAGG-GAAAGAGACGAGAG-3', 5'-GGTGCAATTAGTGGCCAGAG-3', and 5'-GTCCTGCCTAGCCTCCTACCTG-3'. Animals—Tissues were collected immediately after killing the animals. For retinoid extraction, eyes were nucleated; the lens was removed after a small incision made with a surgical blade at the anterior pole of the eye; and the remaining eye globe was immediately frozen in liquid nitrogen. For immunoblotting, all operations were done at 4 °C. After enucleation, each eye was dissected as follows. A small incision was made at the anterior pole to release the intraglobular pressure, and the whole anterior half of the eye was removed using a pair of fine dissecting scissors. The anterior half of the eye and the lens were discarded. The retina was then gently peeled using a pair of fine dissecting forceps and kept in sucrose buffer at 4 °C until homogenization and fractionation. For X-gal staining, anesthetized mice were fixed via transcardial perfusion with 0.2% glutaraldehyde, and tissues of interest were dissected and grossly trimmed prior to embedding in optimal cutting temperature freezing matrix. Specimens were snap-frozen by partial immersion in liquid nitrogen-supercooled isopentane. Cryosections (8 μm thick) were prepared, mounted on silanated microscope slides, and air-dried. Slides were briefly stored at ~80 °C prior to β-galactosidase detection. For paraffin histology, the eyes were enucleated, placed in paraformaldehyde fixative, and processed according to established procedures (22, 23).

**Protein Extraction and Immunoblotting**—Rod outer segment (ROS) and “rest of the retina” (RR) membrane fractions were prepared by discontinuous sucrose gradient centrifugation as described (24). Briefly, retinas were homogenized with a Teflon/glass homogenizer in buffer A (10 mM Tris-HCl (pH 7.0), 100 mM NaCl, 1 mM EDTA, 1.17 g/ml sucrose, and protease inhibitor mixture (1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin A)). Discontinuous sucrose gradients (1.11, 1.13, and 1.17 g/ml) were centrifuged at 105,000 × g for 70 min. The 1.11/1.13 g/ml interfacial band-containing ROS fractions and the 1.13/1.17 g/ml interfacial band-containing RR fractions were collected, separately diluted with 10 volumes of buffer A without sucrose, and centrifuged at 100,000 × g for 30 min to pellet membrane fractions. Membrane fractions were resus-

### Table I

| Name   | Localization | Activity, coenzyme | Disease | Identity to hRDH11 (and mRDH11) | Ref. |
|--------|--------------|--------------------|---------|---------------------------------|------|
| RDH5   | RPE          | 11-cis-Retinol dehydrogenase, NAD | Fundus albipunctatus | 25 (27) | 16, 17, 36 |
| RDH11  | RPE, Müller cells | All-trans-retinol dehydrogenase, NADP |   | 23 (32) | 22, 38 |
| mRDH11 | Photoreceptor (IS) | trans- and cis-retinal reductase, NADPH |   | 100 (84) | 5, 6 |
| mRDH12 | Photoreceptor (IS) | trans- and cis-retinal reductase, NADPH |   | 84 (100) | 3 |
| mRDH13 | Photoreceptor (IS) | None detected | LCA | 71 (70) | 6, 18, 19 |
| mRDH14 | Photoreceptor (OS) | trans- and cis-retinal reductase, NADPH |   | 43 (44) | 6 |
| retSDR1 | Cone (OS) | All-trans-retinal reductase, NADPH |   | 21 (22) | 39 |
| prRDH  | Photoreceptor (OS) | All-trans-retinal reductase, NADPH |   | 22 (22) | 13 |
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**Fig. 1. Disruption of the mrdh11 allele.** A, schematic of the gene-targeting strategy. The map of the wild-type allele spans the seven exons of the mrdh11 gene (black boxes). The 5′- and 3′-untranslated regions of exons 1 and 7 are indicated (white boxes). The sequence replacement bacterial artificial chromosome was constructed as described under “Experimental Procedures.” In the disrupted allele, 15.7 kb of the mrdh11 gene were replaced with the bacterial lacZ gene fused in frame with the first four amino acids of mRDH11 and the neo selection marker. B denotes BamHI restriction sites. PGK, phosphoglycerate kinase promoter. B, representative Southern blot analysis of BamHI-digested tail DNA of the offspring from mating of mrdh11 −/+ and mrdh11 −/− mice. The DNA probe was a 0.7-kb fragment starting 1.5 kb upstream of exon 1. The migration positions of the fragments derived from wild-type and disrupted alleles are indicated. C, immunoblot analysis of mRDH11 from the livers and testes of mrdh11 −/+ and mrdh11 −/− mice. Aliquots (50 μg of protein) of tissue homogenates were subjected to SDS-PAGE and immunoblotted with a 1:1000 dilution of rabbit anti-mRDH11 antisera. The asterisk indicates a nonspecific band.

