A P347L Rhodopsin Transgenic Rabbit Model of Retinitis Pigmentosa

BW Jones[1], RE Marc[1], JR Anderson[1], CB Watt[1], M. V. Shaw[1], J-H Yang[1], H. Terasaki[2], M. Kondo[2] • Moran Eye Center, University of Utah, Salt Lake City, UT[1] • Ophthalmology, Nagoya University, Graduate School of Medicine, Nagoya, Japan.[2]

NIH EY02576 (RM), EY015128 (RM), EY014800 Vision Core (RM), Research to Prevent Blindness (RM), Research to Prevent Blindness CDA (BWJ)

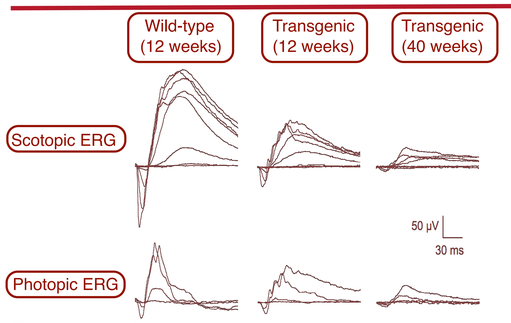
Purpose: The transgenic P347L rabbit model of autosomal dominant retinitis pigmentosa (RP) represents a new and valuable tool in the exploration of RP fulfilling a convenient large eye need. Our goal was to assess the dependence of retinal remodeling on cone survival in the Tg P347L rabbit model of autosomal dominant RP.

Methods: 3 Tg P347L rabbits (3, 4, 10 months) were euthanized via intraperitoneal urethane, eyes enucleated and retinas incubated in vitro for excitation mapping with 1-amino-4-guanidobutane (AGB). Retinas were incubated 10 min at 35 deg C in oxygenated Ames-Hepes medium + 5 mM AGB with/ without iGluR agonists (KA 50 uM, NMDA 1 mM), followed by fixation, embedding in epoxy resins and serial sectioning at 200 nm. Retinal neurons were classified by computational molecular phenotyping and electroretinography was performed to assess the state of rod loss prior to tissue harvest.

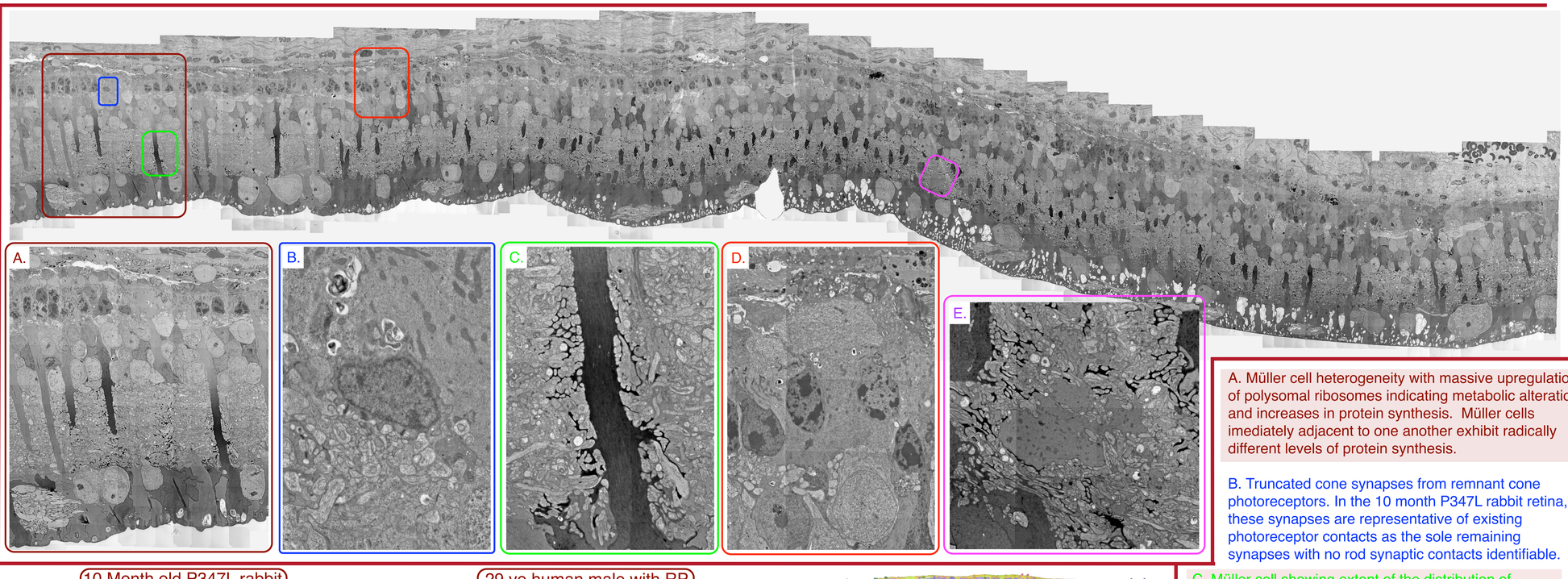
Results: As in human retina, progressive rod-specific degeneration in the Tg P347L rabbit leads to complete loss of rods (and rod signaling) and extensive survival of extremely deconstructed cones that prevent onset of gross remodeling, preserving iGluR-coupled signaling to horizontal and bipolar cells. Also similar to human retina, survivor bipolar cells show evidence of neurite sprouting and potential reprogramming by up-regulation of IGluR expression.

