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The Ubiquitin–Proteasome System in Retinal Health and Disease

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Abstract The ubiquitin–proteasome system (UPS) is the main intracellular pathway for modulated protein turnover, playing an important role in the maintenance of cellular homeostasis. It also exerts a protein quality control through degradation of oxidized, mutant, denatured, or misfolded proteins and is involved in many biological processes where protein level regulation is necessary. This system allows the cell to modulate its protein expression pattern in response to changing physiological conditions and provides a critical protective role in health and disease. Impairments of UPS function in the central nervous system (CNS) underlie an increasing number of genetic and idiopathic diseases, many of which affect the retina. Current knowledge on the UPS composition and function in this tissue, however, is scarce and dispersed. This review focuses on UPS elements reported in the retina, including ubiquitinating and deubiquitinating enzymes (DUBs), and alternative proteasome assemblies. Known and inferred roles of protein ubiquitination, and of the related, SUMO conjugation (SUMOylation) process, in normal retinal development and adult homeostasis are addressed, including modulation of the visual cycle and response to retinal stress and injury. Additionally, the relationship between UPS dysfunction and human neurodegenerative disorders affecting the retina, including Alzheimer's, Parkinson's, and Huntington's diseases, are dealt with, together with numerous instances of retina-specific illnesses with UPS involvement, such as retinitis pigmentosa, macular degenerations, glaucoma, diabetic retinopathy (DR), and aging-related impairments. This information, though still basic and limited, constitutes a suitable framework to be expanded in incoming years and should prove orientative toward future therapy design targeting sight-affecting diseases with a UPS underlying basis.

Keywords Neurodegenerative disorders · Oxidative stress · Proteasome · Retinal diseases · Ubiquitin

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The Proteasome: An Overview

Degradation of proteins can be executed by various proteolytic systems, including lysosomal degradation, chaperone-mediated autophagy, and substrate-specific degradation by the ubiquitin–proteasome system (UPS). These different mechanisms of protein elimination in the cell serve different physiological requirements and allow the organism to adapt to changing environmental and pathophysiological conditions. The main of such mechanisms is the clearance of intracellular proteins carried out by the UPS [1]. This process is highly specific, and the different proteins that are removed through this system have distinct half lives that range from a few minutes (e.g., the tumor suppressor protein p53) to several days (e.g., muscle proteins actin and myosin), and even up to a few years (crystallins) [2]. The UPS is responsible for the elimination of damaged, misfolded, or obsolete proteins that originate in the cell under both normal and pathological conditions [3–6] and, as described in many cell types and tissues, is involved in the modulation of many different biological processes by carrying out the degradation of a huge number of regulatory proteins. Among such processes are cell cycle

[7–11], proliferation [12], differentiation [13], organogenesis, development, signal transduction, transcriptional regulation (histone ubiquitination vs. acetylation) [14], receptor down-regulation, and synaptic plasticity [15].

The degradation of a protein via the UPS involves two successive steps: first, its tagging by the covalent attachment of multiple ubiquitin molecules and second, the degradation of the tagged protein by the proteasome complex with release of free and reusable ubiquitin (Fig. 1). This is a highly evolutionarily conserved 76-residue polypeptide, whose attachment to the protein substrate proceeds via a well-known three-step cascade mechanism. Initially, a ubiquitin-activating enzyme, E1, activates ubiquitin in an adenosine triphosphate (ATP)-requiring reaction to generate a high-energy thiol ester intermediate, E1-S-ubiquitin. Then, one of several E2 enzymes, also called ubiquitin-carrier proteins or ubiquitin-conjugating enzymes (UBCs), transfers the activated ubiquitin moiety from E1 via an additional high-energy thiol ester intermediate, E2-S-ubiquitin, to the substrate protein, which in turn, is specifically bound to an E3 ubiquitin-protein ligase. In this process, E3 enzymes play a key role in the ubiquitin-mediated proteolytic cascade by acting as one of the specific recognition factors of the system. By the successive addition of activated ubiquitin moieties, a polyubiquitin chain is synthesized that is recognized by the downstream proteasome complex (Fig. 1). The minimal tail length for proteasomal targeting is a chain of four ubiquitin monomers linked between them by lysine residue at position 48 (K48) [16]. Depending on the extent and nature of bonds between ubiquitin molecules, these polyubiquitin chains can encode distinct information, tagging proteins not only for their degradation but also for their implication in a variety of cellular functions related to signal transduction, cell division, and DNA repair [17–19], among many others [20]. In the human genome, there are two different encoded E1, at least 38 E2, and 600–1,000 estimated E3 enzymes [21]. There are four known families of E3 enzymes, dubbed as really interesting new gene (RING) finger, homologous to E6AP carboxyl terminus (HECT), Skp1-Cul1-F box (SCF), and anaphase-promoting complex (APC) families [1, 8, 12]. However, it is important to remark the existence of a small, but still growing, number of cases where protein degradation by the UPS occurs in a ubiquitin-independent manner. Proteins exhibiting intrinsic structural determinants [22, 23], mildly oxidized residues [24, 25], or that have been subjected to denaturing conditions [26] are examples of nonubiquitinated polypeptides carrying signals for proteasomal degradation.