A. Schematic of the gene-targeting strategy

B. Southern blot analysis

C. Immunoblot analysis

**RESULTS**

Fig. 1A summarizes the strategy used to eliminate the rdh11 transcript in mice. A 15.7-kb region of the mrdh11 gene was replaced with a sequence encoding bacterial β-galactosidase (lacZ) for expression profiling in tandem with the neomycin resistance gene (neo), allowing the selection of both bacteria and embryonic stem cells. A representative Southern blot of genomic tail DNA prepared from rdh11 +/+ , rdh11 +/− , and rdh11 −/− mice is shown in Fig. 1B. Immunoblot analysis demonstrated the absence of mRDH11 in the livers and testes of...
rdh11−/− mice (Fig. 1C). Mating of male and female rdh11−/− mice resulted in the production of litters of normal size (8–13 pups) with no apparent gross abnormalities or defects in survival or growth, indicating that rdh11−/− mice are viable and fertile.

We investigated the pattern of expression of β-galactosidase in different tissues of rdh11+/− and wild-type mice (Fig. 2). All tissue sections were processed in the same experiment to allow comparison of β-galactosidase activities between tissues. None of the tissues collected from wild-type mice showed X-gal staining (data not shown). In rdh11+/− mice, β-galactosidase activity was high in germ cells in the testis (Fig. 2H). This result was expected because mrdh11 mRNA and protein were previously detected in these cells (3). The mrdh11 promoter was also active at a comparable level in the keratinized squamous epithelium of the stomach and the epithelium of the small intestine (Fig. 2A–D) and in hair follicle-associated sebaceous glands (Fig. 2F). β-Galactosidase activity was detectable at a lower level in chondrocytes (Fig. 2I), the adrenal medulla (Fig. 2J), a subset of renal tubules (Fig. 2K), and the brain (Fig. 2L). The absence of β-galactosidase activity in the liver (Fig. 2G) was unexpected because mrdh11 mRNA and protein were abundantly present in this tissue. A possible interpretation is that expression of mrdh11 in the liver requires intronic sequences that have been removed in the disrupted allele. This expression profile shows that mrdh11 is actively transcribed in a wider variety of tissues than reported previously. The epithelium of the small intestine and hair follicles, as well as the testis and liver, are known to have active retinoid metabolism, consistent with a function of mRDH11 in this metabolism.

mRDH11 has been detected previously in four different layers of the retina, including photoreceptor inner segments; but in contrast to hRDH11, no signal was detected in the RPE (3). To confirm this result, β-galactosidase activity was measured in the retinas of albino mice. Albino mice were chosen to avoid the pigmentation in the RPE and choroid that could potentially mask blue coloration. Fig. 3A shows strong and homogeneous X-gal staining in the photoreceptor inner segment layer, demonstrating that the mrdh11 promoter is active in photoreceptors. No significant X-gal staining was detected in other layers.
Fig. 3. Localization of RDH11 in mouse retina. A, X-gal staining was performed in the sagittal sections of retinas from adult Swiss Jim Lambert albino mice. Wild-type mice (left panel) showed no X-gal staining. rdh11+/− mice (right panel) showed strong and homogeneous X-gal staining in the photoreceptor inner segment layer. Note the absence of X-gal staining in the RPE. S denotes the sclera. Numbers refer to the following layers of the retina: 1, the RPE; 2, the photoreceptor outer segment; 3, the photoreceptor inner segment; 4, the external limiting membrane; 5, the outer nuclear layer; 6, the inner nuclear layer; 7, the inner plexiform layer. Scale bar = 60 μm. B, dissected retinas from the indicated mice were fractionated as described under “Experimental Procedures.” Aliquots of protein (100 μg) from the indicated fractions were subjected to SDS-PAGE and immunoblotted with a 1:1000 dilution of rabbit anti-mRDH11 antiserum (left panel) or anti-GRK1 antibody (right panel).

