Abstract 2986 A327

Computational Molecular Phenotyping and Excitation Mapping in the P347L Rhodopsin Transgenic Rabbit Model of Retinitis Pigmentosa

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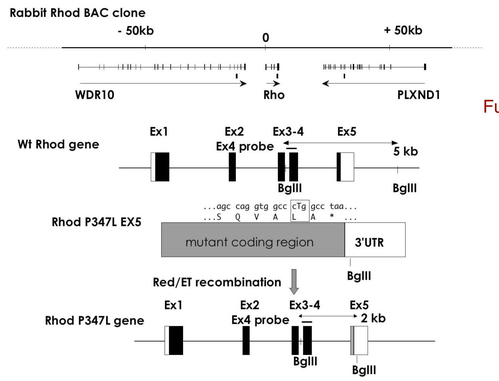
Purpose: Practical large animal models of retinitis pigmentosa (RP) are rare. It is difficult to predict the progression of cone-sparing forms of RP from rodent studies because the cones are so small and usually die along with rods. In human RP however, transformed cones often outlive rods and partially prevent retinal remodeling (Marc et al. 2007 IOVS 48: 3364-3371). Our goal was to assess the dependence of retinal remodeling on cone survival in the transgenic rhodopsin P347L (Tg P347L) rabbit model of autosomal dominant RP.

Methods: Three adult Tg P347L rabbits (ages 3, 4, 10 months) were euthanized via intraperitoneal urethane, the eyes enucleated and retinas incubated in vitro for excitation mapping with 1-amino-4-guanidobutane (AGB). Ocular fragments were incubated 10 min at 35 deg C in oxygenated Ames-Hepes medium + 5 mM AGB with and without iGluR agonists (KA 25 uM, NMDA 1 mM), followed by conventional fixation in buffered aldehydes, embedding in exopy resins and serial sectioning at 200 nm (Marc RE 1999 JCN 407:47-64). Retinal neurons were classified by computational molecular phenotyping (CMP, Marc and Jones 2002 J Neurosci22:413-427) using an array of small molecule signatures (asparate, glutamate, glycine, glutamine, glutathione, GABA, taurine) with the addition of tyrosine hydroxylase, rhodopsin, LWS1 cone opsin, and CRALBP macromolecule signals. Electroretinography was also performed to assess the state of rod loss prior to tissue harvest.

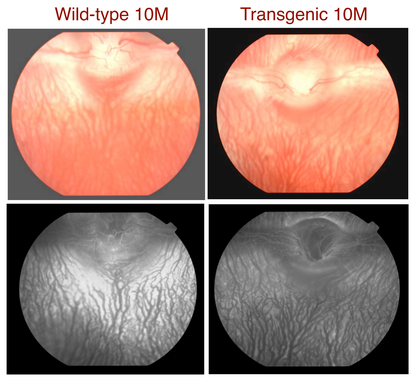
Results: As in human retina, progressive rod-specific degeneration in the Tg P347L rabbit leads, by 10 months, to complete loss of rods (and rod signaling) and extensive survival of extremely deconstructed cones. Importantly, even deconstructed survivor cones clearly prevent the onset of gross remodeling and, importantly, preserves iGluR-coupled signaling to horizontal and bipolar cells. But also similar to human retina, survivor bipolar cells show evidence of neurite sprouting and potential reprogramming by up-regulation of IGluR expression.

Conclusions: Disease progression in the Tg P347L rabbit model closely tracks human disease, including the cone-mediated preservation of bipolar cell signaling and triggering of reprogramming. The relatively fast disease progression makes the Tg P347L rabbit a superior model for cell biological, progenitor cell transplantation and bionic prosthetic studies.

Commercial Relationship: BW Jones, None; RE Marc, Signature Immunologics; H. Terasaki, None; M. Kondo, None.

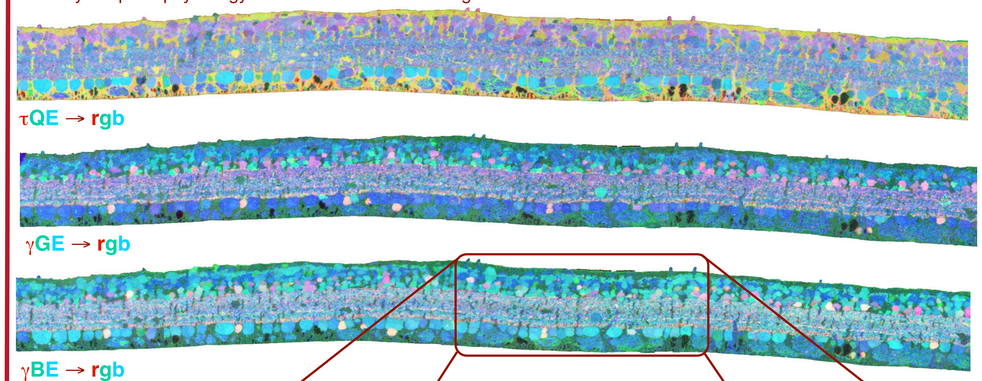


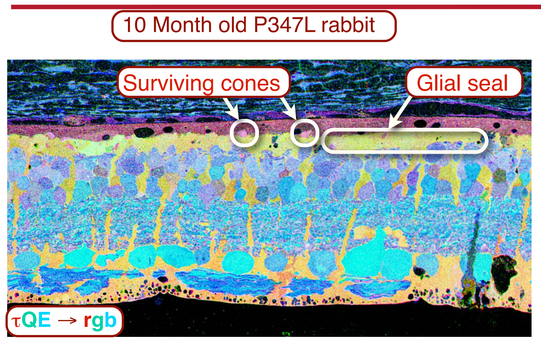
These rabbits were crafted using a BAC construct containing the Pro347Leu mutation of the rhodopsin gene introduced into fertilized rabbit eggs. For specifics, see program # 2200/ Poster # A548, M. Kondo et. al.

