Commentary

Retinitis pigmentosa: Unfolding its mystery

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Retinitis pigmentosa affects 50,000–100,000 people in the United States and about 1.5 million people worldwide. Patients usually report impaired adaptation, night blindness, and loss of mid-peripheral visual field in adolescence. As the condition progresses, they lose far-peripheral visual field and eventually lose central vision as well. Some patients have become blind as early as age 30. The majority are legally blind by age 60, with a central visual field diameter of less than 20°. Findings on ophthalmoscopy include intraretinal pigment around the mid-peripheral retina, for which this condition is named. Histopathologic examinations of autopsy eyes with advanced stages have shown that loss of vision is due to degeneration of both rod and cone photoreceptor cells (1, 2).

Retinitis pigmentosa can be detected in early life by electroretinographic testing. Patients with early stages of this disease have electroretinograms (ERGs) that are reduced in amplitude with delays in their temporal aspects (Fig. 1). ERG amplitudes become smaller as the disease progresses. Abnormal ERGs have been detected in asymptomatic children in some cases a decade before diagnostic changes are seen on routine ocular examination. Individuals, age 6 and older, with normal ERGs and a family history of retinitis pigmentosa have not been observed to develop retinitis pigmentosa at a later time (2, 3).

The common forms of retinitis pigmentosa have yielded to treatment with vitamin A supplementation. In a randomized, controlled trial, the course of retinal degeneration as monitored by the ERG was slower on average among adult patients taking 15,000 international units of vitamin A daily, whereas the course appeared to be hastened by supplementation with 400 international units daily of vitamin E. The mechanism by which vitamin A supplementation slows the progression of retinitis pigmentosa is not known. Vitamin E may have an adverse effect on this condition by reducing the amount of vitamin A reaching the eye, as serum vitamin A levels were observed to be significantly lower in patients taking vitamin E (4).

Retinitis pigmentosa can be inherited by an autosomal dominant, autosomal recessive, X-linked, or digenic mode (5). Substantial genetic heterogeneity has been observed in this condition, with over 20 chromosomal loci mapped (6, 7). Mutations have been identified in seven genes (5, 8–15). Four of these genes encode proteins in the rod phototransduction cascade—namely rhodopsin, the α and β subunits of rod cGMP phosphodiesterase, and the rod cGMP cation-gated channel protein α subunit. Two of these genes encode proteins involved in maintaining photoreceptor outer segment disc structure—namely peripherin/RDS and rod outer segment membrane protein 1. Mutations in the gene encoding myosin VIIa have been found in a form of retinitis pigmentosa with associated profound congenital deafness (Usher syndrome, type I). Mutations in these seven genes together account for about 20–25% of cases of retinitis pigmentosa in the United States.

Mutations in the rhodopsin gene account for about 10% of cases in the United States and, therefore, represent the most common cause of retinitis pigmentosa for which a molecular genetic basis is known. More than 70 mutations have been found in the rhodopsin gene; most are missense mutations altering a single amino acid in the rhodopsin molecule (16–18). Differing severity of disease at a given age has been observed both within and between families, even among patients with the same gene defect (3, 19). The reason for variable clinical expression among patients of comparable age with the same gene defect is not known. The mechanism by which a rhodopsin gene defect expressed in rods leads to cone photoreceptor cell death also is not understood.

Opsin is a seven-transmembrane segment protein with 348 amino acid residues (Fig. 2, *Upper*) (20, 21). It is well known that opsin binds to its vitamin A-derived chromophore, 11-*cis*-retinal, via a Schiff-base linkage at the Lys-296 residue to form rhodopsin. Opsin is normally folded with the first (helix A) and seventh (helix G) transmembrane segments in proximity to form a pocket for 11-*cis*-retinal (Fig. 2, *Lower*). Change in the conformation of rhodopsin in response to light initiates the phototransduction cascade with consequent hydrolysis of cGMP. The decline in cGMP results in closure of the cGMP-gated channels and hyperpolarization of rod photoreceptor cells. The normal functioning of rhodopsin depends on its proper folding and binding to 11-*cis*-retinal (22–25).