Fig. 4. Retina histology of rdh11+/+ and rdh11−/− mice. A, representative sagittal sections of retinas from 18-month-old rdh11+/+ (left panel) and rdh11−/− (right panel) mice. S denotes the sclera, and C denotes the choroid. Numbers refer to the following layers of the retina: 1, the RPE; 2, the photoreceptor outer segment; 3, the photoreceptor inner segment; 4, the external limiting membrane; 5, the outer nuclear layer; 6, the inner nuclear layer; 7, the inner plexiform layer; 8, the ganglion cell layer. Scale bar = 100 μm. B, outer nuclear layer (ONL) thickness (in micrometers) plotted as a function of the retinal location (in millimeters) from the optic nerve head (ONH). Measurements were made at 0.33-mm intervals from the optic nerve head. Error bars represent the S.D. (n = 6).

of the retina, including the RPE. However, it should be noted that the lack of β-galactosidase expression in the RPE could be due to the same reasons given for the lack of expression in the liver.

To investigate the subcellular localization of mRDH11, retinas from light-adapted wild-type and knockout mice were dissected, and ROS and RR fractions were separated in a discontinuous sucrose gradient as described under “Experimental Procedures.” Membranes were isolated from these fractions, and equal amounts of proteins were analyzed by immunoblotting. As shown in Fig. 3B (left panel), mRDH11 was present in RR membranes and undetectable in ROS membranes. GRK1 was used as a positive control for the ROS preparation (Fig. 3B, right panel). This protein is associated with photoreceptor outer segment disc membranes under light-adapted conditions (30) and was detected only in the ROS membrane preparation. This result confirms the accuracy of the ROS and RR fractionation and thereby demonstrates that mRDH11 is not localized in the outer segments of photoreceptors under light-adapted conditions, consistent with the previous immunofluorescence detection of a signal in the inner segments of photoreceptors (3).

To investigate the consequences of mRDH11 disruption at the histological level, eyes from 1-, 3-, 6-, and 18-month-old animals were examined. No disturbance or signs of retinal pathology were observed when mrdh11−/− animals were compared with mrdh11+/+ littermates within the time frame investigated. Fig. 4A shows representative sections from 18-month-old rdh11+/+ and rdh11−/− mice. To quantify the number of photoreceptors, the outer nuclear layer thickness was measured in these sections. As shown in Fig. 4B, no significant difference in outer nuclear layer thickness was found between the two groups of mice. This result demonstrates that mRDH11 disruption does not lead to photoreceptor loss.

ERGs were performed on 3–6-month-old animals to assess the functional consequences of mRDH11 disruption. Fig. 5A shows that ERG a-waves, corresponding to the massed photocurrent response of the rod photoreceptors, were comparable in representative rdh11+/+ and rdh11−/− mice. For each mouse, a-waves are shown for a series of retinal illuminances up to and higher than that producing a-wave amplitude saturation. The dashed curves are best fits of the phototransduction model to the ensemble of leading edges. The thick solid curve is for a retinal illuminance of 1.7 log scot td/s. The recovery from this retinal illumination is shown in the right panels of Fig. 5A. The dashed curves are exponential fits to normalized recovery values determined in paired-flash experiments. The deviation of each curve from saturation defines t sat, the period of complete photoreceptor suppression following the intense flash.
ERG parameters from all mice are summarized in Table II. There were no significant differences between rdh11+/+ and rdh11−/− mice in rod or cone amplitudes or implicit times. There were no significant differences in sensitivity (k) or maximum amplitude (Vmax) for the rod b-wave and no significant differences in gain (S) or maximum amplitude (Rm) for the a-wave. There was a borderline significant difference between groups in treset, suggesting that recovery kinetics were slower in the rdh11−/− mice.

Recovery of rod ERG function following a 5-min exposure to a 400-lux illumination is shown in Fig. 5B. The single responses (Fig. 5B, upper panels) at various times following termination of the bleaching light indicated a very small response at 5 min in the rdh11+/+ mice, with major growth in amplitude by 30 min and virtually full recovery by 60 min. In contrast, the rdh11−/− mice showed little recovery at 20 min, very gradual recovery over the following hour, and incomplete recovery even at 2 h post-bleaching. As shown in Fig. 5B (lower panel), the time course of recovery in rdh11+/+ mice is comparable with that reported previously for wild-type mice (31). In contrast, recovery took over 2 h in rdh11−/− mice.

