

Editorial

Phototransduction in a Rabbit Model with Retinitis Pigmentosa

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Introduction

Retinitis pigmentosa (RP) is a progressive disease that exhibits abnormalities in the photoreceptor cells of the rods and cones that detect light, and is characterized by symptoms such as night blindness, visual field constriction especially in the peripheral field, and reduced visual acuity [1]. In Japan, this disorder is estimated to affect approximately 20 in 100,000 people and is the third leading cause of blindness following glaucoma and diabetic retinopathy [2]. RP appears through a variety of genetic inheritance modes, including autosomal dominant, autosomal recessive, and X-linked recessive patterns, and 40 or more causative genes have been identified [3]. In many patients, however, genetic abnormalities cause this disorder to be under diagnosed.

To overcome these difficulties, transgenic (Tg) rabbits with a P347L rhodopsin mutation were created by Kondo et al. [4]. They displayed symptoms extremely similar to findings in patients with RP and are considered to offer a useful animal model for elucidating the pathology of RP [5]. However, when and where the degeneration begins are unclear, as are its underlying mechanisms and pathologies. There are many other unknown issues as well, such as how light is received and converted to electric signals in the steps leading to blindness, and why organisms with undeveloped vision such as rabbits receive light, and where photoreceptors are located in these animals. In humans, light is received by photoreceptor cells and is converted to electric signals to visualize an object.

In clinical settings, we encounter patients with RP who maintain circadian rhythms in which the body wakes in the morning and becomes sleepy at night, and those who present a residual light response in which the pupil becomes smaller on exposure to light. This is unlike patients who have lost their vision due to other eye diseases. In other words, RP patients show partial response to light even when blind and unable to sense light. Based on these facts, we hypothesized the existence of photoreceptors other than the rods and cones of the photoreceptor cells. In this review based on our previous studies [6,7], we report the mechanism of retinal degeneration and describe the process of phototransduction in Tg rabbits with a P347L rhodopsin mutation.

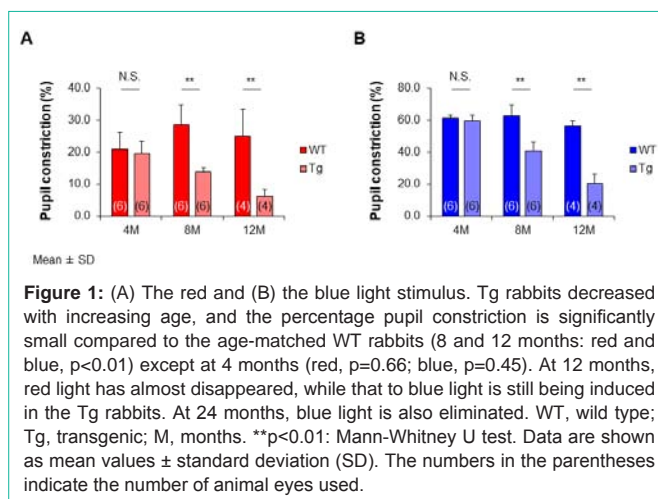


Figure 1: (A) The red and (B) the blue light stimulus. Tg rabbits decreased with increasing age, and the percentage pupil constriction is significantly small compared to the age-matched WT rabbits (8 and 12 months: red and blue, $p < 0.01$) except at 4 months (red, $p = 0.66$; blue, $p = 0.45$). At 12 months, red light has almost disappeared, while that to blue light is still being induced in the Tg rabbits. At 24 months, blue light is also eliminated. WT, wild type; Tg, transgenic; M, months. ** $p < 0.01$: Mann-Whitney U test. Data are shown as mean values \pm standard deviation (SD). The numbers in the parentheses indicate the number of animal eyes used.

