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Photoreceptor Pathology in the X-Linked Retinoschisis (XLRS) Mouse Results in Delayed Rod Maturation and Impaired Light Driven Transducin Translocation

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Abstract

Light-activated movement of transducin-α ($G_{\alpha t1}$) from rod photoreceptor outer segments (ROS) into inner segments (IS) enables rods to rapidly adapt to changes in light intensity. The threshold light intensity at which $G_{\alpha t1}$ translocates from ROS into IS is primarily determined by the rates of activation and inactivation of $G\alpha t_1$. Loss- of- expression of the retina specific cell surface protein, retinoschsin (*Rs1*-KO), led to a dramatic 3–10 fold increase, depending on age, in the luminance threshold for transducin translocation from ROS into IS compared with wild-type control. In contrast, arrestin translocated from IS into ROS at the same light intensity both in WT and Rs1-KO mice. Biochemical changes, including reduced transducin protein levels and enhanced transducin GTPase activity, explain the shift in light intensity threshold for $G_{\alpha t1}$ translocation in *Rs1*-KO mice. These changes in *Rs1*-KO mice were also associated with age related alterations in photoreceptor morphology and transcription factor expression that suggest delayed photoreceptor maturation.

Keywords

Transducin; Arrestin; Translocation; Photoreceptors; Retinoschisis

71.1 Introduction

Vertebrate vision is initiated in the retinal rod and cone photoreceptor outer segments, where light is captured and converted to a neuronal signal that ultimately leads to a reduction in

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cGMP levels [1]. The sequence of events commonly referred to as the phototransduction cascade include: activation of rhodopsin through photoisomerization of chromophore 11-*cis* retinal to all-*trans* retinal (R*) and activation of transducin by GTP/GDP exchange on $G_{\alpha t1}$ subunit which in turn leads to activation of cGMP phosphodiesterase 6 (PDE6) that hydrolyses cGMP. The decrease in cGMP concentration leads to closure of cGMP-gated cation channels (CNG) in the plasma membrane and membrane hyperpolarization. In the photoresponse deactivation phase, R* is shut off by Ca^{2+}/recoverin mediated phosphorylation of R* by rhodopsin kinase and the subsequent binding of arrestin to phosphorylated rhodopsin. $G_{\alpha t1}$* turns itself off by hydrolyzing GTP to GDP (intrinsic GTPase activity) which is accelerated by retinal RGS9 (regulator of G-protein signaling) protein. A drop in calcium levels caused by light exposure stimulates guanylate cyclase and restores cGMP concentration to the resting dark level.

71.2 Light Dependent Translocation of Phototransdction Proteins

The kinetics of phototransduction, i.e. the amplitude and speed of the photoresponse, are critical factors for the function of the visual system allowing it to respond to wide range of light levels from starlight to bright sunlight. There are several different mechanisms involved in light adaptation, but the activities and expression levels of phototransduction proteins and the calcium concentration are the key modulators [1]. Research over the past decade has demonstrated that light driven translocation of signaling molecules, namely, transducin and arrestin, between outer and inner segments contributes to photoreceptor cell adaptation to light [2]. When the light intensity reaches a critical threshold at which the rate of activation of $G_{\alpha t1}$ exceeds the rate of inactivation by GTP hydrolysis, $G_{\alpha t1}$ moves from the ROS into the IS and the cell body. Cone α-transducin, which is compartmentalized in the cone outer segment, does not translocate as cones function in much brighter light than rods and cone $G\alpha_{t2}$ can turn off about a factor of two more rapidly than $G\alpha_{t1}$. Arrestin, which quenches photoactivated rhodopsin, moves in reciprocal manner from the IS to the ROS when the intensity of background illumination approaches the upper limit of rod responsiveness. Diffusion is believed to be the basic principle driving this protein movement. Gat1 translocation is expected to contribute to photoreceptor light adaptation, as it allows rods to escape saturation and extends their range of light responsiveness. Alternatively, the process may reduce metabolic stress in the retina by reducing excess GTP consumption by rods and thereby the activation of Gat1 molecules in cone dominated bright light vision.

