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Molecular genetics of retinal degeneration
A Drosophila perspective

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Abbreviations: ADRP, autosomal dominant retinitis pigmentosa; Arr2, arrestin 2; Arr1, arrestin 1; atg, autophagy related gene; Baz, bazooka; CalX, Na^+/Ca^{2+} exchanger; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; cGMP, cyclic GMP; CNX, calnexin; DAG, diacylglycerol; dpp, deep pseudopupil; GPCR, G-protein coupled receptor; nina, neither-inactivation-nor-afterpotential; norpA, no-receptor-potential A; IAP, inhibitor of apoptosis; ina, inactivation-no-afterpotential; IP_3, inositol trisphosphate; PA, phosphatic acid; PATJ, PALS1-associated tight junction protein; PCD, programmed cell death; PDZ, PSD95, discs-large, ZO-1; PKC, protein kinase C; PIP_2, phosphatidylinositol-4,5-bisphosphate; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; PLD, phospholipase D; rdgA, retinal degeneration A; rdgB, retinal degeneration B; rdgC, retinal degeneration C; TOR, target of rapamycin; TRP, transient receptor potential; TRPL, trp-like; UPR, unfolded protein response

Inherited retinal degeneration in Drosophila has been explored for insights into similar processes in humans. Based on the mechanisms, I divide these mutations in Drosophila into three classes. The first consists of genes that control the specialization of photoreceptor cells including the morphogenesis of visual organelles (rhabdomeres) that house the visual signaling proteins. The second class contains genes that regulate the activity or level of the major rhodopsin, Rh1, which is the light sensor and also provides a structural role for the maintenance of rhabdomeres. Some mutations in Rh1 (*NinaE*) are dominant due to constitutive activity or folding defects, like autosomal dominant retinitis pigmentosa (ADRP) in humans. The third class consists of genes that control the Ca^{2+} influx directly or indirectly by promoting the turnover of the second messenger and regeneration of PIP_2, or mediate the Ca^{2+}-dependent regulation of the visual response. These gene products are critical for the increase in cytosolic Ca^{2+} following light stimulation to initiate negative regulatory events. Here I will focus on the signaling mechanisms underlying the degeneration in *norpA,* and in ADRP-type *NinaE* mutants that produce misfolded Rh1. Accumulation of misfolded Rh1 in the ER triggers the unfolded protein response (UPR), while endosomal accumulation of activated Rh1 may initiate autophagy in *norpA*. Both autophagy and the UPR are beneficial for relieving defective endosomal trafficking and the ER stress, respectively. However, when photoreceptors fail to cope with the persistence of these stresses, a cell death program is activated leading to retinal degeneration.

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Introduction

Drosophila is an excellent model organism to explore the molecular basis of retinal degeneration. Retinal degeneration in the fly is characterized by the initial loss of rhabdomeres, the visual organelles, followed by deterioration of photoreceptor cell bodies. The power of genetics allows the identification of mutants using a simple screening method, the loss of deep pseudopupil (dpp). Dpp represents the autofluorescence of the Rh1 rhodopsin in rhabdomeres of the major photoreceptor cells (R1-R6) from several adjacent unit eyes of the compound eye.[1] The absence of dpp may be due to a loss of Rh1, which likely results from a loss of rhabdomeres contributed by degeneration of photoreceptor cells. Characterization of retinal degeneration mutants is greatly aided by our knowledge of rhabdomere morphogenesis.[2] Moreover, the use of genetic tools including tissue-specific overexpression[3,4] and RNAi-mediated knockdown[5,6] provide additional avenues for uncovering mechanistic insights leading to photoreceptor death. In essence, degeneration of photoreceptor cells is often accompanied by defective development of rhabdomeres or aberrant visual signaling. As an introduction, a brief background concerning the visual signaling pathway, structure of the compound eye, and terminal differentiation of photoreceptor cells will be provided.

Structure of the Compound Eye

The major visual system of Drosophila and dipteran insects consists of two compound eyes, each of which contains approximately 800 unit eyes or ommatidia in Drosophila (**Fig. 1**). Each ommatidium is made up of 22 cells including eight photoreceptor cells, R1-R8, which are arranged in a cylindrical pattern with the outer photoreceptor cells, R1-R6, occupying the peripheral region (**Fig. 1B**), whereas the central photoreceptor cells (R7 and R8) reside in the central region with R7 distally located while R8 proximally. R1-R6 are the major photoreceptor cells

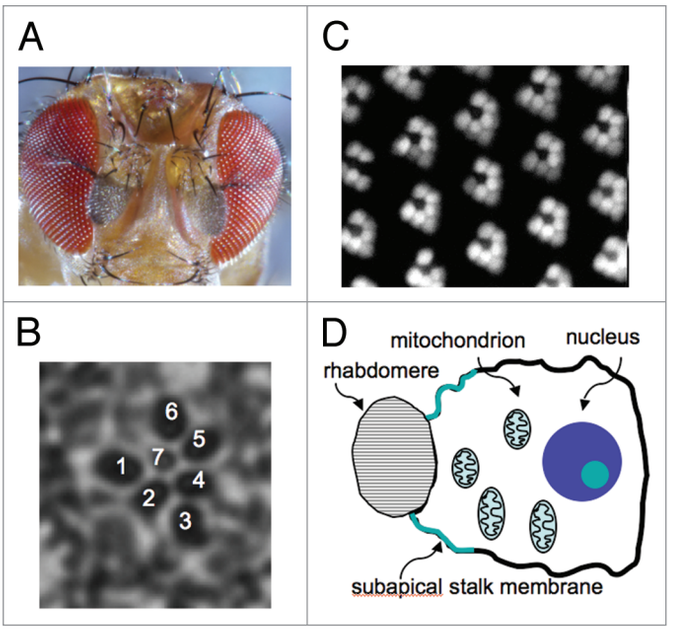

Figure 1. Structure of the compound eye in Drosophila. (A) Each compound eye consists of 600–800 unit eyes (ommatidia). (B) Each ommatidium contains eight photoreceptors, R1-R8. Shown is a cross section of the distal region of an ommatidium, which reveals the R1-R7 photoreceptors with the stereotypical arrangement of rhabdomeres. Rhabdomere is the visual organelle in photoreceptor cells, and consists of densely packed membranes supported by the actin cytoskeleton (C and D). Shown is the rhabdomere of the R1-R6 photoreceptors revealed by epifluorescence of actin tagged with the green fluorescent protein (C). (D) A diagrammatic depiction of a photoreceptor cell (cross section).

in Drosophila. Similar to vertebrate rod photoreceptors in which rod outer segments house the signaling proteins involved in the visual cascade, Drosophila photoreceptor cells possess specialized visual organelles, rhabdomeres, in which visual signaling proteins reside (**Fig. 1D**). Each rhabdomere consists of densely stacked membrane structures of about 60,000 microvilli supported by an actin-based cytoskeleton (**Fig. 1C**).[7]

