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Phenotypic characterization of P23H and S334ter rhodopsin transgenic rat models of inherited retinal degeneration

Matthew M. LaVail[a,*], Shimpei Nishikawa[a,2], Roy H. Steinberg[a,1], Muna I. Naash[b], Jacque L. Duncan[a], Nikolaus Trautmann[a,3], Michael T. Matthes[a], Douglas Yasumura[a,1], Cathy Lau-Villacorta[a], Jeannie Chen[c], Ward M. Peterson[a], Haidong Yang[a,4], John G. Flannery[d]

[a] Beckman Vision Center, University of California, San Francisco, San Francisco, CA 94143-0730, USA
[b] Department of Biomedical Engineering, University of Houston, 3517 Cullen Blvd., Room 2011, Houston, TX 77204-5060, USA
[c] Zilka Neurogenetic Institute, USC Keck School of Medicine, Los Angeles, CA 90089-2821, USA
[d] School of Optometry, UC Berkeley, Berkeley, CA 94720-2020, USA

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ABSTRACT

We produced 8 lines of transgenic (Tg) rats expressing one of two different rhodopsin mutations in albino Sprague-Dawley (SD) rats. Three lines were generated with a proline to histidine substitution at codon 23 (P23H), the most common autosomal dominant form of retinitis pigmentosa in the United States. Five lines were generated with a termination codon at position 334 (S334ter), resulting in a C-terminal truncated opsin protein lacking the last 15 amino acid residues and containing all of the phosphorylation sites involved in rhodopsin deactivation, as well as the terminal QVAPA residues important for rhodopsin deactivation and trafficking. The rates of photoreceptor (PR) degeneration in these models vary in proportion to the ratio of mutant to wild-type rhodopsin. The models have been widely studied, but many aspects of their phenotypes have not been described. Here we present a comprehensive study of the 8 Tg lines, including the time course of PR degeneration from the onset to one year of age, retinal structure by light and electron microscopy (EM), hemispheric asymmetry and gradients of rod and cone degeneration, rhodopsin content, gene dosage effect, rapid activation and invasion of the outer retina by presumptive microglia, rod outer segment disc shedding and phagocytosis by the retinal pigmented epithelium (RPE), and retinal function by the electroretinogram (ERG). The biphasic nature of PR cell death was noted, as was the lack of an injury-induced protective response in the rat models. EM analysis revealed the accumulation of submicron vesicular structures in the interphotoreceptor space during the peak period of PR outer segment degeneration in the S334ter lines. This is likely due to the elimination of the trafficking consensus domain as seen before as with other rhodopsin mutants lacking the C-terminal QVAPA. The 8 rhodopsin Tg lines have been, and will continue to be, extremely useful models for the experimental study of inherited retinal degenerations.

The clinical problem of inherited and age-related retinal degeneration (RD) is enormous. It is estimated that different forms of hereditary retinitis pigmentosa affect as many as 1:3500 worldwide, and the incidence of the much more prevalent age-related macular degeneration rises with age, affecting as many as 1:4 by the age of 75. The loss of vision in both classes of diseases is due to the death of photoreceptor (PR) cells, but the retinal pigment epithelium (RPE) is involved or is the site of mutant gene expression in some forms of each type of disease. Retinitis pigmentosa has currently been associated with 67 different genetic loci, and 60 genes have been identified (Daiger, 2017). More than 150 different mutations have been identified in the rhodopsin gene alone (Farrar et al., 2012). Although major progress has been made in elucidating the genetic and molecular nature of many human forms of retinitis pigmentosa (see below), the key issue of how these gene defects

* Corresponding author. Beckman Vision Center, UCSF School of Medicine, San Francisco, CA 94143-0730, USA.
E-mail addresses: mmlv@sonic.net (M.M. LaVail), shimpeinishikawa@yahoo.co.jp (S. Nishikawa), mnaash@central.uh.edu (M.I. Naash), jacque.duncan@ucsf.edu (J.L. Duncan), nick.trautmann@web.de (N. Trautmann), michael.matthes@sbcglobal.net (M.T. Matthes), clv95@yahoo.com (C. Lau-Villacorta), jeannie@usc.edu (J. Chen), wardmpeterson@gmail.com (W.M. Peterson), yang.harvey@gmail.com (H. Yang), flannery@berkeley.edu (J.G. Flannery).
[1] Deceased.
[2] Current address.1276-20 Kamiyasumatu, Tokorozawa Saitama 359-0025 JAPAN.
[3] Current address.Grosse Gaenseweide 10, 21423 Winsen/Luhe Germany.
[4] Current address.FAAO Eye Care Hawaii, 34 West Kawailani St., Hilo HI 96720 USA.