Ubiquitination is substrate specific, but in addition to E3 ligases, modifying enzymes such as kinases and chaperones or DNA sequences to which substrates may bind also play an important role in the recognition process. In many cases, substrate proteins must be phosphorylated at specific sites before they can be recognized by the E3 ubiquitin ligase, or the activity of the E3 ligase itself may be regulated by post-translational modification, such as phosphorylation. The stability of other proteins may depend on their association with

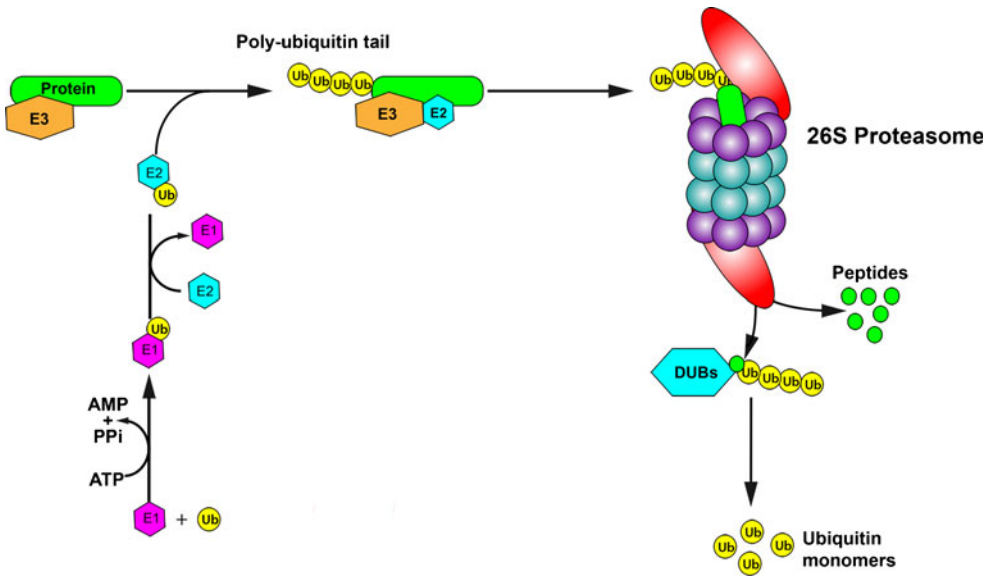

Fig. 1 Ubiquitin–proteasome system (UPS). Intracellular misfolded, damaged, and obsolete proteins are degraded by the UPS in a process in which an E1 ubiquitin-activating enzyme first binds to ubiquitin (Ub) with ATP hydrolysis. The so-activated ubiquitin is then transferred to an E2 ubiquitin-conjugating enzyme, which carries the E2-bound ubiquitin to a substrate protein that is specifically bound to an E3 ubiquitin protein ligase. After several rounds of ubiquitin molecule addition, degradation of the polyubiquitinated substrate occurs inside the proteasome, releasing as products free peptides and reusable ubiquitin moieties. Ubiquitin recycling is carried out by deubiquitinating enzymes (DUBs). The two α- and β-rings of the proteasome 20S core particle are each composed of seven subunits, which are labeled in *purple* and *cyan*, respectively

ancillary proteins, such as molecular chaperones, which act as recognition elements linking them to the appropriate E3 ligase. Many transcription factors must dissociate from their specific DNA-binding sequences in order to be recognized by the system. Finally, the stability of other proteins depends on their hetero- or homooligomerization, with only monomers being degraded [27–29].

The proteasome exists as different oligomeric assemblies, including the 20S and 26S complexes and the immunoproteasome, which is implicated in the processing of antigens for presentation by MHC class I molecules. Each of these proteasomes exhibits a certain specificity in its function that is defined both by the regulatory particle (19S or 11S) associated with its catalytic core and the composition of its catalytic subunits. Binding of the regulators 19S and 11S to the 20S proteasome yields the complexes known as the 26S proteasome and the activated 20S proteasome, respectively. The main difference between them is that the 26S assembly degrades polyubiquitinated proteins in an ATP-dependent manner, whereas the 20S degrades nonubiquitinated proteins and peptides without ATP expenditure [27, 30]. The ability to reconfigure the proteasome during changing cellular stages, together with the multiplicity of E2 and E3 enzymes, allows for further levels of specificity and adaptation of the UPS to varying intra- and extracellular conditions. Furthermore, while most cells contain a heterogeneous population of 20S, 26S, and immunoproteasome assemblies, the relative ratios between the three proteasome subtypes is cell-specific and can be altered under different cellular states [31, 32].