In two papers on the structure and function of rhodopsin in these *Proceedings* (26, 27), Khorana and coworkers report precise methods for separation and characterization of correctly folded and misfolded rhodopsin expressed in cultured COS cells containing synthetic mutant opsin genes. In the first paper, Liu and coworkers (26) judiciously select two rhodopsin mutants, P23H and G188R, known to cause retinitis pigmentosa in humans, as well as two site-specific mutants, D190A and ΔY191-Y192, that would be expected to affect the folding of opsin. The proteins expressed from the P23H and D190A mutants partially regenerated the rhodopsin chromophore with 11-*cis*-retinal and were mixtures of the correctly folded (retinal-binding) and misfolded (non-retinal-binding) opsins. The proteins expressed from G188R and ΔY191-Y192 were composed of totally misfolded non-retinal-binding opsins. They suggest that most, if not all, of the point mutations in the intradiscal domain result in partial or complete misfolding of rhodopsin and that misfolded opsins lead to less compact outer-segment disc structure. In the second paper, Garriga and coworkers (27) study mutations in transmembrane helix C (designated as III in Fig. 2) that would be expected to affect the cysteine disulfide bond that is critical for proper folding of opsin. The mutant opsins showed abnormal dissociation of opsin from 11-*cis*-retinal in response to light and destabilized breakdown products of rhodopsin with reduced transducin activation. They conclude that folding in the transmembrane domain is coupled to that in the intradiscal domain.

It is well known that a normal rod photoreceptor, schematically represented in Fig. 3, sheds about 10% of its outer segment discs at its apex after light onset and renews a corresponding amount of outer segment discs at its base over the course of each day (29). Patients with retinitis pigmentosa and rhodopsin P23H have shown an abnormal rod ERG diurnal rhythm; rod ERG sensitivities are abnormally reduced

Commentary: Berson

Proc. Natl. Acad. Sci. USA 93 (1996)   4527

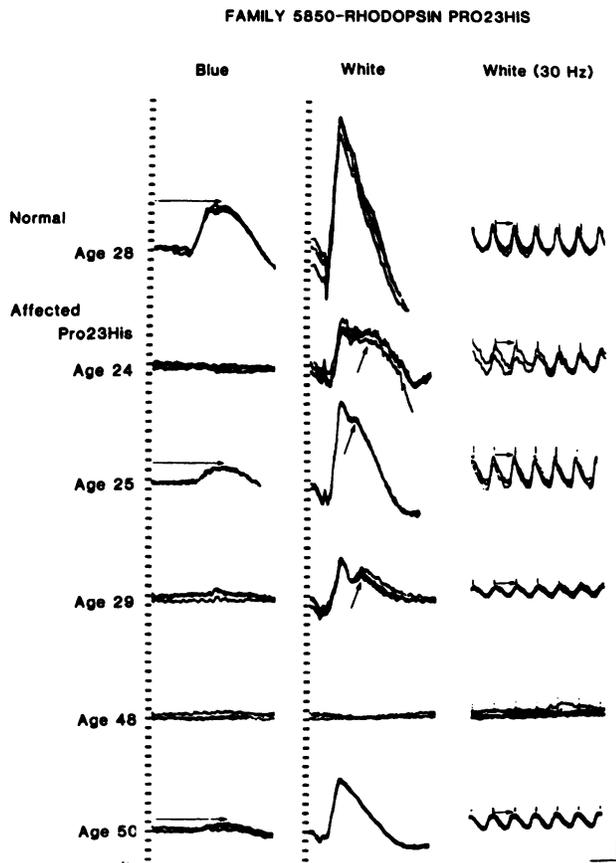**FAMILY 5850-RHODOPSIN PRO23HIS**

FIG. 1. Full-field ERGs from an unaffected patient and five affected relatives in family 5850 with dominant retinitis pigmentosa and rhodopsin P23H. Rod isolated responses are illustrated to blue light, mixed cone–rod responses to white light, and cone isolated responses to 30-Hz white flicker. Stimulus onset is indicated by vertical hatched lines in columns 1 and 2 and vertical spikes in column 3. Two or three consecutive sweeps are illustrated. Cornea positivity is an upward deflection. Horizontal arrows designate rod or cone peak implicit times. Oblique arrows designate delayed rod-dominated peaks. Lower norms for blue, white, and white 30-Hz light are 100 μV, 350 μV, and 50 μV, respectively. Calibration symbol (lower right) designates 50 msec horizontally and 100 μV vertically. (From ref. 3. Copyright 1991, American Medical Association, Chicago, IL.)