The replenishment of 11-cis-retinal through the visual cycle is the limiting factor for the regeneration of photosensitive rhodopsin after submission to a bleaching light. The first step of this cycle, reduction of all-trans-retinal released from bleached rhodopsin, is catalyzed in photoreceptor outer segments. As shown in Fig. 3, mRDH11 was undetectable in ROS fractions. However, the delay in dark adaptation in the knock-out mice led us to measure intermediates of the visual cycle (11-cis-retinal, all-trans-retinal, all-trans-retinol, and retinyl ester) in the dark-adapted state and during bleach recovery. As
shown in Fig. 6, bleaching caused isomerization of 80% of 11-cis-retinal to all-trans-retinal. All-trans-retinal was then reduced and accumulated as retinyl ester in the RPE. Regeneration of 11-cis-retinal was slow, reaching 50% of the dark-adapted level after 1 h of recovery. There was no significant difference in retinoid composition between rdh11+/+ and rdh11−/− mice in the dark-adapted state or at any time during bleach recovery. These results demonstrate that there is no defect in the recycling of the 11-cis-retinal chromophore in knockout mice.

**DISCUSSION**

This study and others (3–5, 7) showed that mRDH11 is expressed in ocular and extraocular tissues, suggesting multiple functions. A possible role of mRDH11 in intestinal absorption of β-carotene has been discussed (7). If this hypothesis is correct, rdh11−/− mice are not expected to develop a vitamin A deficiency caused by a defect in β-carotene absorption because, in the standard rodent diet, vitamin A is mostly supplied as retinyl esters and not as β-carotene. Indeed, rdh11+/+ and rdh11−/− mice have comparable concentrations of circulating vitamin A (data not shown).

In this work, we have described our studies on the ocular function of mRDH11. We created a knockout mouse by replacing the rdh11 coding sequence with the lacZ reporter gene. X-gal staining confirmed active transcription of rdh11 in photoreceptor cells. Photoreceptor RDHs are potentially important enzymes because several lines of evidence suggest that reduction of all-trans-retinal in photoreceptor cells is crucial to maintain the function and integrity of the retina. First, it is the first and limiting step of the visual cycle, which is essential for sustained phototransduction. Second, all-trans-retinal is a highly reactive molecule due to its aldehyde function. Aldehydes are unstable molecules that can form Schiff base bonds with surrounding molecules, leading to the production of toxic adducts. This has been exemplified in mice with a knockout mutation in the abcr gene, which encodes the Rim protein RmP, a retinal ATP-binding cassette transporter (ABCR). RmP disruption leads to an increased level of all-trans-retinal in outer segments following light exposure as well as accumulation of A2E, a Schiff base condensation product of two retinaldehydes with phosphatidylethanolamine, representing the major fluorescent species of toxic lipofuscin pigments (31). Third, all-trans-retinal is a biologically active molecule that can bind and activate opsin, leading to a noisy phototransduction background. All-trans-retinal was also shown to directly and markedly inhibit photoreceptor ion channels at physiological concentrations (32–34). Therefore, disruption of RDH activity in photoreceptors leading to delayed reduction of all-trans-retinal could have a number of important consequences.

We have shown that mRDH11 is not present in ROS fractions, ruling out the possibility that mRDH11 catalyzes the first and limiting step of the visual cycle. No photoreceptor loss was found during aging of RDH11-deficient mice, suggesting that there is no induced toxicity. However, disruption of mRDH11 led to a profound slowing of dark adaptation as shown by the ERG testing of mice during bleach recovery.

The molecular mechanism leading to the defect in dark adaptation in rdh11−/− mice is unknown. After submission to a bleaching light, a number of pathways are activated in photoreceptors to allow their return to the dark-adapted state, which is the state of full sensitivity to light. This relatively slow process comprises the regeneration of 11-cis-retinal through the visual cycle and the shutdown of the phototransduction pathway. mRDH11 disruption does not significantly change the rate of 11-cis-retinal regeneration, suggesting that this enzyme is not involved in the visual cycle. We hypothesize that mRDH11 disruption rather inhibits a step of the phototransduction shutdown that takes place in the inner segment of photoreceptors.

Light-induced hyperpolarization of photoreceptors triggers the closure of L-type voltage-gated Ca2+ channels located in rod inner segments. These L-type voltage-gated Ca2+ channels control the synaptic transmission of visual information by controlling intracellular Ca2+ concentration and neurotransmitter release (35). It has been shown that, in addition to light, all-trans-retinal also inhibits L-type voltage-gated Ca2+ channels at physiological concentrations (34). Thus, delayed clearance of all-trans-retinal in photoreceptor inner segments could mimic light activation (and therefore decrease the kinetics of dark adaptation) by decreasing Ca2+ influx. Further study of the RDH11 knockout mice may provide additional insights into the relationship between dark adaptation, calcium homeostasis, and retinoid metabolism in photoreceptor inner segments.

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