Six Rhodopsins, Rh1-Rh6, are Expressed in Drosophila Photoreceptors

The sensitivity of photoreceptor cells to a particular wavelength of light is governed by the nature of the rhodopsin it expresses.[8,9] Drosophila expresses six distinct rhodopsins, Rh1-Rh6, and senses a wide spectrum of light including ultraviolet (UV) light. Rhodopsin 1 (Rh1) is the major rhodopsin with absorption maximum at 480 nM, and is present in R1-R6 photoreceptor cells.[10,11] The abundance of Rh1 rhodopsin in the compound eye is parallel to that of rod rhodopsin in vertebrate retinas. In Drosophila, two UV-absorbing rhodopsins, rhodopsin 3 and rhodopsin 4, are found in non-overlapping R7 photoreceptor cells.[12,13] In contrast, R8 photoreceptor cells express either rhodopsin 5,[14] or rhodopsin 6 [15] conferring the sensitivity of these cells to either blue or green light, respectively. Lastly, rhodopsin 2 is found in the ocelli,[16] the three simple eyes located on the vertex of the head, and is critical for visual guidance. All Drosophila rhodopsins belong to the class A subfamily[17] of G-protein coupled receptors (GPCRs), and are the most abundant membrane proteins in the rhabdomere of the photoreceptor cell.

Morphogenesis of Rhabdomeres

The shape and content of the rhabdomere support the critical elements of photoreceptor cell's function and survival. Without the completion of the morphogenesis program, photoreceptor cells will not achieve the desired shape. In particular, malformation of the rhabdomere renders it unable to accommodate the signaling machinery, affecting both visual transduction and maintenance of photoreceptor cells, defects in which eventually bring about retinal degeneration.

In Drosophila, morphogenesis and terminal differentiation of photoreceptors start at the mid-pupal stage, resulting in the elaboration of rhabdomeres (reviewed in ref. 2). These events are orchestrated by the Crumbs complex [Crumbs, Stardust and DPATJ (PALS1-associated tight junction protein)], which first initiates the subdivision of the apical membrane into two distinct regions, and subsequently controls the specialization of these regions into subapical stalk membranes and rhabdomeres (**Fig. 1D**). This initial division of the apical domain is mediated by Bazooka (Baz),[18] a scaffolding protein containing three PDZ (PSD95, discs-large, ZO-1) domains. Baz participates in the recruitment of PTEN (Phosphatase and Tensin Homolog deleted on chromosome 10), a lipid phosphatase that converts phosphatidylinositol-3,4,5-trisphosphate (PIP_3) into phosphatidylinositol-4,5-bisphosphate (PIP_2).[19] PIP_2 is critical for actin polymerization[20] whereas PIP_3 activates AKT/protein kinase B.[21] Mutants missing PTEN display defects in rhabdomeres and stalk membranes, indicating that specialization of photoreceptor cells is sensitive to the relative level of these two phosphoinositides.[22]

As indicated, the Crumbs protein complex is implicated in the expansion of the subapical stalk membrane, and the elongation of rhabdomeres. Indeed, mutants devoid of components of the Crumbs complex display a reduction of the stalk membrane and a failure in the rhabdomere elongation.[18,23-25] The elaboration of rhabdomeres enables photoreceptor cells to increase the amount of the membrane in order to accommodate about 10^8 molecules of Rh1 rhodopsin.[26] This high density of rhodopsin in rhabdomeres makes the visual signaling exquisitely sensitive. Moreover, compartmentalization of the signaling molecules within the rhabdomere facilitates fast kinetics of the visual signaling by promoting protein-protein interactions, thus enhancing the kinetics of the photoresponse.

Visual Signaling in Drosophila

Photoreceptors in the eye are highly specialized neurons that provide a window for the brain to see the world. These neurons utilize a set of signaling proteins to detect the light signal and convert it into an electrical impulse, which then propagates to neurons in the visual brain. Specifically, activation of the visual signaling cascade generates a second messenger that regulates the ion flux,

resulting in a change of membrane potential of photoreceptors. In essence, the visual signaling mechanism operates in a similar way to that mediated by a prototypical GPCR.

In the visual cascade, rhodopsin is the critical GPCR that senses the light signal by the covalently bound chromophore, 11-cis retinal (11-cis 3-hydroxy-retinal in the fly rhodopsin). The energy of light causes the isomerization of 11-cis retinal to an all-trans configuration, which activates rhodopsins (reviewed in ref. 33). In Drosophila, activated rhodopsin promotes the exchange of GTP for GDP in the α-subunit of the heterotrimeric Gq protein,[27] which switches on NORPA (no-receptor-potential A). NORPA[28] is a phospholipase Cβ that catalyzes the hydrolysis of phospholipids PIP_2 to generate inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). It appears that DAG and its metabolites including polyunsaturated fatty acids[29,30] are critical for the gating of the downstream TRP (transient receptor potential) and TRPL (TRP-like) cation channels, whereas IP_3 is not required for the light-mediated membrane depolarization (**Fig. 2**) (reviewed in refs. 31–33).

The light-dependent depolarization of Drosophila photoreceptor cells is one of the fastest GPCR-mediated signaling events. The fast kinetics is partly contributed by the compartmentalization of the signaling molecules in rhabdomeres. Moreover, several signaling proteins including TRP, NORPA and eye-PKC (eye-specific protein kinase C) are organized into a macromolecular signaling complex (transduciosome or signalplex) (**Fig. 2**) through the association with INAD (inactivation-no-afterpotential D),[34-39] a multivalent scaffolding protein containing five PDZ domains. INAD is critical for the subcellular localization as well as the stability of its interacting proteins in vivo, and it promotes fast activation and deactivation of the visual response.[37]

In vertebrates, by contrast, light hyperpolarizes photoreceptor cells (rods and cones) by closing cGMP channels (reviewed in ref. 40). In the dark, the cGMP level is higher, which opens the cGMP channel to generate the 'dark currents'. Upon light stimulation, activated phosphodiesterase lowers the cGMP level, allowing fewer cGMP channels to remain open leading to hyperpolarization of rods or cones. In short, light switches on phosphodiesterase via the heterotrimeric G-protein transducin, resulting in a reduction of cGMP and hyperpolarization of vertebrate photoreceptor cells.