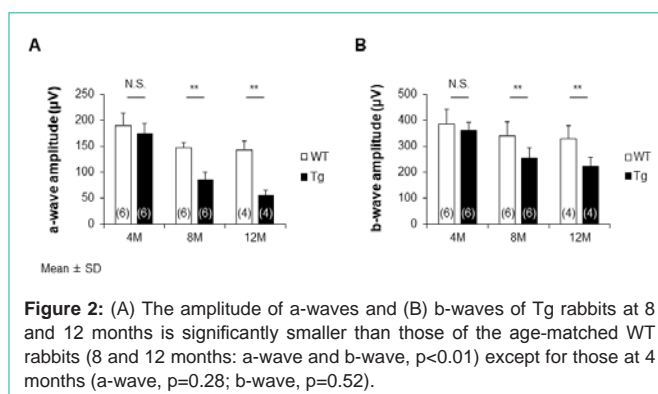
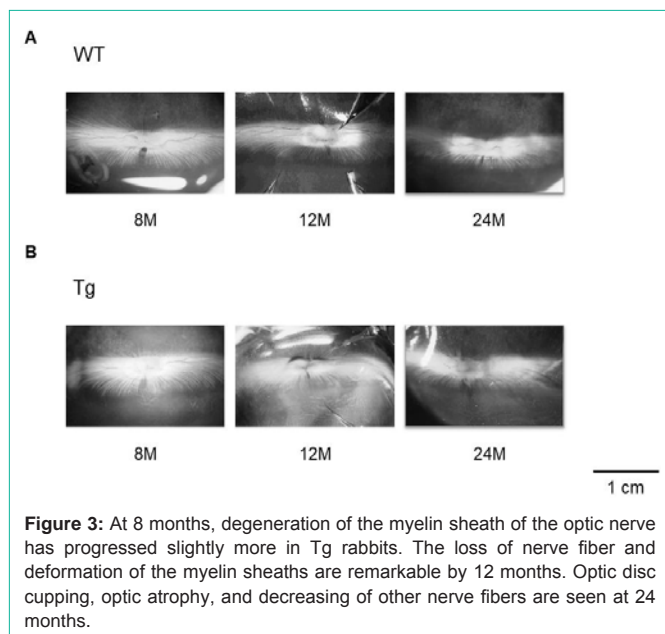


Figure 2: (A) The amplitude of a-waves and (B) b-waves of Tg rabbits at 8 and 12 months is significantly smaller than those of the age-matched WT rabbits (8 and 12 months: a-wave and b-wave, $p < 0.01$) except for those at 4 months (a-wave, $p = 0.28$; b-wave, $p = 0.52$).

Pupil Response

The photoreceptor cells control the constriction and dilation of the pupil in response to change in light stimulus, namely the pupil response. Figure 1 shows (A) the percentage pupil constriction results to red light (635nm) and (B) blue light (470nm) with intensity of 2.3 log cd/m² for 60s in the Wild-type (WT) and Tg rabbits. The mean values at 4 months was 20.8% for red light and 61.1% for blue light in the WT rabbits and 19.6% for red light and 59.4% for blue light in the Tg rabbits. The differences between the two types were not significant (red, $p = 0.66$; blue, $p = 0.45$, Mann-Whitney U test). At 8 months, the red light was slightly reduced in the Tg rabbits (13.8%). This value was significantly lower than that of the WT rabbits (28.5%; $p < 0.01$). The values for the WT rabbits at 12 months were 25.0% for red light and 56.2% for blue light, whereas the Tg rabbits indicated a loss of response to red light (6.1%); however, pupil constriction to blue light was still induced (20.3%). The values for the Tg rabbits were also significantly smaller than those of the WT rabbits (red and blue, $p < 0.01$). At 24 months, pupil constriction even in blue light stimulus



was eliminated (7.9%).

Electroretinogram

The electroretinogram (ERG) is a valuable tool for understanding retinal function (a-wave: photoreceptor cells component, b-wave: bipolar cells component) in both human patients and animal models of RP, even in early stage. Figure 2 shows the ERG with a full visual field stimulation at 2.3 log cd s/m² of emission intensity results of the (A) a-wave and (B) b-wave for the WT and Tg rabbits. At 4 months, there was no significant difference between the Tg and WT rabbits (a-wave, $p=0.28$; b-wave, $p=0.52$). The mean amplitude of the a-waves in the WT rabbits at 4 months was 190 μV and of the b-waves, 385 μV . These values of WT rabbits at 8 months were 147 μV and 339 μV . In 12 months, a-waves were 142 μV and b-waves were 328 μV . Meanwhile, in the Tg rabbits, the a-wave decreased from 174 μV at 4 months to 86 μV at 8 months, and the b-waves decreased from 360 μV to 255 μV . At 12 months, the ERG response had considerably decreased for both the a-wave (56 μV) and b-wave (221 μV). The a-wave and b-wave were significantly lower in the Tg rabbits than in the WT rabbits (8 and 12 months: a-wave and b-wave, $p<0.01$).