71.3 X-Linked Retinoschisis (XLRS)

Retinoschisin (RS1), a discoidin domain family member, is a retina specific cell surface protein expressed predominantly in photoreceptor IS and bipolar cells and functions in retinal cell adhesion and lamination processes [3]. Loss of function mutations in the X-linked RS1 gene causes XLRS a form of macular degeneration seen in young males [3, 4]. The disease phenotype is mimicked in the mouse model (*Rs1*-KO), which has delamination of inner retinal layers with decreased ERG b-wave amplitudes and diminished bipolar cell signaling. Because of the robust expression of RS1 in photoreceptors and its role in maintaining the photoreceptor inner segment stability and architecture [5, 6], it is surprising

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that only a third of XLRS patients display photoreceptor pathology with reduced a-wave amplitude [4]. By comparison, Rs1-KO mice display reduced ERG a-wave amplitude and have shortened rod outer segment (ROS) length as early as 1 month. The mice also have slow photoreceptor loss that progresses over more than a year [7]. To address the role of RS1 in photoreceptor function, we determined the threshold light intensities for transducin and arrestin translocation in *Rs1*-KO mice at postnatal days 21 (P21) and 60 (P60) when retinal degeneration is minimal [8]

71.4 Translocation in *RS1*-KO Mice

Rs1-KO mice required higher light intensity for $G\alpha_{t1}$ translocation than age-matched WT mice at both P21 and P60 (Fig. 71.1) [8]. Complete translocation of $G\alpha_{t1}$ from ROS to the IS, ONL and OPL in adult P60 *Rs1*-KO retinas required 2.5-fold higher light intensity compared with P60 WT retinas (1 h exposure at 30 sc. cd/m^2 vs. 12 sc.cd/m^2). However, at P21, complete movement of $G\alpha_{t1}$ into the OPL was seen in *Rs1*-KO retinas only at 10-fold higher light intensity (300 sc.cd/m^2) compared with P21 WT retinas (30 sc.cd/m^2) (Fig. 71.1). Exposure for 3 h to 60 sc. cd/m^2 failed to cause $G\alpha_{t1}$ translocation in P21 *Rs1*-KO retinas, thus ruling out the possibility of slow movement of $G\alpha_{t1}$ as the reason for the elevated translocation threshold. In contrast to $G\alpha_{t1,}$ arrestin translocated from IS to the ROS at the same light intensity (between 1 and 2 sc.cd/m^2) both in WT and *Rs1*-KO retinas at P21 (Fig. 71.2). Loss of RS1 protein did not impair the axial diffusion of $G\alpha_{t1}$ and arrestin between the photoreceptor compartments because re-translocation of $G\alpha_{t1}$ in the dark (from IS to the ROS) and arrestin (from ROS to IS) occurred similarly in WT and *Rs1*-KO retinas at P21.

71.5 Photoreceptor Maturation and Translocation Threshold Light Intensity

Another interesting finding was the progressive decrease in luminance threshold for transducin translocation in WT mice as they mature from P18 to P21 to P60 indicating that changes in the sensitivity of transducin translocation are part of normal rod maturation [8]. *Rs1*-KO mice also showed a decrease in luminance threshold for transducin translocation from p21 to p60. However, *Rs1*-KO mice had a much higher threshold relative to WT at P21 (10X) than at P60 (2.5X, Fig. 71.1), suggesting a delay in maturation of the translocation threshold in *Rs1*-KO mice. The age related biochemical and morphological changes seen in *Rs1*-KO mice also suggest a delay in rod cell maturation processes. ROS length normally reaches adult levels in WT mice at P21 [9], but in *Rs1*-KO mice ROS length was significantly shorter at P21 but it reached adult levels by P60. *Rs1*-KO mice had reduced levels of the photoreceptor development specific transcription factors NRL and CRX at P21, but the levels were similar to WT at P60. Taken together, this indicates a delay in photoreceptor maturation in the *Rs1*-KO mouse.

71.6 Phototransduction in *Rs1*-KO mice

Kinetics of rhodopsin and transducin deactivation are key factors that set the speed and sensitivity of the photoresponse. Transducin levels were decreased 15–30 % relative to rhodopsin in *Rs1*-KO mice at P21 but not at P60 (Fig. 71.3a) [8]. Whereas, RGS9, the GTPase-accelerating protein (GAP) for $G\alpha_{t1}$, was elevated 1.7- to 2.5-fold above WT at

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P21, PDEα and PDEγ were slightly elevated relative to rhodopsin in *Rs1*-KO photoreceptors (Fig. 71.3b–d). Consistent with this observation, the rate of $G\alpha_{t1}$ inactivation by GTP hydrolysis was nearly two fold higher in ROS of *Rs1*-KO mice (Fig. 71.3e) than in WT retinas at P21 but indistinguishable from WT at P60 (Fig. 71.3f). The increased inactivation rate in *Rs1*-KO mice at P21 results in a shorter lifetime of activated transducin, which could shift the light intensity threshold for transducin translocation to higher intensity by reducing the amount of activated transducin present during exposure.