Modulation of the Visual Signaling by Ca^{2+}

Photoreceptor cells are capable of responding to a wide-range of light intensities by adjusting degrees of amplification in the visual response. This is accomplished by several feedback mechanisms. In Drosophila, for example, the influx of Ca^{2+} via the TRP and TRPL channels generates localized Ca^{2+} transients which activate several Ca^{2+}-dependent regulatory events including those initiated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), eye-PKC and RDGC (retinal degeneration C) (**Fig. 2**). RDGC catalyzes the dephosphorylation of rhodopsin,[41,42] whereas CaMKII is involved in the phosphorylation of arrestin 2 (Arr2),[43,44] the major visual arrestin critical for the inactivation of activated rhodopsin.[45] Eye-PKC is a conventional PKC[46] activated by both

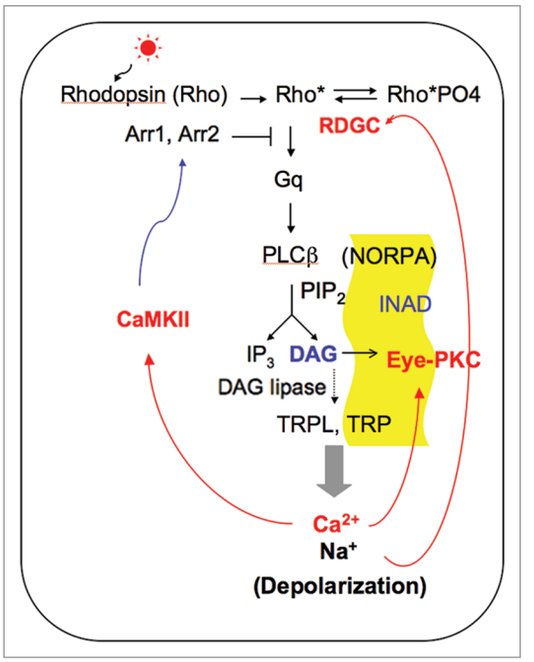

Figure 2. Visual signaling in Drosophila. Light stimulation results in depolarization of Drosophila photoreceptors, as activated rhodopsin (Rho*) couples to Gq leading to the activation of PLCβ (NORPA), and the eventual opening of the TRP and TRPL channels. The INAD complex, highlighted in yellow, consists of INAD, eye-PKC, NORPA and TRP. The Ca^{2+}-dependent regulatory events employ CaMKII, RDGC and eye-PKC partly to regulate Arr2, rhodopsin and TRP for the deactivation of the visual response.

DAG and Ca^{2+}. Importantly, eye-PKC is tethered to the INAD signaling complex to regulate INAD and TRP;[47] *inaC* mutants devoid of eye-PKC display defective light adaptation and slow deactivation of the visual response.[48,49]

Genes Affecting Retinal Degeneration in Drosophila can be Divided into Three Classes

The class I genes regulate the specialization of rhabdomeres. Class I encompasses genes involved in the terminal differentiation of photoreceptor cells including those controlling the morphogenesis of rhabdomeres (**Table 1**). As detailed earlier, rhabdomeres are the visual organelles that house most of the visual signaling machinery. The specialization of rhabdomeres is orchestrated by the Crumbs protein complex including Crumb, Stardust and dPATJ: mutations in each component of this protein complex, and their interacting proteins result in defective morphogenesis with shortened stalk and aberrant rhabdomeres.[2] Photoreceptors with abnormal rhabdomeres undergo light-dependent retinal degeneration.[2] Significantly, the degeneration phenotype can be prevented when rhodopsin synthesis is greatly decreased by feeding flies with vitamin A-deficient food, presumably because it reduces the demand for active elongation of rhabdomeres to accommodate massive Rh1 rhodopsin.[23]

The class II genes are involved in the biosynthetic pathway and/or regulating Rh1 rhodopsin. The Class II gene

Table 1. Three major classes of retinal degeneration mutations in Drosophila

Class	Genes	Proteins (Activities)	Mechanisms#	References
I	**Regulate terminal differentiation of photoreceptors**			
	Crumbs	transmembrane protein with EGF-like and laminin G repeats	1	24, 25
	Stardust	membrane associated guanylate kinase (MAGUK)	1	18, 23
	Dpatj	cytosolic protein with PDZ and L27 domains	1	18
	Baz	scaffolding protein	1	18
	WASp	rhabdomere morphogenesis (actin polymerization)	1	7
II	**Control the level or activity of the Rh1 rhodopsin**			
	Structural gene for Rh1			
	NinaE (type I)	Rh1 rhodopsin	2	75
	NinaE (type II, LOF)	Rh1 with folding defects	3	84–87
	NinaE (type II, GOF)	Rh1 with constitutive activity	4, 5	77
	Rh1 folding and maturation			
	ninaA	cyclophilin-like; chaperone	3	65, 99
	cnx99A	calreticulin-like, lectin chaperone (Rh1 maturation)	3	50
	Trafficking of Rh1			
	rab6	small GTPase (trafficking of Rh1)	2	52
	rab11	small GTPase (post-Golgi trafficking of Rh1)	2	53
	ninaC	unconventional myosin III (anchoring Rh1 in rhabdomeres)	2	54
	Inactivation of Rh1			
	Arr2	arrestin 2 (inactivation of Rh1)	5	45, 55
	Arr1	arrestin 1 (inactivation and internalization of Rh1)	5	55
	rdgC	rhodopsin phosphatase	4	41, 56
	Tsp42Ej (sun)	tetraspanin (degradation of Rh1)	4	57
	TADR	Rh1 interacting protein	4, 5	149
	Endocytosis of Rh1			
	car	sec-1 like protein (endosome to lysosome trafficking)	4	58, 128, 129
	lt	vacuolar protein sorting 41 (endosome to lysosome trafficking)	4	58, 130
	cm	clathrin adaptor mu subunit (endosome to lysosome trafficking)	4	58
III	**Modulate Ca^{2+} level or control Ca^{2+} influx**			
	Modulate cytosolic Ca^{2+} level			
	cnx99A	calreticulin-like (cytoplasmic Ca^{2+} buffering)	4, 5	50
	calx	Na^{+}/Ca^{2+} exchanger (cytoplasmic Ca^{2+} homeostasis)	5	104
	Participate in the synthesis/recycling of PIP_2			
	rdgA	diacylglycerol kinase (inactivation of second messenger)	5	29, 109
	cdsA	CDP-DAG synthase (regeneration of PIP_2)	4	111
	pis	phosphatidylinositol synthase (regeneration of PIP_2)	4	111
	rdgB	phosphatidylinositol transfer protein (regeneration of PIP_2)	4	107, 111
	cerk	ceramide kinase (regulates norpA, PIP_2)	4	108
	Regulate TRP Ca^{2+} channel			
	trp	Ca^{2+} channel	4	120
	Trp	constitutive TRP activity	5	118
	inaC	photoreceptor-specific protein kinase C	4	46
	inaD	scaffolding protein (regulates the stability of TRP)	4	109
	norpA	phospholipase Cβ (generates second messenger)	4	113, 127

#Five mechanisms that contribute to retinal degeneration are (1) defective rhabdomere formation, (2) reduced Rh1 levels, (3) misfolded Rh1, (4) trafficking defects associated with internalization of activated Rh1 and (5) the Ca^{2+}-mediated excitatoxicity due to overactive signaling. Abbreviations: LOF, loss-of-function; GOF, gain-of-function.

products participate in the biosynthesis or inactivation of the Rh1 rhodopsin (**Table 1**). Mutants affecting the biogenesis result in a reduced Rh1 level, which impacts the elaboration of rhabdomeres (see below, Structural Role of Rhodopsin). These gene products include CNX (calnexin),[50] and NINAA (neither-inactivation-nor-afterpotential A),[51] that controls the maturation of Rh1, rab6,[52] and rab11[53] that take part in the trafficking and delivery of Rh1 following its de novo synthesis, NINAC that anchors Rh1 in the rhabdomere,[54] and *ninaE* (neither-inactivation-nor-afterpotential E), the structural gene for Rh1.[10,11] Mutations in *ninaE* can be further subdivided into two types, based on whether it is recessive (type I) or dominant (type II) in promoting retinal degeneration (see below).