Dissecting Microscopy

The WT fundus has an opaque myelinated portion that extends horizontally from the optic nerve head (Figure 3A). The optic nerve of Tg rabbits at 8 months was characterized by a slight degeneration of the myelin sheath, and the degeneration was characterized by the loss of nerve fiber. Deformation of the myelin sheath became remarkable by 12 months. Optic disc cupping and optic atrophy were observed at 24 months (Figure 3B).

Light Microscopy

At 8 months, the overall photoreceptor structure appeared almost normal. At 12 months, the whole retina thickness became thinner because of loss of the outer nuclear layer (ONL) and the photoreceptor layer in the peripheral portions, and the rods and cones disappeared.

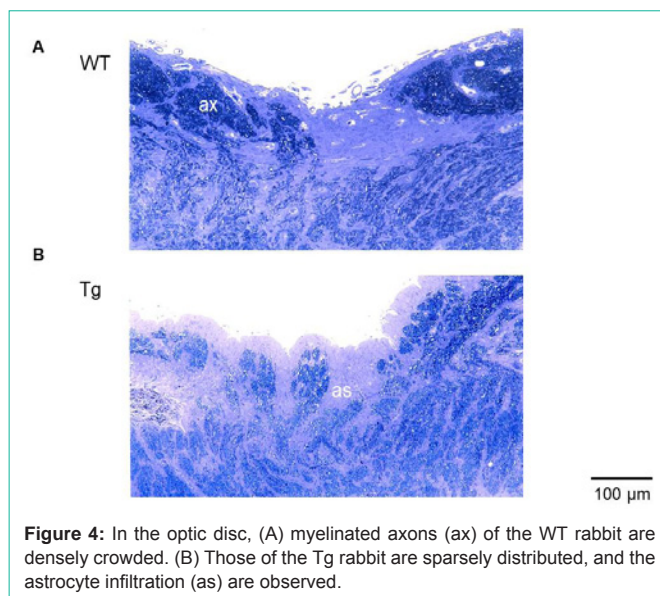


Figure 4A shows the WT rabbits' optic disc at 24 months. In the Tg rabbits' optic disc, myelinated axons were sparsely distributed, and growth of astrocytes, i.e., astrocyte infiltration was observed (Figure 4B).

Electron Microscopy

Comparing the WT rabbits at 24 months (Figure 5A) with the Tg rabbits (Figure 5B), the number of nuclei from the ONL had decreased at 8 months. Simultaneously, the cell pyknosis in the nucleus of the ONL was observed in the visual streak. At 12 months, the retinal cells of the inner nuclear layer were observed to make direct contact with the portion of the retinal pigment epithelium in the peripheral area. The rods had disappeared, and the remaining cones exhibited ballooning degeneration in the central area (Figure 5C). At 24 months, in the periphery of the retina, there is a vacuolar degeneration of retinal ganglion cells (RGCs), and hypertrophy and proliferation of Müller cells (Figure 5D). Degeneration of myelinated axons in the optic disc and damage of the myelin sheath of small axons in the optic nerve were observed (Figure 5E).

Immunofluorescent Staining with Specific Antibody

Alexa Fluor[®] labeled neuronal class III beta-tubulin (TUJ-1; Molecular Probes, Eugene, OR, USA) 1:500 monoclonal antibodies were used for immunofluorescent staining of the RGCs. Even at 12 months, subtype of the RGCs showed both large and small cells in the WT rabbits (Figure 6A). In the Tg rabbits, the remaining large cells, which have an extensively branching dendritic structure with long dendrites, were more clearly observed, whereas the number of small cells has decreased (Figure 6B).