The most common form of the proteasome is known as the 26S proteasome, a huge ~2.5-MDa multicatalytic protease assembly highly conserved among all eukaryotes. It is composed of two subcomplexes: a 20S core particle that carries out its catalytic function through its protease subunits [33] and two 19S regulatory particles that modulate the core function. The 20S core particle is a barrel-shaped structure composed of four stacked rings: two identical outer α-rings and two identical inner β-rings. The eukaryotic α- and β-rings are each composed of seven distinct subunits (Fig. 1). The two inner β-rings contain the proteolytic active sites facing inwards into a sequestered proteolytic chamber [34, 35]. Each extremity of the 20S barrel is capped by a 19S regulatory particle [36] composed of ≥19 different subunits [37] and that can be further dissociated into two subcomplexes: the base, which binds directly to the 20S core particle, and a peripheral lid [36]. An important function of the 19S regulatory particle is to recognize and bind to polyubiquitinated proteins and other potential proteasome substrates. This regulatory particle also exhibits deubiquitinating activity, having the ability to reduce the length of the polyubiquitin chain (editing) or even to remove it completely, in order to render proteins as better substrates for the peptidase activity of the 20S core particle [30, 38]. Another role of this particle includes channel opening, which allows entry of the substrate into the proteolytic chamber and its unfolding (reverse chaperone activity) in such a way that it fits through the narrow proteasomal channel. These two actions require a high expenditure of energy, which is provided by six different ATPase subunits contained in the 19S regulatory particle [34, 39–42]. After degradation of the substrate, short peptides are released, together with reusable ubiquitin. Despite the possibility that this particle might exhibit intrinsic chaperone-like activity, increasing evidence links chaperones to proper proteasome function [43, 44]. In this light, misfolded and aggregated proteins in cells are exposed to chaperone-mediated refolding and are degraded by proteasomes if refolding is impossible [45, 46]. In particular, to recognize misfolded, unfolded, and impaired proteins, the UPS uses HSP90 and HSP70 chaperones, together with cochaperones with E3 ubiquitin ligase activity, such as C-terminus of HSC70-interacting protein (CHIP), which constitute a bridge between the UPS and chaperones. By interacting with these and E2 enzymes, CHIP catalyzes the ubiquitination of chaperone-bound proteins [47]. It has been suggested that Kelch-like protein 7 (KLHL7), a substrate-specific adaptor of the cullin 3 (CUL3)-based E3 ligase complex, participates as a chaperone in the ubiquitin–proteasome protein degradation pathway, like other Kelch-like family members such as KLHL9, KLHL12, and KLHL13 [48, 49]. Chaperone activity has also been unveiled for β- and γ-synucleins, which can protect cultured photoreceptors from mutant opsin accumulation [50].

Following an inflammatory stimulus, the constitutive catalytic subunits of the 26S proteasome β1, β2, and β5 can be replaced in nascent proteasomes by the inducible subunits LMP2 (β1i), MECL-1 (β2i), and LMP7 (β5i), respectively [51–53]. The subunit composition of proteasomal core particles becomes thus altered to form the immunoproteasome. This generates a spectrum of peptides different from that released by the standard, 26S proteasome, producing a set of antigenic peptides that bind to MHC class 1 molecules for presentation to the immune system. While this role in generating immunogenic peptides is clearly established [54, 55], the expression of the immunoproteasome in noninflamed immune-privileged tissues and organs, such as the retina [56, 57], brain [58, 59], and lens epithelium [60], implies that this complex might have other nonimmune functions.

After proteolytic degradation by the core particle of ubiquitinated proteins, a series of deubiquitinating enzymes (DUBs) come into action to provide ubiquitin monomers for recycling. These enzymes are a large group of thiol esterases that specifically cleave ubiquitin-linked molecules at the terminal carbonyl group of the last residue of ubiquitin (Gly76). The largest and most diverse DUBs are those

comprising the ubiquitin carboxyl-terminal hydrolases (UCHs) or type 1 and ubiquitin-specific processing proteases (UBPs, USPs) or type 2 families. Known members of the UCH family are mammalian UCH-L1/PGP9.5, UCH-L2, and UCH-L3 [61–63] as well as BAP1 [64]. Mammalian UCH-L1 [63, 65] is specifically found in central and peripheral neurons [66, 67], where it accumulates in inclusion bodies characteristic of neurodegenerative diseases [68] but does not appear to have any associated factors [69]. UCH-L2 is widely distributed in all kinds of tissues, suggestive of a housekeeping function, whereas UCH-L3 is abundantly expressed in hematopoietic cells [66]. Members of the UBP family include deubiquitinating isopeptidases ISOT1, ISOT2/T and ISOT3, as well as DUB1 and DUB2.