Transducin and arrestin translocation defects were demonstrated in mouse models mimicking phototransduction gene linked diseases. One such example is bradyopsia (slow vision), a condition that results from mutations in genes encoding RGS9 or the RGS9 anchor protein (R9AP) [10]. Patients with bradyopsia have trouble adjusting to changing light conditions because of delay in the recovery from light responses (reduced rate of transducin GTPase activity). In the mouse model of bradyopsia, Gat1 translocated at lower (~ 2.3-fold) light intensity than in WT mice [11]. On the other hand, light activation threshold for Gat1 translocation was shifted to a higher light intensity in *Shaker1* mice, an animal model for Usher syndrome (USH1B) with mutations in *MYO7A* [12]. MYO7A is expressed in melanosomes of retinal pigment cells and in photoreceptor cilium, the region that links inner segments to outer segments (OS) and the sole route for delivering the proteins from IS to OS. Although this study did not show evidence of a mechanism for the shift in translocation threshold, it might be linked to decreased rhodopsin in rods, (Shaker 1 mice have been shown to have diminished ERG a-wave amplitudes) or to changes in melanosomes altering the effective light intensity in photoreceptors. Neither RS1 nor MYO7A is a member of the phototransduction cascade or is linked directly to phototransduction. Nevertheless, loss of their function affected light responses in photoreceptors. These results suggest that any defect intrinsic to photoreceptor function could in principle modulate photoresponses and thereby light adaptation.

Acknowledgments

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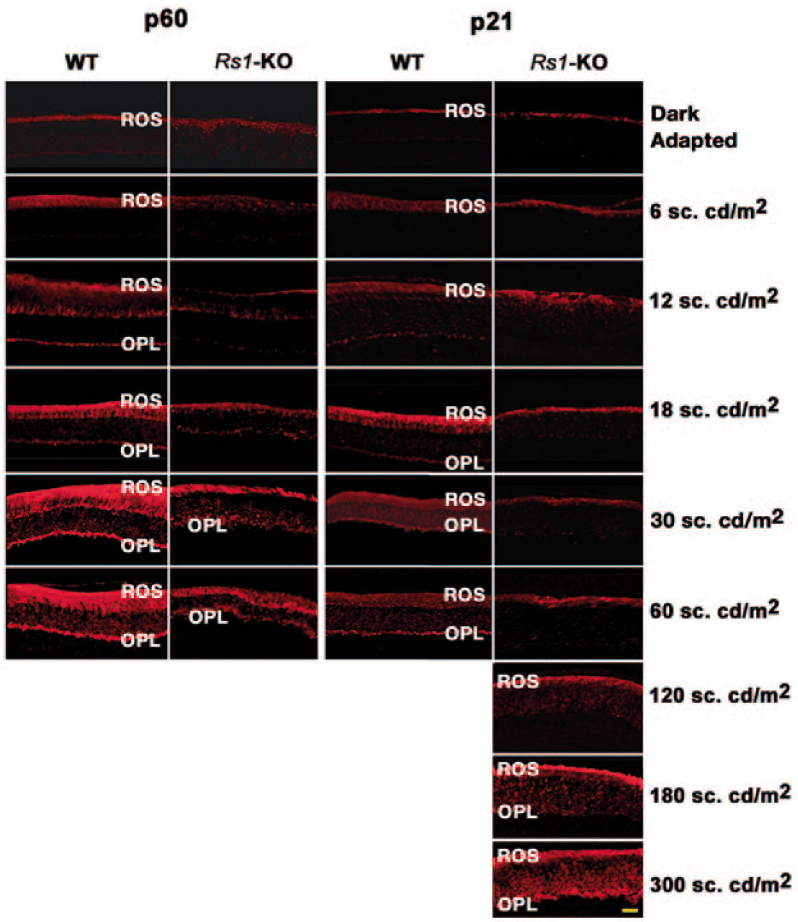

Fig. 71.1.

