

SUPPLEMENTAL MATERIAL

Generation of photoreceptor-specific conditional PTP1B knockout mice

To determine the functional role of PTP1B on rod photoreceptors, we disrupted PTP1B gene specifically in mouse photoreceptors using Cre-*loxP* technology (Rajala et al., 2008). For this purpose, mice expressing the floxed PTP1B (Rajala et al., 2010) allele was bred with rod-expressing Cre driven by 0.2-kb mouse rod opsin promoter-controlled Cre (opsin-*cre*) recombinase mouse line (Rajala et al., 2010). We have mated opsin-*cre* to homozygous PTP1B floxed mice (PTP1B^{lox/lox}) and screen the resulting offspring by PCR to identify mice that were heterozygous for both (*cre*^{+/-}/PTP1B^{lox}). We have crossed the *cre*^{+/-}/PTP1B^{lox} mice to homozygous PTP1B^{lox/lox} mice and used PCR to identify mice that are *cre*^{+/-}/PTP1B^{lox/lox}. The resultant mice were heterozygous for opsin-*cre* and homozygous for PTP1B^{lox}. The genotype of the photoreceptor-specific PTP1B knockout mice (ie. animals carrying the *Cre* transgene and homozygous for the PTP1B floxed allele) was confirmed by using PCR analysis of tail DNA. To identify opsin-*Cre*, PCR was done with 1 µl of genomic DNA and the sense primer 5'-AGG TGT AGA GAA GGC ACT TAG C-3', and the antisense primer: 5'-CTA ATC GCC ATC TTC CAG CAG G 3' to amplify a 411-bp product. To identify PTP1B-floxed mice, we used the sense primer 5'-TGC TCA CTC ACC CTG CTA CAA -3', and the antisense primer 5'-GAA ATG GCT CAC TCC TAC TGG-3'. The wild-type allele generates a 206-bp product, and the floxed allele generates 327-bp product. We have characterized this mouse line earlier and the knockout had approximately 2 to 4% of PTP1B protein content compared to that from wild-type (Basavarajappa et al., 2011). All animal work

was in strict accordance with *the NIH Guide for the Care Use of Laboratory Animals* and the Association for Research in Vision and Ophthalmology on the Use of Animals in Vision Research. All protocols were approved by the IACUC at the University of Oklahoma Health Sciences Center and the Dean A. McGee Eye Institute.

Localization of PTP1B in the Mouse Retina

On the day of an experiment, mice were dark-adapted overnight and half were subjected to normal room light (~300 lux) for 30 min. Mice were euthanized by CO₂ asphyxiation and the eyeballs were placed in Prefer solution (Anatech Ltd, Battle Creek, MI) for 15 min at room temperature followed by 70% ethanol overnight. The tissue was paraffin embedded and 5 μm thick sections were cut and mounted onto slides. The slides were then subjected to immunohistochemistry with anti-PTP1B and anti-arrestin antibodies as described (Rajala et al., 2013).

Exposure of animals to light stress

Wild type (PTP1B^{flox/flox}) and PTP1B knockout (PTP1B^{flox/flox} Cre^{+/-}) mice were born and raised in dim cyclic (5 lux) light. Pigmented mice were exposed to constant light for 7 days at an illuminance level of 14,000 lux using white fluorescent light bulbs that are suspended 50 cm above the floor of the cage. During light exposure, animals were maintained in transparent polycarbonate cages with stainless-steel wire bar covers. Drinking water was supplied by a bottle attached to the side of the cage and food was placed on bedding in the bottom of the cage so that there was no obstruction between the light and the animal. Mice were maintained in 100-lux cyclic light for 7 days after light exposure. Morphologic analyses in mice were then completed.

REFERENCES

Basavarajappa, D.K., Gupta, V.K., Dighe, R., Rajala, A., and Rajala, R.V. (2011). Phosphorylated Grb14 Is an Endogenous Inhibitor of Retinal Protein Tyrosine Phosphatase 1B, and Light-Dependent Activation of Src Phosphorylates Grb14. *Mol Cell Biol* 31, 3975-3987.

Rajala, A., Dighe, R., Agbaga, M.P., Anderson, R.E., and Rajala, R.V. (2013). Insulin Receptor Signaling in Cones. *J Biol Chem* 288, 19503-19515.

Rajala, A., Tanito, M., Le, Y.Z., Kahn, C.R., and Rajala, R.V. (2008). Loss of neuroprotective survival signal in mice lacking insulin receptor gene in rod photoreceptor cells. *J Biol Chem* 283, 19781-19792.

Rajala, R.V., Tanito, M., Neel, B.G., and Rajala, A. (2010). Enhanced retinal insulin receptor-activated neuroprotective survival signal in mice lacking the protein-tyrosine phosphatase-1B gene. *J Biol Chem* 285, 8894-8904.