In contrast, mutations that cause a delay in the inactivation of activated Rh1 resulting in either prolonged visual signaling and/or defective trafficking/degradation. These include arrestin 1 (Arr1),[55] Arr2,[45] *rdgC*,[41,56] *sun* (sunglass),[57] and the 'granule group genes'.[58] While both Arr1 and Arr2 contribute to the inactivation of Rh1,[45] Arr1 also is critical for promoting Rh1 endocytosis.[55] The internalized Rh1 can be detected in multivesicular bodies and appears targeted for degradation.[55] The 'granule group gene' products play a role in the endosome to lysosome trafficking,[58] while the *sun* gene encodes a novel tetraspanin that may modulate the degradation of Rh1 in the lysosome.[57] Conceivably, these class II mutations result in light-dependent retinal degeneration because activation of Rh1 is required.[57,58] The light-dependent degeneration of these mutants can be prevented by a reduction of Rh1 through dietary vitamin A restriction.

Biosynthesis of Rh1 Rhodopsin: Opsin and the Retinal Chromophore

Functional rhodopsin consists of a protein moiety, opsin and a retinal chromophore. The opsin serves to fine-tune the absorption spectrum of the covalently linked retinal chromophore resulting in a unique spectral sensitivity for each rhodopsin.[8,9] Opsin, like other integral membrane proteins, is translated in the ribosome of the rough endoplasmic reticulum (ER). Following translation, Rh1 undergoes N-linked glycosylation at Asp(20),[59,60] rendering it sensitive to endoglycosidase H. However, the carbohydrate moiety is subsequently removed when Rh1 exits the ER, resulting in a mature Rh1 without glycosylation.[61] In the ER, the carbohydrate moiety is required for folding and maturation of Rh1,[61] which is aided by two chaperones, NINAA[51] and CNX.[50] CNX is a resident protein in the ER involved in the quality control of protein synthesis, and it interacts with both the sugar moiety and the peptide chain of newly glycosylated polypeptides.[62] A lack of CNX results in defects in Rh1 maturation.[50] In contrast, NINAA is a chaperone with sequence homology to prolyl cis-trans isomerase,[63,64] and it regulates the export of Rh1 out of the ER.[51] Mutations in *ninaA* lead to ER retention of Rh1 in R1-6 photoreceptors.[65]

The chromophore of rhodopsin in the fly, 11-cis 3-hydroxyretinal, is derived from vitamin A of the diet. Vitamin A is taken up and processed to 11-cis retinal through multiple biochemical reactions, which are catalyzed by the gene products of *ninaB*,[66] *ninaD*,[67,68] *ninaG*[69] and *santa maria* (scavenger receptor acting in neural tissue and majority of rhodopsin is absent).[70] The stability of Drosophila Rh1 rhodopsin is dependent on its incorporation of the retinal chromophore:[71] flies defective in proteins that control the uptake or processing of vitamin A, contain a reduced level of Rh1. Despite having a low Rh1 level, both *ninaB* and *ninaD* flies do not undergo retinal degeneration.[72]

Mature Rh1 is transported through the Golgi. The post-Golgi trafficking is orchestrated by monomeric G-proteins including Rab 6[52] and Rab11.[53] Finally, Rh1 is delivered to and inserted into the rhabdomeric membrane. The rhabdomeric localization of Rh1 appears dependent on the helix 8 in its carboxyl terminal sequence.[73] Moreover, the carboxyl tail of Rh1 is required for the maintenance of rhabdomere structure.[74]

Role of Rh1 in the Morphogenesis of Photoreceptors

Beside functions as a light detection molecule, Rh1 is also critical for the differentiation of R1-R6 photoreceptor cells. The incorporation of newly synthesized Rh1 into the rhabdomeric membrane coincides with its rapid elongation during terminal differentiation of R1-R6 cells. Consistently, the expansion of rhabdomeres is significantly compromised in *ninaE* flies unable to produce sufficient amount of Rh1. Leonard et al. demonstrated that a drastically reduced level of Rh1 resulted in smaller rhabdomeres at the eclosion when flies emerge. Moreover, photoreceptor cells that lack Rh1 display age-dependent degeneration with concomitant deterioration and disappearance of rhabdomeres.[75] It appears that the severity of retinal degeneration is in proportion to the degree of reduction in the Rh1 level, indicating a structural role of Rh1 in the morphogenesis of photoreceptor cells.[76] Interestingly, the size of rhabdomere is not solely dependent on the level of Rh1, as $ninaE^{P332}$ flies with about 1% of the wild-type level actually contain about 65% of the normal rhabdomeric membrane area. It is likely that insertion of other membrane proteins also contributes to the expansion of rhabdomeres. Surprisingly, rhabdomeres are barely formed in a null allele ($ninaE^{117}$),[75] suggesting that Rh1 also serves to initiate the morphogenesis of rhabdomeres. Thus Rh1 may have dual roles in orchestrating the elongation of rhabdomeres by serving as a signal as well as by providing the Rh1-enriched membrane. Similarly, mutations in the class II genes that greatly reduce either the biosynthetic capacity or the transport of Rh1 may lead to degeneration of photoreceptor cells because of a decreased Rh1 content that restricts the rhabdomeric expansion.

Mutations in Rh1 (*ninaE*) Elicit Either Dominant or Recessive Retinal Degeneration

In general, mutations in *ninaE* that trigger retinal degeneration fall into two categories. The first (type I) consists of recessive *ninaE* alleles in which degeneration is observed only in homozygotes containing two mutant alleles. Type I *ninaE* mutations include hypomorphic (e.g., $ninaE^{P332}$) and null alleles (e.g., $ninaE^{117}$), which generally produce unstable Rh1 rhodopsin resulting in a drastically reduced level of Rh1. In contrast, the second group

(type II) displays dominant degeneration as heterozygotes also exhibit a degeneration phenotype. There are two mechanistically distinct type II *NinaE* mutations: one that produces active Rh1 (e.g., $NinaE^{pp100}$) leading to constitutive signaling and persistent Arr2 binding,[77] and the other that produces misfolded Rh1 (e.g., $NinaE^{D1}$) that negatively impacts the expression of the wild-type *ninaE* allele.[78] The degeneration phenotype of $NinaE^{D1}$ is lessened when the expression of *ninaE* is reduced by dietary vitamin A restriction, while that of constitutively active $NinaE^{pp100}$ can be eliminated only when both Arr2 and Gqα are absent.[77] Similar dominant mutations are found in human rod rhodopsin gene, which cause either stationary congenital night blindness or ADRP (autosomal dominant retinitis pigmentosa).[79,80]

Defect in Human Rod Rhodopsin Is One of the Major Causes of ADRP

ADRP is primarily characterized by the progressive loss of rods in the retina. Mutations in human rod rhodopsin, the visual pigment expressed in rod photoreceptors, constitute to 25–30% of ADRP.[80] Importantly, these mutant rod rhodopsins display either an increased activity or defective protein folding, similar to dominant *NinaE* alleles. Moreover, some mutant rod rhodopsins exhibit defective transport to the outer segment or abnormal endocytosis (reviewed in ref. 81).

