Retinitis Pigmentosa 2 protein regulates transport of isoprenylated proteins to photoreceptor outer segments

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Purpose: X-linked retinitis pigmentosa (XLRP) is a devastating form of retinal degeneration, manifesting early in life with symptoms of night blindness, visual field defects, and decreased visual function. In vitro, RP2 functions as a GAP for the small GTPase ARL3, a GDP/GTP exchange catalyzed by a guanine nucleotide exchange factor (GEF) (5). Mutations in the Rp2 gene account for approximately one quarter of all XLRPs. The purpose of this study was to investigate the consequences of Rp2 deletion and identify mechanisms causative of XLRP.

Methods: Immunofluorescence of Rp2 in photoreceptors was determined by confocal microscopy. A B6 mouse line containing a gene trap in intron 1 of the Rp2 gene was used for electroporation of an Rp2h correction vector. The knockout mice were harvested after one month of age to analyze the localization of Rp2 in photoreceptors.

Results: Rp2h-GFP was localized to the plasma membrane of inner segments, axons and synaptic terminals in photoreceptors, but not in outer segments. The Rp2h knockout mice were viable and developed normally. Ablation of Rp2 gene expression led to slowly progressing degeneration of cone and rod photoreceptors as indicated by ERG recordings. Scotopic a-wave and photopic b-wave amplitudes were reduced as early as one month of age in the knockout mice. The Rp2h-VEGF amplitudes were further reduced at 6 months of age. Trafﬁcking of transmembrane phototransduction proteins, including cone opsins, to photoreceptor outer segments was not affected at 1 month of age. While targeting of transducin α to the photoreceptor outer segments was not affected in the knockout, transport of rod and cone PDE6 as well as GRK1 to outer segments was impaired.

Conclusions: RP2 is distributed to plasma membrane of inner segments and synaptic terminals in photoreceptors. RP2 is not essential for trafﬁc cone opsins and transducin to photoreceptor outer segments, but regulates transport of isoprenylated proteins to photoreceptor outer segments. Our results suggest that RP2/RK1 may allosterically release prenylated proteins from their soluble complex with PDE6D and extend them to donor membranes (e.g., TGN vesicles). In the knockouts, this process is impaired.

Figure 1. The mouse Rh1 gene knockout. A, the mouse Rh1 gene contains five exons. A gene trap was introduced into exon 1 of mouse Rh1 (WtR1) by the strategy described (3, 20). The transgene expressed in retinal neurons. B, the Ez, gp-selected chimeric mice expressing the gene trap were backcrossed to C57BL/6 mice to generate the WT and knockout strains. C, the WT and knockout mice were examined by western blotting using an anti-RP2 antibody. D, the WT and knockout mice were examined by western blotting using an anti-RP2 antibody. E, the WT and knockout mice were examined by western blotting using an anti-RP2 antibody.

Figure 2. Scotopic and photopic electroretinography. A, Representative scotopic ERG traces recorded from wild-type and Rh1 knockout mice at one month of age. B, Scotopic a-wave amplitudes from wild-type and Rh1 knockout mice at one month of age. Each data point represents mean ± SD. C, Single-flash scotopic a-wave amplitudes of wild-type and Rh1 knockout mice at one month of age. Each data point represents mean ± SD. D, Single-flash scotopic a-wave amplitudes of wild-type and Rh1 knockout mice at one month of age. Each data point represents mean ± SD. E, Single-flash scotopic a-wave amplitudes of wild-type and Rh1 knockout mice at one month of age. Each data point represents mean ± SD. F, Single-flash scotopic a-wave amplitudes of wild-type and Rh1 knockout mice at one month of age. Each data point represents mean ± SD.

Figure 3. Localization of transmembrane proteins in photoreceptors. A, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-RP2 antibody. B, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-RP2 antibody. C, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-RP2 antibody. D, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-RP2 antibody. E, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-RP2 antibody. F, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-RP2 antibody.

Figure 4. Normal trafficking of rhodopsin in Rh1 knockout mice. A, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-rhodopsin antibody. B, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-rhodopsin antibody. C, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-rhodopsin antibody. D, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-rhodopsin antibody. E, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-rhodopsin antibody. F, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-rhodopsin antibody.

Figure 5. Trafficking defects of peripheral membrane proteins in Rh1 knockout mice. A, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-opsin antibody. B, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-opsin antibody. C, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-opsin antibody. D, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-opsin antibody. E, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-opsin antibody. F, WT and Rh1 knockout mouse retinal sections (age of one month) were stained with anti-opsin antibody.

Summary of Results

1. An RP2 gene mouse line was generated.
2. RP2 is primarily localized to plasma membrane of photoreceptor inner segments.
3. RP2h-GFP mouse displays a cone-rod dystrophy as early as one month of age and the retina.
4. Rhodopsin and GC1 traffics normally to Ret2+/- ROS cones develop, and form short rods.
5. Distribution of rhodopsin and GC1 is normal and return of rhodopsin to ROS during dark-adaptation after light-adaptation is not affected in the RP2 knockout mice.
6. Transport of rod and cone PDE6 as well as GRK1 to outer segments was impeded in the Ret2+/- retina.

Figure 6. Inhibition of PDE6 by an inhibitor of exchanger catalyzed by GMPPNP (11). addition of GMPPNP to wild-type ERG records from wild-type and Rh1 knockout mice at one month of age. Each data point represents mean ± SD. F, Single-flash scotopic a-wave amplitudes of wild-type and Rh1 knockout mice at one month of age. Each data point represents mean ± SD.

Figure 7. CARP trapping in the ER to the destination membrane. A, model of ARL3/GTP-dependent trafficking in WT photoreceptors. B, VAP and GRK1 are expressed in the ER, the CARP (1). CARP is disulfide bond in the presence of ARL3/GDP and GTP. PDE6 is in a closed cytosolic compartment that is less in the presence of CARP (1). CARP is released to begin another round transport (8). GMPPNP/GTP exchange catalyzed by a guanine nucleotide exchange factor (GEF) regulates ARL3/GTP (7).