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lead to PR cell death is largely unanswered.

Many vertebrates and invertebrates have gene defects that lead to RD. Vision scientists have taken advantage of RD mutations in laboratory animals, which have played a prominent role as experimental models in vision research in the past several decades and from which much has been learned about the cellular mechanisms of PR degeneration (Baehr and Frederick, 2009; Chader, 2002; Colley et al., 1995; Stuck et al., 2016; Sung and Tai, 2000; Tanna et al., 2017; Veleri et al., 2015). Among the various species with RDs, mice and rats have been used most extensively, primarily because of the experimental advantages of small animal size with relatively low costs, short gestation time, powerful genetic control in the form of several readily available RD mutants, multiple inbred and congenic strains with genetic controls and different eye pigmentation types, and the potential to carry out certain embryological genetic procedures such as the production of experimental chimeras, transgenic (Tg) and gene knock-in animals (Baehr and Frederick, 2009; Flannery, 1999; Sakami et al., 2011; Veleri et al., 2015).

Among rodents, the number of mouse mutants that may serve as relevant animal models for human diseases is remarkable (Baehr and Frederick, 2009; Chang et al., 2002; Chen et al., 1999; Hafezi et al., 2000; Veleri et al., 2015). Some naturally occurring mouse mutants have human counterparts with orthologous gene defects, such as the *rd1*, *rd2* and *Mertk* mutants. In addition, many Tg mouse mutants carry constructs that lead to overexpression or disruption of candidate genes for RDs (Chader, 2002; Fauser et al., 2002; Hafezi et al., 2000), as well as knock-in rhodopsin models (Price et al., 2011; Sakami et al., 2011).

The goal of RD research is ultimately to develop therapeutic means to prevent or slow the rate of RD. At present, no generally accepted treatment exists for most of the RDs. However, in the past 2-3 decades, many areas of experimental therapy have arisen and continue to expand significantly to prevent PR degeneration or restore visual function. These include: 1) neuroprotective therapy with direct application of various survival-promoting factors (Abed et al., 2015; Faktorovich et al., 1990; LaVail et al., 1992; Wen et al., 2012), 2) gene-based therapy of recessively and dominantly inherited RDs, as well as viral vector delivery vehicles (Acland et al., 2001; Bennett et al., 1996; Dalkara et al., 2016; Dalkara and Sahel, 2014; Farrar et al., 2012; Laemmli, 1970; Lau et al., 2000; Lewin et al., 1998; Thompson et al., 2015; Trapani et al., 2015; Yang et al., 2015), 3) nanoparticles that act as antioxidants and biodegradable microspheres as non-viral delivery vectors for drug, gene and trophic factor delivery (Adijanto and Naash, 2015; Fernandez-Sanchez et al., 2017; Trapani et al., 2014; Wong et al., 2015; Zarbin et al., 2013; Zulliger et al., 2015), 4) transplantation and cell-based therapy with the use of retinal, RPE and stem cells (Aramant and Seiler, 2002; Li and Turner, 1988; Seiler et al., 2017; Thompson et al., 2015; Yang et al., 2015; Zarbin, 2016), 5) the development of visual prostheses using silicon chip technology (da Cruz et al., 2016; Duncan et al., 2017; Marc et al., 2014; Stingl and Zrenner, 2013), and 6) the field of optogenetics (Dalkara and Sahel, 2014; Duebel et al., 2015; Marc et al., 2014; Zarbin et al., 2013). The need for animal models has increased concomitantly with this research.