Light-intensity-dependent transducin translocation in WT and *Rs1*-KO mice at P21 and P60. Animals were either dark adapted or exposed to 1 h of light of different intensities (indicated on the *right*). Comparison of Gα_{t1} distribution in WT and *Rs1*-KO mice retinas shows that, in P21 and P60 WT retinas, Gα_{t1} translocates from *ROS* and distributes into OPL at much lower light intensities compared with *Rs1*-KO retinas. Immunofluorescence of Gα_{t1} in P21 *Rs1*-KO retinas shows persistent staining only in the ROS. Only exposure to very bright light (180–300 sc.cd/m^2) caused Gα_{t1} distribution into the OPL. Scale bar, 20 μm

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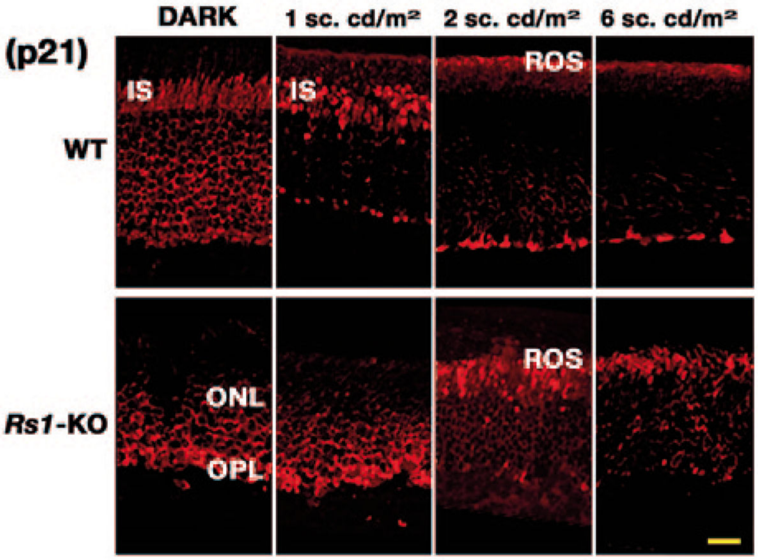

Fig. 71.2.

Arrestin movement in response to light in P21 WT and *Rs1*-KO mice: Animals were either dark adapted or exposed to 1 h of light of different intensities as indicated. Light of 2 sc.cd/m^2 was able to mobilize arrestin from the IS to the ROS both in WT and *Rs1*-KO mice. Scale bar, 15 µm

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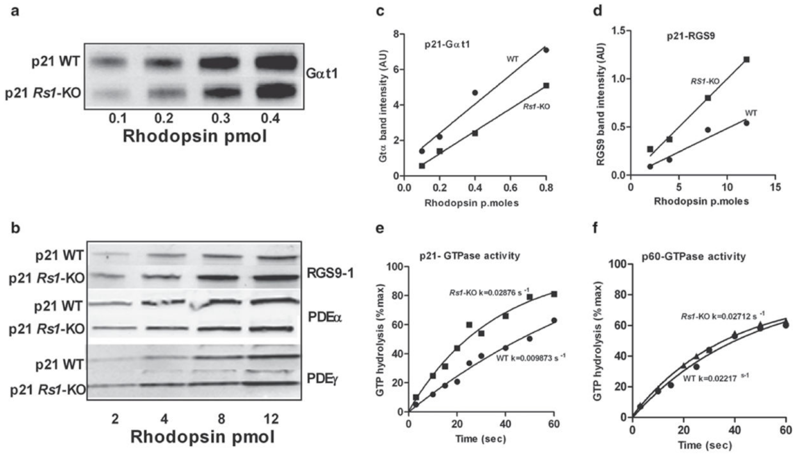

Fig. 71.3.

Quantitative immunoblot analyses (Odyssey imaging system; LI-COR) of key phototransduction protein subunits, transducin (Gα_{t1}), RGS9, PDE6α, and PDE6γ, in dark-adapted outer segment extracts from P21 WT and *Rs1*-KO mice. Gα_{t1} levels relative to rhodopsin were 15–30 % lower in *Rs1*-KO mice than in WT (**a, c**). RGS9, the GAP for Gα_{t1} was 1.7- to 2.5- fold higher in *Rs1*-KO than in WT (**d**). Both PDE6α and PDE6γ protein levels were marginally elevated in *Rs1*-KO retinas (**b**). The time course of phosphate formation during hydrolysis of [γ-32P] GTP by Gα_{t1}* in ROS: In single-turnover GTPase activity measurements in isolated ROS, GTP hydrolysis by Gα_{t1} was nearly twofold higher in *Rs1*-KO than in WT at P21 (**e**). resulting in a shorter lifetime of activated Gα_{t1} in *Rs1*-KO ROS. The rates were not different at P60 (**f**). The data were fitted to a single-phase exponential decay curve using GraphPad Prism (GraphPad Software)