Conclusions: The Tg P347L rabbit model mimics human disease, including cone-mediated preservation of bipolar cell signaling and triggering of reprogramming and thus represents superior model for cell biological, progenitor cell transplantation and bionic prosthetic studies.

Commercial Relationship: BW Jones, None; RE Marc, Signature Immunologics; JR Anderson, None; CB Watt, None; MV Shaw, None; J-H Yang, None; H. Terasaki, None; M. Kondo, None.

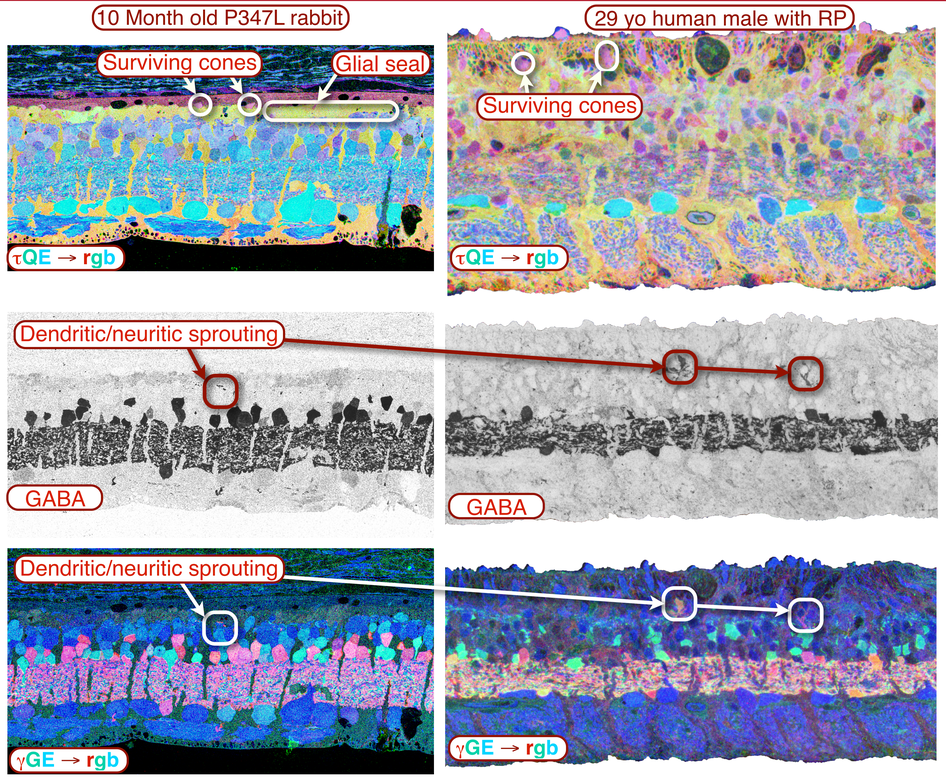


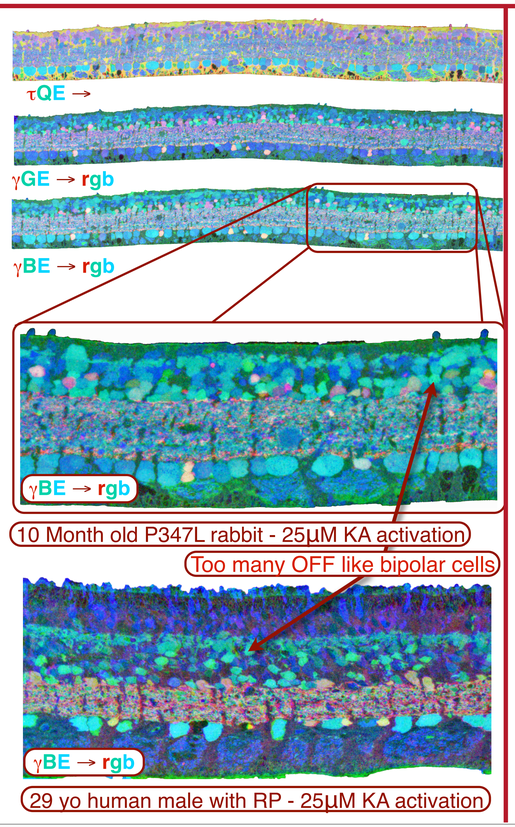
ERGs recorded from 12-weeks-old wild-type and 12- and 40-weeks-old Pro347Leu transgenic rabbits. Upper trace: scotopic ERGs elicited by eight different stimulus intensities ranging from -4.8 to 2.2 log cd s m^{-2}. Lower trace: photopic ERGs elicited by four different stimulus intensities ranging from -0.8 to 2.2 log cd s m^{-2} on a rod-suppressing white background of 1.3 log cd m^{-2}.



A. Müller cell heterogeneity with massive upregulation of polysomal ribosomes indicating metabolic alteration and increases in protein synthesis. Müller cells imediately adjacent to one another exhibit radically different levels of protein synthesis.

B. Truncated cone synapses from remnant cone photoreceptors. In the 10 month P347L rabbit retina, these synapses are representative of existing photoreceptor contacts as the sole remaining synapses with no rod synaptic contacts identifiable.





C. Müller cell showing extent of the distribution of processes and upregulation of polysomal ribosomes within the cytosol. Not shown in this figure is the mass migration of mitochondria in the Müller cells towards the outer portion of the retina towards the external limiting membrane.

D. Müller cell growth cone protruding above the external limiting membrane illustrating the beginning of a glial seal that ultimately will entomb the retina.

E. Likely Müller cell expansion within the inner plexiform layer possibly representing proliferating Müller cells.

Summary:

The P347L rabbit mimics the autosomal dominant focal, cone sparing RP found in human, the pde6b^{rd1} mouse and P347S porcine models. Rods survive up to 3 months and cones survive up to 10 months, but rod bipolar cells lose rod contacts and make ectopic cone contacts, expressing iGluRs, effectively switching phenotype from ON to OFF. By 10 months, no expression of opsin can be found in cones, yet their presence prevents gross retinal remodeling. The P347 rabbit model will allow the continued exploration of axonogenesis, neuritogenesis and retinal remodeling. Additionally, the large eye allows for a more convenient model for cell transplantation, surgical interventions and exploration of bionic prosthetic studies.

To the best of our knowledge, this is the first transgenic rabbit model of retinal degeneration. Like other retinal degenerative diseases, the speed of retinal degeneration is dependent on the expression level of the transgene. Because of the large eye, ease of handling and substantial history in circuitry, anatomy and ophthalmology, the P347L rabbit is a powerful model to study the pathophysiology and treatment of retinal degeneration.