Small ubiquitin-like modifier (SUMO) proteins display similarities to ubiquitin in their structure, their ability to be reversibly ligated to other proteins, and their mechanism of ligation. In contrast to ubiquitin, which mainly tags proteins for proteasome-mediated degradation, covalent modification by SUMO can also affect the regulation of protein–protein interactions, subcellular localization, and protein stability. Even, in some cases, SUMO can antagonize the proteasome pathway by competing with ubiquitin [70–72] and can target proteins not only for degradation but also to other cellular fates. Enzymes involved in SUMO conjugation (SUMOylation) resemble those in charge of ubiquitin conjugation [73–75]. Actually, SUMOylation requires the E1 ubiquitin-activating heterodimeric enzyme AOS1/UBA2 and the E2 enzyme UBC9 [76]. In contrast, several SUMO E3-like factors have been identified in mammalian cells, such as RanBP2 and the protein inhibitor of activated STAT (PIAS). RanBP2 is clearly distinct from E3 ligases of the PIAS family [77–80], both in its amino acid sequence and in its independence of a RING-like domain in its molecule for catalytic activity.

Known Components of the Retinal Ubiquitin–Proteasome System

Ubiquitin is present throughout the retina, although it is particularly prominent in ganglion cells, whose axons form the optic nerve, and in the retinal pigment epithelium (RPE). The majority of endogenous ubiquitin in the retina is found covalently attached to many different target proteins [3, 81–84].

A systematic, comprehensive study of the UPS components in the retina has not been undertaken. However, numerous studies exist in which the detection of a subset of proteasomal subunits and/or other elements of the UPS has been achieved in retinal homogenates, tissue slices, or retina-derived cell lines. Additionally, the analysis of the UPS structure and function in the retina under both physiological and pathological conditions has revealed the presence of a good number of proteins belonging to this system, which are summarized in Table 1 and will be dealt with separately in subsequent sections of this review.

Retinal Ubiquitinating Enzymes: Ubiquitin-Activating (E1), Ubiquitin-Conjugating (E2), and Ubiquitin Ligases (E3)

E1 activity, at least four E2 enzyme activities ($E2_{14K}$, $E2_{20K}$, $E2_{25K}$, and $E2_{35K}$), proteasome activity, and deubiquitinating activity have been reported in rod outer segments, and both ubiquitinated rhodopsin and transducins have been isolated from this source [83]. As for the RPE, ubiquitin conjugates can be formed de novo with exogenous histone 2A, oxidized RNase, transducin, and β-lactoglobulin using a human RPE cell supernatant as the source of UBCs [81, 82]. Recombinant human E2 enzymes UbcH5 and UbcH7 selectively catalyze ubiquitination in vitro of γ-transducin isolated from rod outer segments, which leads to degradation of the βγ-transducin heterodimer [83, 85]. Of the enzymes that comprise the mouse class III of E2 enzymes, UbcM2 appears to be the most highly expressed in the retina, followed by UbcM3 and then by UBE2E2 [86]. Also, UbcM2 is the only one of these three E2 enzymes that localizes to photoreceptors and to the nuclei of RPE cells [86].

Despite the discovery of a number of E3 ubiquitin ligases in the retina, not all their substrates have been identified to date in this tissue, and the function(s) of most of these enzymes remain(s) an open question. Although E3 RING finger enzymes are the most abundant and studied ubiquitin ligases in the retina (Table 1), the E3 enzymes HERC6 and NEDD4, belonging to the HECT family, have been detected in retinal ganglion cells [87, 88] and KLHL7, a substrate adaptor for the CUL3-based E3 ligase complex, is expressed in rod photoreceptors [89]. Topoisomerase I-binding RS protein (TOPORS) is an E3 RING finger ubiquitin ligase that is expressed in the human retina [90] and can ubiquitinate and regulate the level of the transcription factor p53 by targeting it for proteasome degradation [91]. UBR1, another E3 RING finger enzyme, is ubiquitously expressed in the adult, with the highest levels found in skeletal muscle and heart [92], although it has been detected at low levels in the outer segments of photoreceptor cells [93]. Tripartite motif-containing protein 2 (TRIM2) is an E3 ubiquitin ligase whose ubiquitination activity is confined to its RING finger domain [94]. In the mouse retina, a high expression of this protein is found in the outer plexiform, inner nuclear and ganglion cell layers. TRIM2 is an UbcH5a-dependent enzyme that binds to and regulates neurofilament light subunit (NF-L) metabolism by ubiquitination. In this regard, mice deficient in TRIM2 exhibit swollen axons in several brain areas and in the retina, an axonopathy that is characterized by the disorganization of intermediate filaments and accumulation of NF-L in axons, followed by progressive neurodegeneration. Retinas of TRIM2-deficient