Although some therapeutic studies can take advantage of the mouse as an animal model, the small size of the eye is severely limiting for some approaches, particularly when surgical procedures are required. The problem is exacerbated by the early onset of many rodent RDs, thus requiring the use of a particularly small, young mouse eye. Indeed, even the relatively simple delivery of neurotrophic factors by intravitreal injections can be inconsistent or ineffective with very small mouse eyes (LaVail et al., 1998). By contrast, the rat eye is 6–12 times the volume of the mouse eye, depending upon age (LaVail et al., 1998), so the larger eye size of a rat is highly desirable or necessary for many types of therapeutic RD research. The RCS rat is a widely studied model of RD (LaVail, 2001; Strauss et al., 1998), but for decades it had been the only rat model with an inherited RD. Although the RCS rat has an orthologous human gene defect (Gal et al., 2000), and it is particularly interesting because of its mutant gene expression in the RPE (Mullen and LaVail, 1976; Vollrath et al., 2001), there had been no rat model with an RD gene defect intrinsic to the PR cell.

With the goal of creating rat models with gene defects expressed in rod PR cells, like some human forms of retinitis pigmentosa, and to expand the number of rat models available for RD therapeutic research with different rates of RD, we developed a number of Tg rat lines with mutant opsin genes identified in (or similar to) autosomal dominant retinitis pigmentosa (adRP) in human patients (Steinberg et al., 1996). We chose to produce transgenic rats that carry one of two different rhodopsin mutations. The first, P23H, had a proline to histidine substitution at codon 23. This was the first and most common rhodopsin mutation identified in patients with adRP (Dryja et al., 1990), accounting for approximately 12% of adRP cases in the United States (Berson et al., 1991; Sung et al., 1991). We produced 3 lines of P23H rats with different levels of transgene expression and different rates of RD. Second, we produced 5 lines of rats with a mouse opsin transgene bearing a termination codon at residue S334 (S334ter), resulting in a C-terminal truncated opsin protein lacking the last 15 residues. The S334ter opsin lacks all of the phosphorylation sites (Chen et al., 1995) and the QVAPA residues needed for rhodopsin trafficking (Concepcion and Chen, 2010; Sung et al., 1994). We chose these different genetic constructs because they represent two of the different classes of rhodopsin mutations with respect to molecular conformation, cellular processing and localization, and probable pathogenic function in PRs (reviewed by Sung and Tai, 2000), and they generally have different degrees of clinical expression in human adRP patients (Sandberg et al., 1995). In general, the P23H mutation, as a member of several other mutations located in or near the N-terminus of rhodopsin, is considered to result in RD due to defective rhodopsin folding in the endoplasmic reticulum (ER), aggregation and retention within the ER, with resulting ER stress and activation of the unfolded protein response (UPR) (Chiang et al., 2012; Illing et al., 2002; Lin et al., 2007). The RD resulting from truncation of the rhodopsin C-terminus, such as in the S334ter lines, is considered to result in RD due to defective rhodopsin trafficking to the ROSs and mis-localization of the protein (Green et al., 2000; Sung et al., 1994). This truncated protein with an absence of phosphorylation sites also fails to deactivate the rhodopsin molecule and has prolonged responses to light absorption (Chen et al., 1995). Having both type of mutations on the same Sprague-Dawley (SD) background allows for controlled comparison of the different pathological processes.

These Tg rat models were produced in 1996 (Steinberg et al., 1996) and since have been made available to be used by various studies by numerous vision scientists and have used them for various studies (see Discussion). Although some aspects of the RDs in these animals have been described in these studies, only 4 of the 8 lines have been described to any extent; most of these have only been described for a short period of the degeneration; and only a few phenotypic features have been presented or assayed for each line. In the current study, we present relatively comprehensive data on many structural and functional phenotypic characteristics of each of these lines up to at least one year of age. These data should allow vision scientists to use the Tg rats effectively to gain insight into mechanisms of RD caused by rhodopsin mutations and to have rat models with different mechanisms and rates of degeneration with which to carry out experimental therapeutic studies that may be applicable to many different forms of RD. In addition, we also present ultrastructural data that confirms the presumed cytopathological process in the S334ter lines that results from defective rhodopsin transport does not occur in the P23H lines. Moreover, we demonstrate that injury-induced endogenous neuroprotection that occurs in some RDs, and which can interfere with results from utilizing intraocular injections, do not occur in the rhodopsin Tg lines, at least not at ages in which PR degeneration occurs.