FIG. 2. (*Upper*) Model of normal opsin in a rod outer segment disc membrane with seven transmembrane segments. Site of cysteine disulfide bond between Cys-110 in the third transmembrane segment and Cys-187 in the second intradiscal loop is illustrated. (*Lower*) Representation of a normal rhodopsin molecule folded in three dimensions to form a pocket to hold the vitamin A-derived chromophore (11-*cis*-retinal), which is covalently attached to Lys-296, designated by "K" in the seventh transmembrane segment. (Modified from ref. 2. Copyright 1993, Association for Research in Vision and Ophthalmology, Bethesda, MD.)

1.5 h after light onset and are slow to return to prelight onset levels over the course of each day (3, 30). This observation is compatible with the idea that patients with this mutation shed abnormally large fractions of rod outer segments after light onset and that their rods are slow to renew their prelight onset outer segment length. If discs containing misfolded mutant opsins are less compact and, therefore, shed more easily *in vivo*, then the *in vitro* findings of Khorana and coworkers may provide an explanation for this clinical observation. Perhaps it is the excess daily shedding of outer segment discs that eventually leads to rod photoreceptor cell death.

Immunocytochemical studies of transgenic mice with the P23H mutation have shown labeled mutant opsin not only in the outer segments and cell bodies but also in the synaptic layer of rod photoreceptors (31). This observation suggests that some mutant opsin molecules that would normally be transported to the outer segments are misrouted and, thereby, may interfere with synaptic transmission. In this regard, it is interesting to note that patients with the early stages of rhodopsin P23H have abnormally delayed b-waves in rod responses to blue light and biphasic mixed cone–rod responses to single flashes of white light (see Fig. 1), possibly due to a delay in signal transmission between rod photoreceptors and more proximal retinal cells.
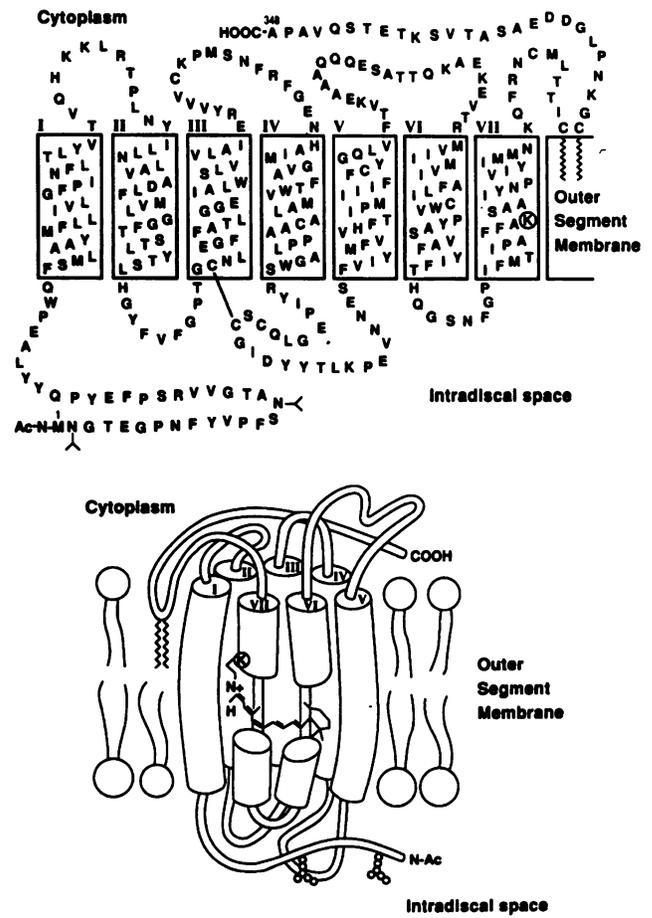
The finding by Khorana and coworkers of mixtures of correctly folded and misfolded opsins in cultured COS cells containing the synthetic P23H mutant gene raises the possibility that rod photoreceptors with this mutation contain different ratios of folded and misfolded opsins. This awaits confirmation in transgenic animal models. Varying amounts of misfolded opsin perhaps could lead to the variable amount of rod malfunction seen among patients of comparable age with the P23H mutation (see Fig. 1).