Table 1 Retinal known UPS components

Protein	Retinal UPS component	References
E1	E1 ubiquitin-activating enzyme	[83]
$E2_{14K}$/UBE2B	E2 ubiquitin-conjugating enzyme	[83]
$E2_{20K}$/UBE2H	E2 ubiquitin-conjugating enzyme	[83]
$E2_{25K}$/UBE2K	E2 ubiquitin-conjugating enzyme	[83]
$E2_{35K}$	E2 ubiquitin-conjugating enzyme	[83]
UbcM2/UBE2E3	E2 ubiquitin-conjugating enzyme	[86]
UbcM3/UBE2E1	E2 ubiquitin-conjugating enzyme	[86]
UbcH8/UBE2E2	E2 ubiquitin-conjugating enzyme	[86]
TOPORS	E3 RING finger ubiquitin ligase	[90]
UBR1	E3 RING finger ubiquitin ligase	[93]
TRIM2	E3 RING finger ubiquitin ligase	[94]
Parkin	E3 RING finger ubiquitin ligase	[99]
SIAH1	E3 RING finger ubiquitin ligase	[95]
MDM2	E3 RING finger ubiquitin ligase	[124]
HERC6	E3 HECT ubiquitin ligase	[87]
NEDD4	E3 HECT ubiquitin ligase	[88]
KLHL7	Substrate adaptor for the CUL3-based E3 ligase	[89]
RanBP2	E3 SUMO ligase	[76]
PIAS3	E3 SUMO ligase	[115]
UCH-L1/PGP9.5	DUB (deubiquitinating enzyme)	[99–103]
UCH-L3	DUB (deubiquitinating enzyme)	[106]
UBH1/USP12	DUB (deubiquitinating enzyme)	[107]
UHX1/USP11	DUB (deubiquitinating enzyme)	[108]
α6	Structural proteasome subunit	[147]
β1	Structural proteasome subunit	[110]
β5	Structural proteasome subunit	[110]
LMP2/β1i	Inducible immunoproteasome subunit	[56, 57, 110, 111]
LMP7/β5i	Inducible immunoproteasome subunit	[56, 57, 110, 111]
MECL1/β2i	Inducible immunoproteasome subunit	[142]
PA28	Proteasome 11S regulatory subunit	[110]
P112	Proteasome 19S regulatory subunit	[113]

mice display a thinning of the inner nuclear layer and a decreased number of ganglion cells. Consistently, the thickness of the outer plexiform layer is also reduced, whereas the size of the photoreceptor layer is not altered [94]. Seven in absentia homolog (SIAH) is another E3 RING finger enzyme that is present in retinal Müller glial cells [95].

Parkin, encoded by the *PARK2* gene, is an E3 RING finger ubiquitin ligase [96, 97] involved in Parkinson's disease (PD) [98]. Parkin interacts with E2 enzymes by virtue of its RING finger domains and with the 26S proteasome (RPN10 subunit) by its N-terminal ubiquitin-like domain (UBL) and is also able to ubiquitinate itself and promote its own degradation. Results from our group have evidenced expression of *PARK2* at the mRNA and protein levels in the neural retina of all mammals studied, including mouse, rat, bovine, monkey, and human [99]. By immunohistochemical methods, we have reported parkin to localize in photoreceptors and in the inner nuclear layer, including many, but not all horizontal, bipolar and amacrine cells [99]. As well, parkin was present in most neurons of the ganglion cell layer in the murine retina, where its levels were especially prominent.

Retinal Deubiquitinating Enzymes

The first DUB found in the retina was the product of the *PGP9.5* gene, the neuron-specific protein UCH-L1 [100–102], which localizes to horizontal and ganglion cells in vertebrate retinas [103]. We have shown expression of *UCHL1* at the mRNA and protein levels in the neural retina of a number of mammalian species, where UCH-L1 distributes through horizontal, bipolar, and amacrine cells in the inner nuclear layer as well as in ganglion cells [99]. This protein is also found through the entire length of cone

photoreceptors, its localization also extending to RPE cells [104, 105]. A UCH-L1 homolog, the ubiquitous ubiquitin C-terminal hydrolase UCH-L3, is present as well in the retina and is enriched in the inner segments of mouse photoreceptors [106].