In short, most mutations of rod rhodopsin implicated in ADRP affect post-translational events including folding and trafficking, which result in the accumulation of mutant rhodopsin in the ER. Pharmacological chaperones such as 11-cis 7-ring retinal have been shown to rescue misfolded rhodopsin for its trafficking to the cell surface.[82] However, misfolded opsins may form aggregates[83] causing dysfunction of the ER. The ER stress triggered by the expression of mutant rhodopsin may initiate the unfolded protein response (UPR) in order to relieve the accumulation of misfolded proteins (see below).

Dominant *NinaE* Mutations: ADRP in Drosophila

As indicated before, dominant *NinaE* mutations can be caused by either dominant active Rh1 possessing constitutive activity, or dominant negative Rh1 that interferes with the biosynthesis of wild-type Rh1. A great number of dominant negative *NinaE* alleles (e.g., $NinaE^{G69D}$, $NinaE^{D1}$) were originally recovered by genetic screens as suppressors of *rdgB* (retinal degeneration B),[84] or *rdgC*.[85] These *NinaE* mutants having either mis-sense mutations or premature stop codons, were shown to produce predominantly immature forms of Rh1 that are glycosylated and sensitive to endoglycosidase H. The mutant Rh1 appears to be retained in the ER.[84]

Interestingly, misfolded Rh1 tends to oligomerize, and mostly exists as dimers.[78,84] It is very likely that dimers represent the conformation of the newly translated Rh1, because dimers can be detected following the heat-shock induced transcription in flies expressing Rh1 under the control of the *hsp70* promoter.[78,84] Importantly, *NinaE* heterozygotes, but not homozygotes, display over-proliferation of the ER cisternae,[84] strongly suggesting defects associated with the ER accumulation of the non-productive Rh1 dimers containing both wild-type and mutant Rh1. Consistently, retinal degeneration is more severe in *NinaE* heterozygotes than in homozygotes,[78] supporting the notion that the non-productive Rh1 dimers are more stable and exert a more profound effect than those containing only mutant Rh1. Similarly, folding defects associated with the expression of modified Rh1 ($ninaE^{N201}$) that lacks N-linked glycosylation,[62] also lead to ER retention of non-glycosylated Rh1, and consequently dominant retinal degeneration.[59]

Mutations in *NinaE* that correspond to human ADRP also promote dominant retinal degeneration. This occurs, for example, in transgenic flies expressing modified Rh1 ($Rh1^{P37H}$) containing a His at Pro(37),[86,87] which is equivalent to human rod rhodopsin(P23H), the most common ADRP mutation in North America.[88] In several fly models of ADRP, misfolded Rh1 is localized in the ER, and appears to trigger the ER stress that progresses to cell death. Besides folding defects, some ADRP rhodopsin mutations are shown to display constitutive activities,[89] which may promote internalization of rhodopsin-arrestin complexes, similar to that occurring in gain-of-function *NinaE* alleles.

ER Stress Induces the Unfolded Protein Response

A folding deficiency often results in accumulation of misfolded proteins in the ER, which triggers ER stress or the unfolded protein response (UPR).[90,91] The UPR is initiated by sensing misfolded proteins by three type I transmembrane proteins including ATF6 (activating transcription factor 6), IRE1 (inositol requiring 1), and PERK (PKR-like ER kinase), which are kept in inactive states by an ER chaperone Grp78 (BiP).[91] As the level of misfolded proteins increases, which occupy Grp78, the inhibition of these three proteins is removed.[92,93] Specifically, ATF6 becomes active following the protease cleavage in the Golgi.[93] Protease-cleaved ATF6 is translocated to the nucleus and regulates the transcription of XBP1 (X box protein 1),[94] a transcription factor that controls the expression of several genes involved in the retrograde transport and the degradation of misfolded proteins.[95] The expression of XBP1 is also regulated by mRNA splicing, which requires IRE[94] that contains an endoribonuclease domain and is activated following oligomerization.[96] Similarly, PERK is switched on upon oligomerization and autophosphorylation. Activated PERK phosphorylates and inactivates eIF2α (eukaryotic initiation factor 2α),[97] thereby shutting down protein synthesis, and reducing the protein load in the ER. In short, the UPR leads to a reduction of protein synthesis, yet an increased transcription and translation of chaperones that promote re-folding or degradation of misfolded proteins.

Protective Effect of the UPR Towards ADRP-Like *NinaE* Mutations

Studies on ADRP-like *NinaE* mutations indicate that the UPR helps reduce degeneration of photoreceptor cells. In flies expressing $Rh1^{P37H}$, the misfolded Rh1 becomes a substrate of

VCP/ter94, a chaperone that extracts misfolded proteins for proteasomal degradation. A reduction of VCP/ter94 elevates the level of misfolded $Rh1^{P37H}$, which triggers the UPR that ameliorates death of photoreceptors.[87] Likewise, administration of inhibitors of the VCP/ter94 or proteasomes offers protection against photoreceptor degeneration. In contrast, suppression of the ER stress response by decreasing the *xbp1* gene dosage accelerates degeneration.[98]

The beneficial effect of the UPR is also observed in *ninaA* photoreceptors. NINAA is a resident chaperone critical for folding and maturation of Rh1.[51] Rh1 accumulates in the ER in the absence of NINAA, which triggers the UPR.[99] Significantly, the UPR prevents cell death initiated by several apoptotic signals including RPR (reaper), p53 and death caspase-1.[99] This protective effect appears to be contributed by the inhibition of caspases.[99] Taken together, upregulation of the UPR triggered by ADRP-like Rh1 or a loss of NINAA prevents death of photoreceptors, while inhibition of the UPR accelerates.

Somewhat paradoxically, persistent activation of the UPR has been linked to cell death (reviewed in refs. 90, 91 and 100). This may be promoted by recruiting ASK1 (Apoptosis Signal-Regulating Kinase 1) via IRE1, which is critical for the activation of JNK (c-Jun N-terminal Kinase) and cell death programs.[101] Indeed, expression of $Rh1^{P37H}$ in Drosophila results in the activation of JNK and p38, which initiate stress-induced cell death pathways. Importantly, $Rh1^{P37H}$-dependent retinal degeneration is suppressed by the expression of p35, an anti-apoptotic protein that inhibits caspases.[86]

Cell Death Caused by Recessive *nina* Mutations

A loss of Rh1 also leads to retinal degeneration. How does a lack of Rh1 affect survival of photoreceptors? Rh1 serves dual roles as it not only acts as the light receptor that triggers rhythmic Ca^{2+} influx, but also supports the morphogenesis of rhabdomeres. Interestingly, transgenic expression of a dominant active Rac, the small Rho GTPase involved in the formation of the actin cytoskeleton, prevents retinal degeneration in $ninaE^{117}$ flies.[102] Therefore defects in rhabdomere expansion can be overcome by the active elongation of the actin cytoskeleton. Retinal degeneration in *ninaE* also can be rescued by vitamin A deprivation, suggesting that accumulation of free retinal is toxic to photoreceptors.[72] Consistently, both *ninaB* and *ninaD* flies that are incapable of generating 11-cis retinal, exhibit no overt degeneration phenotype.[72] Free retinal may covalently modify both proteins and lipids. Indeed, a lipid-retinal adduct, N-retinylidene-N-retinyl ethanolamine, has been implicated in human retinal disease.[103] It is likely that defective rhabdomere morphogenesis due to a lack of Rh1 rhodopsin results from accumulation of free actin and 11-cis retinal, which may initiate apoptotic pathways.