Discussion

Fundamental treatment methods for RP are currently lacking. To delay progression, patients are sometimes given helenien, vitamin A, and medications that improve circulation, but the effects of these oral therapeutics have yet to be confirmed [8]. In some countries, clinical

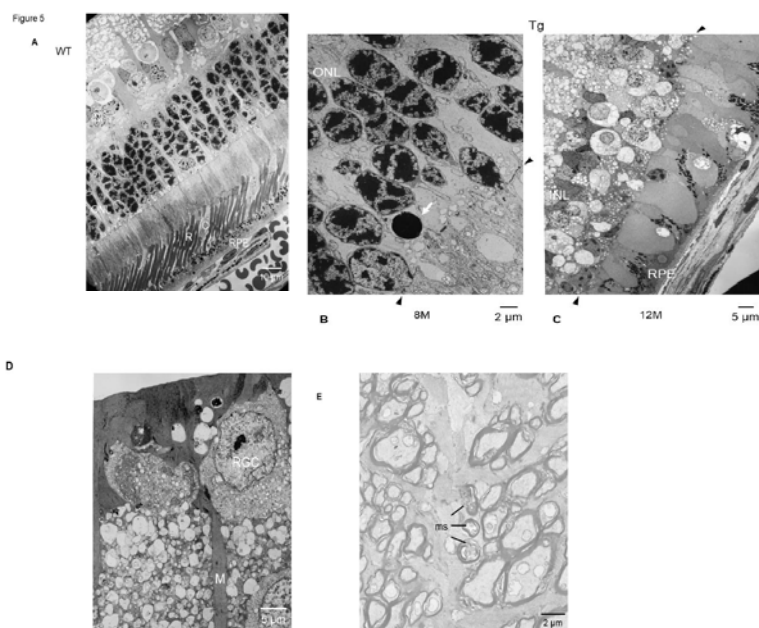


Figure 5: (A) Comparison of the WT rabbits at 24 months, with Tg rabbits; (B) cell pyknosis (white arrow) in the nucleus of the outer nuclear layer (ONL) is observed in the central area of the visual streak at 8 months. Rods (R) have disappeared, and the remaining cones (C) exhibit ballooning degeneration. (C) The retinal cells of the inner nuclear layer (INL) are in direct contact with the retinal pigment epithelium (RPE) at 12 months. Two black arrows show the location of the outer limiting membrane. (D) Vacuolar degeneration of retinal ganglion cell (RGC) and hypertrophic proliferation of Müller cells (M) are observed. (E) Degeneration of myelinated axons in the optic disc. Damage of the ring-like structure of the myelin sheath (ms) of small axons in the optic nerve.

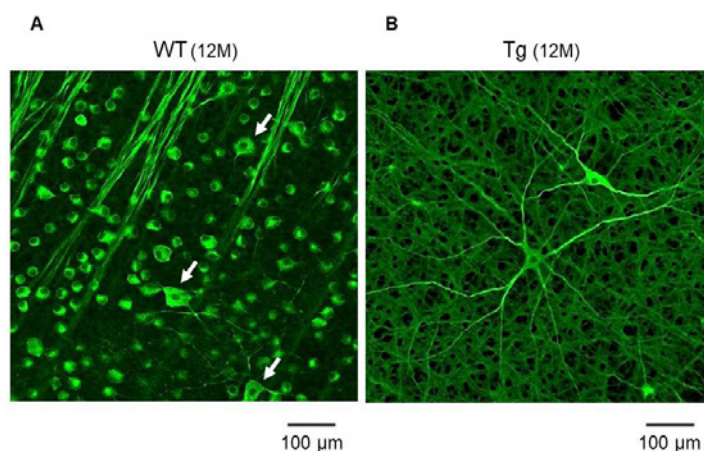


Figure 6: (A) TUJ-1 immunofluorescent staining of the RGCs shows large (white arrows) and small cells in the WT rabbits at 12 months. In the Tg rabbits, the number of small cells is decreased compared to the WT rabbits. (B) A few remaining RGCs have large cell bodies, which have an extensively branching dendritic structure with long dendrites, are more clearly observed even in Tg rabbits at 12 months.