Regarding UBP enzymes, ubiquitin-hydrolyzing enzyme 1 (UBH1), also called ubiquitin-specific protease 12 (USP12), has been detected in the neural retina [107]. Also, a gene located in the human X chromosome was cloned and named *UHX1* (ubiquitin hydrolase on the X chromosome), which codes for the so-called ubiquitin-specific protease 11 (USP11), a protein exhibiting high levels of expression in the human retina [108]. Interestingly, *UHX1* has regions of similarity over its entire length to the *fat facets* gene of *Drosophila*, which encodes two proteins involved in fly retinal development [109]. This makes envisionable that USP11 could be involved in retinal cell fate determination in mammals as well.

Retinal Immunoproteasome

The immunoproteasome subunits LMP2 (β1i) and LMP7 (β5i) have been detected in both rat and human retinas [56, 57, 110] (Table 1). In this tissue, immunoproteasome labeling was heaviest in the RPE and in specific layers of the neural retina, such as the inner segments of photoreceptors and the outer and inner plexiform layers [111]. Besides, the immunoproteasome content is approximately twofold higher in the normal retina than in the brain, suggesting a role in maintaining retinal homeostasis [111].

Retinal SUMOylating Enzymes

Concerning the SUMOylation pathway, expression of the E3 SUMO-protein ligase RanBP2 [76] is highly restricted to the retina, being present at especially high levels in cone photoreceptors [112, 113], where it is involved in the biogenesis of opsins [114], i.e., the light-sensitive G protein-coupled receptors that initiate visual signal transduction. Also found in the retina is PIAS3, an E3 SUMO ligase that functions as well as a transcriptional coregulator. This protein is selectively expressed in developing photoreceptors in the mouse retina and is involved in their cell-fate specification [115].

Function of the Ubiquitin–Proteasome System in the Maintenance of Retinal Health

The proposed functions of the UPS in the retina include roles in differentiation, development, signal transduction, and response to a variety of stresses. It is important to emphasize that ubiquitination of a protein, in addition to targeting it for proteasomal degradation, can alter its stability, activity, interactions with other macromolecules, and/or intracellular localization, and instances of all these effects can be found in retinal proteins.

Role of the Ubiquitin–Proteasome System in the Neural Retina

Retinal Development

Proteasomes can promote either cell death or survival, depending on the stage of cell development, through the degradation of regulatory proteins that inhibit or promote, respectively, the apoptotic process [116, 117]. During the first week after birth, the rat retina contains cells at various stages of development, including proliferating and postmitotic cells, and among the latter, both undifferentiated and differentiated cells [118]. In this regard, chemical inhibition of the proteasome does not prevent programmed death of differentiated retinal cells, whereas it induces apoptosis mainly in postmitotic cells within the neuroblastic, proliferative layer of the retina [119]. These results show that sensitivity to cell death induced by proteasome inhibitors defines a window of development from the last round of cell division to early (postmitotic) stages of differentiation in the retina.

During development of the visual system, new ganglion cell axons extend along earlier formed axons of this same cell type, which constitute the only area in the environment of the growth cone providing DM-GRASP, a cell adhesion molecule that serves as a substrate pathway. The interactions of DM-GRASP present on the substrate axons with DM-GRASP molecules on the advancing growth cones are critical for proper axonal orientation during retinal morphogenesis [120]. In this process, the ubiquitin-mediated endocytosis of DM-GRASP is crucial in modulating its concentration at the cell surface and thereby its function in axon navigation [121].

SUMOylation of photoreceptor-specific transcription factors appears to be a key mechanism for normal differentiation of cones and rods. In this light, the transcriptional coregulator with E3 SUMO ligase activity, PIAS3, interacts physically with and SUMOylates the NR2E3 retinogenic transcription factor when it is bound to the promoters of cone-specific genes, converting it into a potent repressor in order to ensure that only rod-specific genes are expressed in rod photoreceptors [115].

Visual arrestin plays a key role in the regulation of rhodopsin signaling in rod photoreceptors [122]. Subcellular localization of signaling molecules is vital for their biological function, and their distribution between the nucleus and the cytoplasm is particularly important for proteins that directly or indirectly regulate transcription. This is the case

for the protein kinase JNK3, which phosphorylates and activates the transcription factor c-Jun, a component of the proapoptotic transcription complex AP-1 in photoreceptors, and also the E3 ubiquitin ligase MDM2, which ubiquitinates p53 protein and targets it for proteasomal degradation, thereby suppressing p53-mediated apoptosis [123, 124]. Visual arrestin has the ability to bind JNK3 and MDM2 and to relocalize them from the nucleus to the cytoplasm. In this manner, the ability of visual arrestin to dramatically change the nucleocytoplasmic distribution of JNK3 and MDM2 may play an important role in neuronal survival.