The observation by Khorana and coworkers that misfolded opsins abnormally dissociate from 11-*cis*-retinal *in vitro* may have its clinical correlate in the delayed rates of rod dark adaptation that have been found in patients with the P23H mutation as well as patients with the T17M or T58R mutations (32). Stated in another way, abnormal rods with misfolded opsins may not bind or hold 11-*cis*-retinal so that patients with these mutations have slower than normal rates of dark adaptation. A defect in binding to 11-*cis*-retinal would be expected to contribute to instability of opsin and consequent loss of outer segment disc structure, which has been observed in vitamin A nutritional deficiency (33).

Molecular genetics studies of human retinitis pigmentosa have revealed a wide spectrum of mutations in the rhodopsin gene that result in disease; this research, in turn, indicates that

APICAL DISCS

OUTER SEGMENT

INTRADISCAL SPACE

PLASMA MEMBRANE

CYTOPLASM

BASAL OPEN DISCS   D

CONNECTING CILIUM

MITOCHONDRIA

POST GOLGI VESICLES   C

GOLGI   B

ROUGH ENDOPLASMIC RETICULUM   A

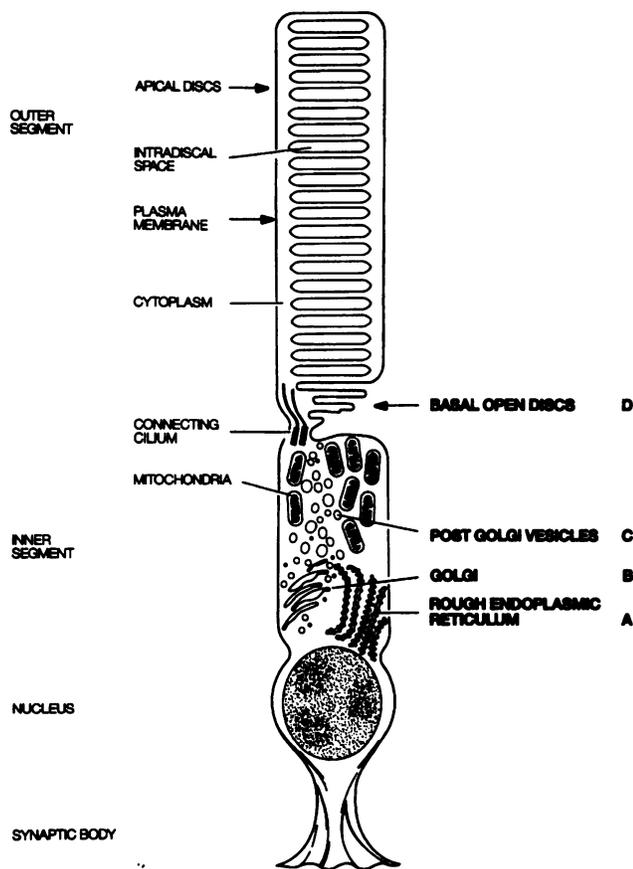INNER SEGMENT

NUCLEUS

SYNAPTIC BODY

FIG. 3. Schematic representation of a normal human rod photoreceptor to illustrate steps in delivery of opsin to outer segments. Opsin is synthesized and glycosylated in ribosomes of the rough endoplasmic reticulum (*A*), further modified in the Golgi complex (*B*), and transported toward the cell surface by post-Golgi vesicles (*C*), where it is thought to be incorporated into the plasma membrane at the level of the connecting cilium. The transfer of opsin from the inner segment to the outer segment is not fully understood. New disc formation (*D*) appears to involve evagination of the plasma membrane of the distal connecting cilium. Opsin appears to acquire vitamin A (i.e., 11-*cis*-retinal) at the base of the outer segment, and probably in the inner segment as well, and this confers light sensitivity on this protein now called rhodopsin. (From ref. 28. Copyright 1990, American Ophthalmological Society, Durham, NC.)

many amino acids in the rhodopsin molecule are critical for the normal functioning of this molecule. *In vitro* and *in vivo* models containing mutant gene constructs provide new opportunities to investigate the structure and function of rhodopsin. The papers by Khorana and coworkers not only provide us with a greater understanding of the mechanisms involved in the correct folding of opsin but also represent an important contribution to unfolding the mystery of some forms of retinitis pigmentosa.

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