trials of artificial retina with embedded electrodes are beginning, but concerns remain that transplantation surgery is highly invasive and expensive [9]. While elucidation of the mechanisms of degeneration and pathology of this disorder will not immediately lead to improved diagnoses and treatment modalities, we anticipate that these results will eventually facilitate prevention and the further development of therapies. Moreover, if this study can elucidate the mechanisms of degeneration and the process of phototransduction in a new photoreceptor other than photoreceptor cells, then the results may be applicable to the provision of alternative vision not only for patients with RP but also for patients who have lost their vision due

to impairment of photoreceptor cells, such as age-related macular degeneration.

In the present review, the pupil response and the ERG components in the Tg rabbits decreased with increasing age, and differences were found compared to the age-matched WT rabbits. At 12 months of age, pupil constriction to red light had disappeared; however, it was still induced during exposure to blue light.

The pupil response has two main pathways: an afferent and an efferent limb. The afferent pathway leaves the optic tract before the lateral geniculate body and terminates in the midbrain. Therefore,

retinal photoreceptor cells as the origin of the pupil response were regarded as the rods and cones for more than 100 years. Why then do rabbits with damage to retinal photoreceptor cells maintain the pupil response to blue light?

Provencio et al. isolated melanopsin, an intrinsically photosensitive visual pigment, from dermal melanophores in *Xenopus laevis* and reported that melanopsin was present in RGCs of inner retina [10]. These RGCs are known as melanopsin-containing RGCs (mRGCs). mRGCs account for only about 1% of the total number of RGCs, but have large cell bodies and an extensively branching dendritic structure with long dendrites [11]. Berson et al. found that mRGCs have projections to the suprachiasmatic nucleus as master clock of the circadian rhythm [12]. They also project to the olivary pretectal nucleus and Edinger-Westphal nucleus in the midbrain and play a role in the pupil response [13,14]. These photoreceptions by mRGCs have a selective sensitivity to blue light with a wavelength of 460–480 nm [15–17].

Morphologically, light and electron microscopy showed a progressive loss of rods over time from 8 months of age, especially in the peripheral retina. Additionally, pyknotic nuclei of the ONL were observed at 8 months. Our images of pyknotic nuclei in the ONL, which most likely represent dying apoptotic photoreceptors, do not adequately explain the underlying mechanism of the disease process that results from the mutant protein expressed in rod photoreceptor cells. Other mechanisms of photoreceptor cell death may be involved, including rhodopsin overproduction, prolonged phototransduction events or mislocalized opsin [18–20]. In the future, these evaluations should be included to confirm this pathogenic event. However, the cell pyknosis suggests that it is an early change associated with retinal photoreceptor cell degeneration in Tg rabbits.

In the late stages of RP, the small type of RGCs and nerve fibers were particularly damaged and hypertrophic proliferation of Müller cells was observed. They subsequently lead to optic disc excavation and atrophy of the optic nerve. The reason was that the bipolar cells and RGCs lost association with the loss of rods and cones because of the degeneration of cells in the periphery of the retina, and those degenerated cells underwent phagocytosis due to Müller cells being subject to hypertrophic growth [21]. We also observed the disruption of the myelin sheath of small axons that occurred with the degeneration of axons and atrophy of the optic nerve. Optic disc cupping could closely be associated with optic atrophy [22]. Therefore, these morphological findings indicated that RP is caused by irreversible degeneration of neural cells. Of note, some remaining RGCs had large cell bodies with long branching dendritic structures as that with the mRGCs, even in a rabbit. Consequently, our findings indicated that the phototransduction of Tg rabbits after complete retinal photoreceptor loss is mediated by mRGCs.

In conclusion, the existence of these residual mRGCs despite advanced degeneration of rods and cones, indicate the fact that mRGCs are involved in synchronization of the circadian rhythm and are primitive photoreceptors in the sleep-wake cycle.

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