Modulation of the Visual Cycle

Phosducin is a regulator of G protein-coupled signal transduction found at high levels in photoreceptor outer segments [125], where it binds with high affinity to and inhibits the active $\beta\gamma$ subunits of the heterotrimeric G protein transducin [126]. In this fashion, phosducin hinders signal transmission from light-activated rhodopsin via transducin in rods, thus playing a role in dark/light adaptation in the retina [127, 128]. Additionally, phosducin, together with phosducin-like proteins, has been shown to interact with a 26S proteasome ATPase subunit, SUG1 [129, 130], suggesting that phosducin function could be post-translationally regulated by ubiquitination. It is also noticeable that SUMOylation maintains phosducin stability in the bovine retina by preventing its ubiquitin-mediated proteasomal degradation. SUMOylation also completely disrupts phosducin binding to the $G\beta\gamma$ subunits of transducin [131], thereby allowing activation of the latter and consequently modulating positively the visual phototransduction cascade.

It has been shown that the C-terminal supradomain of the E3 SUMO ligase RanBP2, composed of the Ran-binding domain 4 (RBD4) plus the cyclophilin domain (CY), selectively associates in cones with red/green opsins [132]. This RBD4-CY supradomain of RanBP2 is responsible for its chaperone activity, avoiding opsin self-aggregation and enhancing in vivo production of functional opsin receptors [114, 132]. Another RanBP2 domain, the cyclophilin-like domain (CLD), interacts with the P112 subunit of the 26S proteasome 19S regulatory complex in retinal extracts [113]. This interaction is thought to constitute an excellent surveillance mechanism for the proper function of opsins, which upon misprocessing may be driven by RanBP2 to the proteasome pathway.

The content of rhodopsin in the outer segments of rod photoreceptors is maintained by two processes involving (1) the shedding of outer segment tips and their phagocytosis by RPE cells and (2) the de novo membrane synthesis at the outer segment base [133]. The adjustment to a new lighting environment is, in part, achieved by changes in disc-shedding patterns and is associated with the modulation of rhodopsin levels within outer segment disks [134, 135]. The ubiquitin-dependent pathway is involved in regulating the content of phototransduction protein levels not only in rod outer segments [83] but also in inner segments, where the bulk degradation process, termed autophagy, occurs as well [136]. In the rat retina, autophagic vacuoles at this location contain rhodopsin and ubiquitin, [137], suggesting a possible mechanism to regulate the rhodopsin content of outer segments through its ubiquitin-regulated degradation by autophagy in the inner segments [138]. This idea is corroborated by the dramatic increase in autophagic vacuoles taking place when an abrupt increase in light intensity occurs and is associated with a downregulation of rhodopsin levels [137]. Thus, the removal of excess rhodopsin through ubiquitination can constitute a protective mechanism against light-induced retinal damage.

Melatonin functions locally in the retina as a neuromodulator, regulating circadian rhythms and physiology in the retinal network [139, 140]. Binding of the chaperone 14-3-3 to the melatonin-synthesizing enzyme arylalkylamine N-acetyltransferase (AANAT) in photoreceptors is regulated by light, with relevant functional consequences [141]. In this context, light disrupts the complex formed by AANAT and 14-3-3, leading AANAT to its catalytic inactivation, dephosphorylation, and proteasomal degradation [141]. Therefore, light acts directly on retinal photoreceptors to decrease melatonin synthesis and protein levels by a mechanism involving proteasomal degradation [141].

It has been recently reported that the immunoproteasome has a previously unrecognized role in visual signal transmission. In this context, deficiency of retinal immunoproteasome is associated with both rod and cone pathway defects, causing impairments in bipolar cell response and consequently affecting signal transmission [142].

Oxidative and Nitrosative Stress Responses

The capacity of the UPS to degrade proteins in cells and tissues becomes altered following oxidative and/or nitrosative stress(es), and this response can be different depending on the magnitude of the stress condition. Although research focusing on the degradation of oxidized proteins in the neural retina and RPE is sparse, it is known that mild to moderate oxidative stress increases susceptibility of proteins to degradation and enhances the proteolytic capacity of the UPS, thereby promoting intracellular protein degradation. In this context, E1 and E2 activities of the ubiquitin conjugation system increase in human lens and RPE cells in response to mild oxidative stress [4]. The increase in the level and activity of the retinal immunoproteasome detected in age-related macular degeneration (AMD) disease might also be related to mild oxidative stress [110]. In contrast, extensive, but nonlethal, oxidative stress impairs UPS