The class III genes are critical for either Ca^{2+}or phospholipid-dependent modulation of visual signaling. The class III gene products include those mediating the distal events of the visual signaling, which can be subdivided into three groups (**Table 1**). The first group encodes proteins that modulate cytosolic Ca^{2+} levels including *calx* and *cnx*. CALX is the Na^+/Ca^{2+} exchanger[104] that exports Ca^{2+}, and CNX[50] controls the buffering of intracellular Ca^{2+}, both of which allow re-establishment and maintenance of a low cytosolic Ca^{2+} level. A lack of CALX or CNX results in Ca^{2+} overload, and light-dependent retinal degeneration.[50,104] CNX is also involved in Rh1 maturation (class II).

The second group encompasses genes that regulate the biosynthesis/recycling of PIP_2 including those well known retinal degeneration genes such as *rdgA* (retinal degeneration A),[29,105] and *rdgB* (retinal degeneration B).[106,107] This group also includes genes required for functional NORPA activity, such as ceramide kinase.[108] Mutations in the second group result in retinal degeneration because of either a lack of Ca^{2+}-dependent feedback modulation or the uncontrolled Ca^{2+} entry.

The recycling of PIP_2 is orchestrated by a series of enzymatic reactions catalyzed by the DAG kinase RDGA,[109,110] CDS (CDP-DAG synthase),[111] PIS (phosphatidylinositol synthase),[111] RDGB (a phosphatidylinositol transfer protein),[107,111] and SKTL (phosphatidylinositol-4-phosphate-5-kinase).[112] Perturbation of these enzymes may reduce the level of PIP_2 affecting both the visual signaling and morphology of photoreceptors. Both *rdgB* and *cds* display light-dependent degeneration, and the molecular mechanism involved in $rdgB^{113}$ appears similar to that in *norpA* (see below), while *rdgA* exhibits light-independent retinal degeneration.[110]

RDGA is critical for the conversion of DAG into phosphatic acid (PA).[105,114] A lack of RDGA thus extends the half-life of DAG leading to constitutive TRP channel activity,[29] and degeneration of photoreceptors.[105] Retinal degeneration caused by the persistence of DAG was also observed upon overexpression of *pld* (phospholipase D) in fly photoreceptors.[115] PLD generates PA, which can be further converted into DAG by LAZA (lazaro), a lipid phosphate phosphatase.[116,117] Similarly, persistent activation of TRP in dominant *Trp* alleles (e.g., Trp^{365}) leads to light-independent retinal degeneration.[118] In all cases, death of photoreceptors is triggered by excessive Ca^{2+} influx, which may result in the activation of Ca^{2+}-regulated proteases, phospholipases and endonucleases leading to destruction of cellular contents, and apoptosis.[119]

The third group of the class III includes *trp*,[120] *inaD*,[109] *inaC*,[46] and *norpA*,[28] whose encoded proteins directly or indirectly control the activity of the TRP Ca^{2+} channel (**Table 1**). Loss-of-function mutations in this group fail to support rhythmic Ca^{2+} entry affecting Ca^{2+}-dependent feedback regulation, which gives rise to light-dependent degeneration of photoreceptors. Degeneration in *norpA* has been especially well explored, serving as a model for the molecular events leading to retinal cell death.

Retinal Degeneration in Flies that Lack NORPA

In Drosophila photoreceptors, NORPA breaks down PIP_2 to generate the second messenger for gating the TRP Ca^{2+} channel. The rise of cytoplasmic Ca^{2+} orchestrates feedback regulation,[121] which is achieved in part by activating both CaMKII and RDGC. CaMKII regulates the phosphorylation of Arr2,[43,122] while RDGC catalyzes the dephosphorylation of Rh1,[41,42,123] both contributing to the inactivation of activated Rh1. Activated

Rh1 may become phosphorylated by rhodopsin kinase,[124] and Rh1 interaction with arrestins (Arr1 or Arr2) greatly diminishes its ability to turn on Gq.

In *norpA* photoreceptors, both RDGC and CaMKII fail to become activated resulting in hyperphosphorylated Rh1 yet unphosphorylated Arr2, both of which promote the formation of Rh1/Arr2 complexes leading to light-dependent retinal degeneration.[113] Similarly, *rdgC* photoreceptors have an elevated level of hyperphosphorylated Rh1, which also results in photoreceptor degeneration.[41,56] Retinal degeneration in *rdgC* and *norpA* appear to share similar mechanisms as degeneration depends on both Rh1 and Arr2.[41,56,113] Significantly, the interaction between Rh1 and Arr2 appears to be more persistent, as Arr2 is not readily released from the membrane when Rh1 becomes inactivated.[113] Based on these findings, it has been proposed that formation of a stable Rh1/Arr2 complex, and its internalization triggers retinal cell death.[56,113,125]

Internalization of the Rh1/Arr2 is the Initial Event Leading to Degeneration of Photoreceptor Cells

The mechanism that regulates the internalization of activated Rh1 appears similar to that of a prototypical GPCR in response to agonists, a phenomenon termed homologous desensitization. Here, activation of GPCR results in its phosphorylation, and phosphorylated GPCR recruits β-arrestin. Binding of β-Arrestin downregulates GPCR activity, and also promotes GPCR endocytosis thus reducing the receptor level in the plasma membrane. Internalized GPCR, depending on its affinity with β-arrestin, is sorted in the endocytic compartment for either recycling/reinsertion into the plasma membrane, or degradation and subsequent removal.[126]

The internalization of Rh1/Arr2 that initiates retinal degeneration may employ the clathrin-dependent endocytic pathway. Indeed, when dynamin is rendered inactive (as in *shibire* mutants), the degeneration phenotype in either *norpA* or *rdgC* is eliminated.[56,113] The interaction with the endocytic machinery may be regulated by phosphorylation of Arr2 as phosphorylated Arr2 fails to associate with purified clathrin cage in vitro.[56] However, internalization of the Rh1/Arr2 complex may occur via direct binding of Arr2 to the AP-2 adaptor complex, as a reduced AP-2 interaction in flies expressing modified Arr2 suppresses photoreceptor death in *norpA*.[127] Significantly, a lack of AP-2 interaction also results in retinal degeneration, indicating that endocytosis of Rh1 is required for the maintenance of photoreceptors.[127]

Endocytosed Rh1 is often destined for degradation, and is trafficked to the lysosome. This trafficking may become a rate-limiting step during massive internalization of Rh1 resulting in the accumulation of activated Rh1 in the Rab7-positive late endosome.[58] The endosome to lysosomal trafficking is regulated partly by the so-called 'granule group' genes including *car* (carnation),[128,129] *lt* (light),[130] and *cm* (carmine). Importantly, mutations in one of the 'granule group' genes lead to light-dependent degeneration, which appears to result from defective trafficking of internalized Rh1.[58] Indeed, by reducing the Rh1 level the degeneration phenotype of these trafficking mutants is greatly suppressed.[58] These findings strongly support the notion that defects in trafficking and degradation of photoactivated Rh1 are detrimental to R1-R6 photoreceptors.