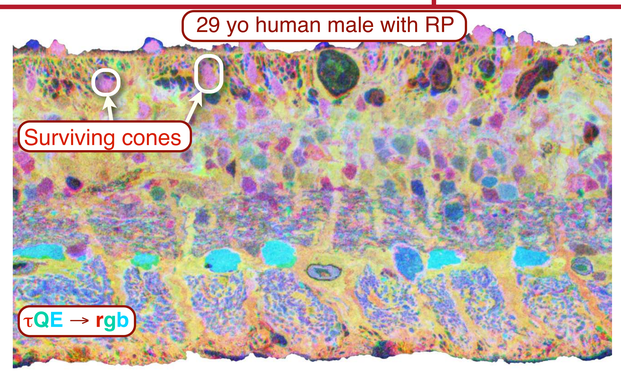


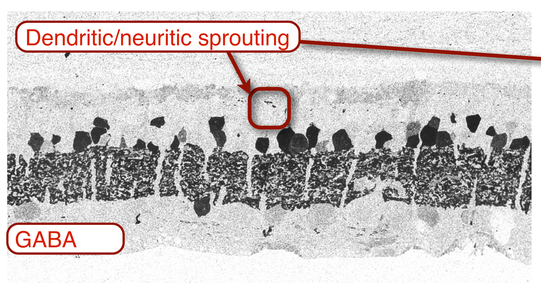
Though no changes are evident by fundoscopy in the albino P347L rabbit, clinically occult changes can be identified histologically through Computational Molecular Phenotyping, CMP.

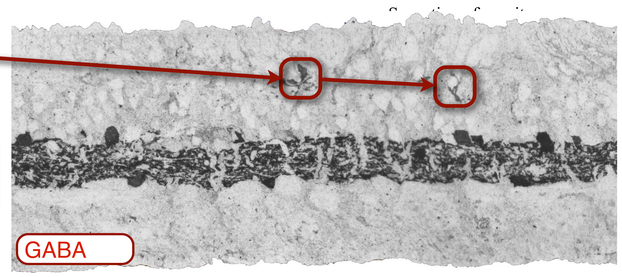
To the best of our knowledge, this is the first transgenic rabbit model of retinal degeneration. Like other retinal degenerative diseases, the speed of retinal degeneration is dependent on the expression level of the transgene. Because of the large eye, ease of handling and substantial history in circuitry,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

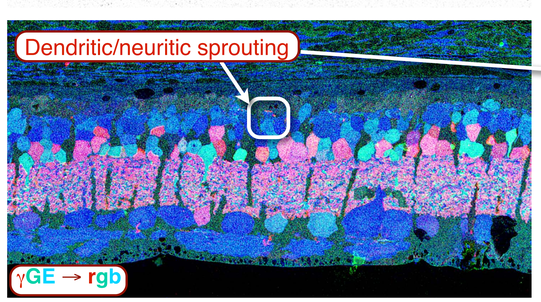


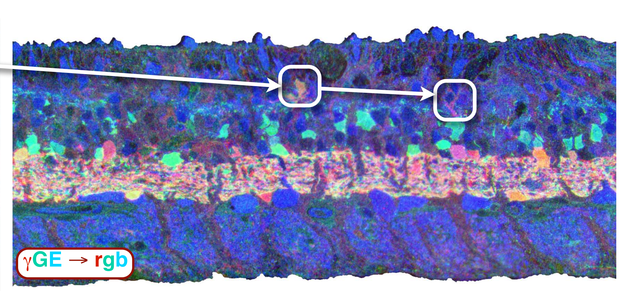


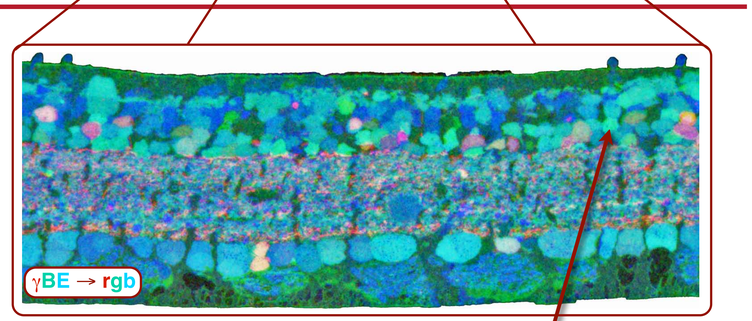






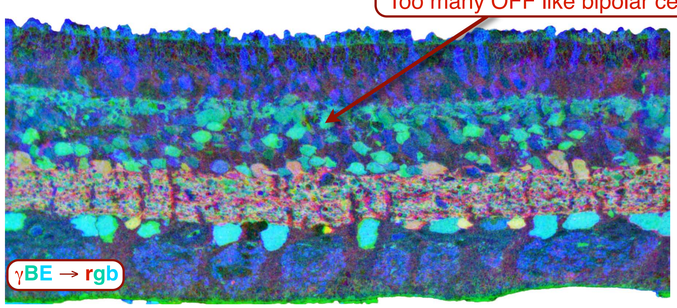






10 Month old P347L rabbit - 25µM KA activation

Too many OFF like bipolar cells



29 yo human male with RP - 25µM KA activation

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, effectivly 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.