It is likely that the endosomal retention of activated Rh1 initiates a cell death program. However, it is also possible that endocytic accumulation of Rh1 interferes with the uptake and/or trafficking of secreted proteins such as growth factors involved in photoreceptor survival. Interestingly, the endocytic turnover of activated Rh1 requires ceramidase secreted from neighboring cells.[131] Ceramidase is involved in the breakdown of ceramide to generate sphingosine; both sphingosine and ceramide are bioactive lipids that mediate diverse signaling including cell proliferation, differentiation and apoptosis.[132] Significantly, photoreceptor cells that are devoid of ceramidase undergo light-dependent degeneration, while overexpression rescues retinal degeneration of *norpA* flies.[133]

Role of Programmed Cell Death

The degeneration phenotype observed in *norpA* is consistent with inappropriate initiation of programmed cell death (PCD). To date, PCD can be subdivided into two common forms, apoptotic cell death (type I) and autophagic cell death (type II),[134] based on the underlying mechanisms. Type I PCD is characterized by the activation of caspases (cysteine-dependent aspartate-directed proteases).[135] In healthy cells, caspases (e.g., death caspase-1) are kept in an inactive state by the inhibitor of apoptosis (IAP, e.g., DIAP1), which inactivates caspases through ubiquitination and proteasomal degradation of pro-caspases.[136] In response to cell death signals, activation of RHG (RPR, HID and GRIM) results in proteosomal degradation of DIAP1, leading to increased levels of active caspases that initiate apoptosis (reviewed in refs. 137 and 138).

Type I PCD may be involved in the degeneration of *norpA* photoreceptors. However, genetically removing or reducing the cell death inducers from photoreceptors including RHG fails to prevent death of *norpA* photoreceptors.[139] Moreover, overexpression of DIAP1 is not able to suppress degeneration, nor is the expression of a dominant negative caspase (Dronc). Thus, retinal degeneration observed in *norpA* is not attributed to the caspase-dependent PCD, unlike that of *NinaE* mutants expressing ADRP-like Rh1 rhodopsin. The cell death of these dominant *NinaE* mutants can be abrogated by the expression of p35, a caspase inhibitor.[86,140] While the molecular events leading to apoptotic cell death are known, the mechanisms underlying type II autophagic cell death are less understood.

Morphologically, type II PCD is characterized by the presence of excess vacuoles or autophagosomes, which are formed during autophagy.[141] Autophagy is defined as a lysosome-dependent degradation process, which is a protective and self-renewal mechanism that allows cells to degrade damaged proteins and organelles, as well as to recycle obsolete cellular constituents (reviewed in ref. 142). Autophagy requires the participation of many *atg's* (autophagy related genes).[142] Several of the ATG proteins interact and collaborate to form the 'core machinery' to promote autophagosome assembly. Subsequently, most of the

core machinery is retrieved, and autophagosomes then fuse with lysosomes for initiating degradation of the encapsulated contents by lysosomal hydrolases (reviewed in ref. 142).

Role of Autophagy in Retinal Degeneration

Autophagy is an evolutionarily conserved mechanism for maintaining cellular homeostasis, and for multicellular development, and it can be upregulated by intracellular (e.g., ER stress) or extracellular stresses (e.g., pathogen infection, growth factor deprivation) (reviewed in ref. 143). Defective autophagy has been implicated in several pathological conditions including cancer, microbial infection and neurodegeneration.[144] Importantly, the activity of some ATG proteins is negatively regulated by TOR (target of rapamycin):[145] Inhibition of TOR promotes autophagy, whereas hyperactivation of TOR suppresses.

Inhibition of autophagy results in light-dependent photoreceptor death[146] while induction greatly reduces retinal degeneration in *norpA* flies. Thus, autophagy appears to have a protective role towards photoreceptor death caused by endosomal accumulation of activated Rh1. Consistently, a reduction of essential components including atg7/atg8, Psd (phosphatidylserine decarboxylase) and Ept (CDP-ethanolamine:diacylglycerol ethanolamine phosphotransferase) results in retinal degeneration,[147] which may be contributed by the accumulation of activated Rh1 in Rab7-positive late endosomes. Importantly, overexpression of Rab7 prevents death of photoreceptors in these *atg's* knockdown flies.[147] These results support the notion that induction of autophagy protects photoreceptor cells from degeneration possibly by promoting autophagic degradation of activated Rh1 in late endosomes. Although autophagy has been shown to relieve the ER stress, it fails to suppress retinal degeneration caused by the ER accumulation of misfolded Rh1 in ADRP-type *NinaE* mutants.[146]

A Summary of Diverse Degeneration Mechanisms

Many mutations lead to retinal degeneration. In general, the downstream events leading to death of photoreceptor cells fall into five distinct mechanisms contributed by (1) defective rhabdomere formation (class I), (2) a reduced Rh1 level [*ninaE* (class II)], (3) misfolded Rh1 [*NinaE* (LOF), *ninaA*, *cnx* (class II)], (4) trafficking defects associated with internalization of photoactivated Rh1 [some of class III; *NinaE* (GOF) (class II)], and (5) the Ca^{2+}-mediated excitatoxicity due to overactive TRP [*Trp*(GOF), *rdgA* (class III); *arr2*, *NinaE*(GOF) (class II)] (**Table 1**). It is important to note that some mutations may initiate multiple mechanisms to trigger cell death. For example, expression of constitutively active Rh1 (*NinaE*) promotes massive endocytosis of activated Rh1, and also orchestrates the Ca^{2+}-dependent excitatoxicity due to un-regulated Ca^{2+} influx.

During the terminal differentiation of photoreceptors, elaboration of rhabdomeres provides the specialized compartment for housing the visual signaling proteins. Misformed rhabdomeres fail to support the subcellular localization, affecting both the visual signaling and maintenance of photoreceptors. The most abundant membrane protein in the rhabdomere is Rh1, which is critical for rhabdomere assembly and elongation. Loss-of-function mutations affecting Rh1 biosynthesis usually lead to photoreceptor degeneration. Photoreceptor death may be caused by defective rhabdomere biogenesis and by the accumulation of cytotoxic 11-cis retinal.

Newly translated Rh1 may form oligomeric complexes to assist its folding and maturation. Mutations in Rh1 or proteins involved in its post-translational maturation lead to accumulation of Rh1 in the ER resulting in degeneration of photoreceptors. In *NinaE* heterozygotes, co-expression of misfolded with wild-type Rh1 has more debilitating consequences as it causes overproliferation of the ER cisternae, and a more severe degeneration phenotype than in *NinaE* homozygotes.

Mutants affecting the intermediate steps of the visual signaling cascade may lead to accumulation of photoactivated Rh1, due to a lack of the Ca^{2+}-dependent feedback regulation. Activated Rh1 forms a stable complex with Arr2 resulting in its rapid internalization. Internalization of massive Rh1 may overwhelm the endocytic pathway, and persistence of activated Rh1 in the endosome may activate pro-apoptotic signaling leading to the demise of photoreceptors.

Unregulated visual signaling also contributes to the pathogenesis of retinal degeneration, which could be due to either gain-of-function mutations in key signaling proteins involved in the activation of visual cascade (e.g., Rh1 rhodopsin or TRP) or loss-of-function mutations involved in either negative regulatory mechanisms (e.g., *arr2*), or inactivation of the second messenger (e.g., *rdgA*). In all cases, the activity of the downstream TRP Ca^{2+} channel fails to become properly regulated leading to constitutive Ca^{2+} entry and the Ca^{2+}-mediated excitatoxicity.

Conclusions

Based on the proximal events that initiate death of photoreceptors, here I divide most of the known retinal degeneration mutations into three classes. It appears that defects associated with trafficking of Rh1 are the major cause of retinal dystrophy (**Fig. 3**). This may be due to the fact that Rh1 is the most abundant membrane protein in photoreceptors (65% estimated by freeze fracture EM analysis[148]). In ADRP-type *NinaE* mutants, misfolded Rh1 hinders the maturation of the wild-type Rh1 by forming oligomeric Rh1 complexes. The ER accumulation of Rh1 complexes orchestrates the UPR by activating the transcription of several proteins to promote re-folding and degradation of misfolded proteins. Conceivably, the UPR is protective as its induction suppresses photoreceptor death. Persistent ER stress caused by the accumulation of misfolded Rh1, however, negatively impacts the survival of the R1-R6 photoreceptors. The links between the UPR and programmed cell death remain to be explored.

While photoactivated Rh1 may be taken up and targeted for degradation, the sorting at the late endosome may become a rate-limiting step to cope with the massive internalization of Rh1. The endosomal accumulation of Rh1 may trigger retinal degeneration. Significantly, induction of autophagy facilitates removal and degradation of internalized Rh1, and protects retinal degeneration

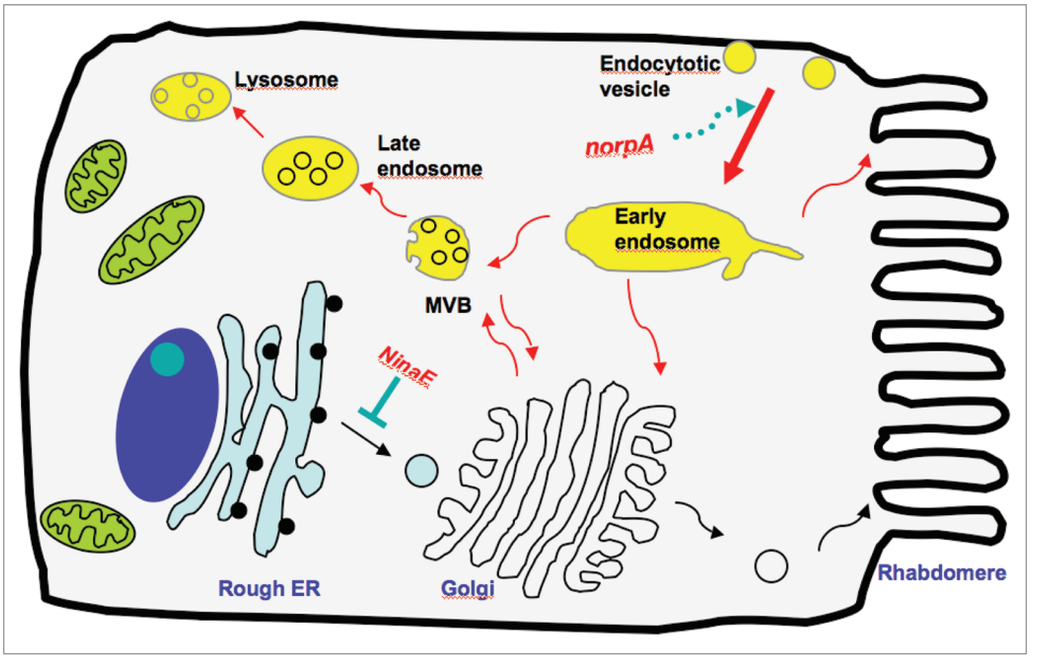

Figure 3. Retinal degeneration contributed by defective trafficking of Rh1 in *NinaE* and *norpA* photoreceptors. Shown is a diagram depicting trafficking of newly translated Rh1 rhodopsin (black arrows) and of internalized photoactivated Rh1 rhodopsin (red arrows) in photoreceptors. Rh1 opsin is translated, and undergoes glycosylation and maturation in the rough ER. Mature opsin is conjugated with 11-cis 3-hydroxy-retinal to become rhodopsin, which is transported through the Golgi for its delivery to the rhabdomere. In *NinaE* heterozygotes, folding deficient Rh1 may form oligomeric complexes with wild-type Rh1 affecting the transport of wild-type Rh1, and resulting in degeneration of photoreceptors. In *norpA* photoreceptors, photoactivated Rh1 becomes rapidly internalized. However, the endosome to lysosome trafficking of endocytosed Rh1 becomes a rate-limiting step leading to accumulation of activated Rh1 in the late endosome, which leads to death of photoreceptor cells. MVB, multivesicular body.

of *norpA* flies. In both *norpA* and *NinaE* photoreceptor cells, the trafficking defects associated with activated or misfolded Rh1 can be corrected by reducing the expression of Rh1 via dietary vitamin A restriction. Vitamin A deprivation is also beneficial for mutants affecting rhabdomere morphogenesis (class I), as a low level of Rh1 alleviates the demand of the compromised rhabdomere. Moreover, in flies containing a reduced level of Rh1, vitamin A restriction limits the cytotoxicity of free 11-cis retinal.

Retinal degeneration in Drosophila occurs via diverse mechanisms, some of which also are involved in that of humans. Our knowledge in the fly will provide a better understanding into the molecular basis of prevalent eye diseases such as ADRP. The distinct mechanistic insights uncovered in Drosophila may bring new avenues towards rationale therapeutic interventions for treating human retinal dystrophy. For example, administration of the UPR activators may alleviate death of photoreceptors in ADRP patients that express misfolded rod rhodopsin. Likewise, drugs that induce autophagy through the inhibition of mammalian target of rapamycin, mTOR, may slow down the progression of degeneration contributed by mutant rod rhodopsin that displays abnormal endocytosis. The promise of these pharmacological agents is also enhanced by the accessibility of the eye for drug application: drugs can be formulated as eye drops for topical application, thus minimizing their systemic toxicity.

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