function in tissues, in general, ameliorating protein degradation and promoting the intracellular accumulation and aggregation of damaged or abnormal, potentially cytotoxic, proteins [3, 4, 143, 144]. The susceptibility of ubiquitinating enzymes to oxidative stress is envisionable from the presence in E1, E2, and some E3 enzymes of an essential cysteine residue in their active sites. In this context, data from retinal tissue indicate that the oxidative stress generated upon treatment with H_2O_2 parallels a rapid, dose-dependent depletion of reduced glutathione (GSH), concomitant with an elevation of oxidized glutathione (GSSG) [3, 143–145]. This results in disulfide formation at the active sites of E1 and E2 enzymes, transiently blocking their ability to form a thiol ester with ubiquitin and hence to complete ubiquitination [3]. In addition, other types of covalent modifications, such as S-nitrosylation, can also inactivate these enzymes, as is the case for parkin [146]. Upon oxidative stress cessation, a hyperactivation of E1 occurs coincidently with an increase in ubiquitination and intracellular proteolysis levels [4, 143, 145]. Presumably, this enhancement of the UPS functions to remove the oxidized proteins generated during the stress situation and the GSSG/GSH ratio would be the molecular redox signal modulating UPS activity. An additional protective mechanism against oxidative stress involves upregulation of proteasomal proteins. This is the case for the proteasome subunit α6, whose levels become elevated as a compensatory antioxidant mechanism to glutamate-induced lipid peroxidation in the porcine retina [147]. Constant light exposure increases the production of reactive oxygen species (ROS) in the retina, resulting in an increase of ubiquitin conjugate levels [84] probably due to impairment of the proteasome. Oxidative stress is inseparably linked to a dysfunction of mitochondria, which are both generators of and targets for ROS [148]. Under conditions of mitochondrial damage elicited by high levels of these species, the E3 ubiquitin ligase parkin contributes to mitochondrial integrity quality control. This is exerted upon parkin translocation into this organelle and activation of the mitochondrial UPS for widespread degradation of outer membrane proteins, thereby promoting the removal of dysfunctional mitochondria by autophagy [149–151]. In a linked fashion, a role for parkin in neuronal protection against oxidative stress, although not addressed in the retina, has been demonstrated in the brain of *Park2* knockout mice, where an overall reduction in the respiratory activity of mitochondria is found together with a decreased antioxidant capacity to respond to ROS generation [149].

The classical glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is also able to act as an apoptosis mediator in the retina. Thus, an increase of both HECT E3 ubiquitin ligase HERC6 and GAPDH levels occurs in response to cell death induced by treatment with S-nitrosoglutathione (GSNO), an NO donor, in rat RGC-5 retinal ganglion cells, a complex being formed between both proteins that is believed to act as a cell death signal [87]. This treatment also results in the translocation of GAPDH from the cytosol to the nucleus, an event that is associated with interaction between S-nitrosylated GAPDH and SIAH1, another E3 ubiquitin ligase whose nuclear localization signal mediates the translocation of GAPDH to the nucleus. Here, SIAH1, stabilized by GAPDH, degrades a series of nuclear protein targets, thereby promoting apoptosis [152–155].

Nitration of tyrosine residues in proteins is sufficient to induce their accelerated removal by the retinal proteasome. In this context, degradation of nitrated Cu,Zn superoxide dismutase by a purified mixture of 20S/26S proteasomes from bovine retina occurs at a rate nearly twice higher than that of the non-nitrated enzyme in vitro [156]. Therefore, this increase in proteasome activity could be a cellular defense mechanism in charge of removing proteins with an altered structure and impaired function following nitrosative stress.

Retinal Injury

Degeneration of ganglion cells following axon transection occurs in explants from rat retina, and in this process, Max, a transcription factor that regulates apoptosis, is rapidly redistributed from the nucleus to the cytoplasm, triggering cell death [157]. The proteasomal degradation of Max within the nucleus, together with the blockade of nucleocytoplasmic transport, accounts for cytoplasmic Max accumulation [158].

A role for the immunoproteasome in protection from injury and/or damage repair in the central nervous system (CNS) (including brain and retina) has been proposed. In this context, the immunoproteasome undergoes a significantly upregulation in the mouse retina following the injury of photoreceptors by cytotoxic T lymphocytes, a damage that mimics that occurring in autoimmune retinitis. In this process, the immunoproteasome subunits LMP2/β1i and LMP7/β5i experience an increase in their levels of ca. fourfold compared to uninjured retinas [111].

Role of the Ubiquitin–Proteasome System in the Retinal Pigment Epitelium

The microphthalmia-associated transcription factor (MITF), belonging to the basic helix-loop-helix/leucine zipper (bHLH/ZIP) family, regulates the expression of genes encoding enzymes essential for melanin biosynthesis in melanocytes and RPE cells. The UPS-mediated degradation of MITF is required for retina/RPE differentiation, a process which depends on its interaction with the ubiquitinating enzyme E2I (Ubc9) [159, 160].

The UPS plays an important role in modulating the activities of other transcription factors, such as the hypoxia-inducible factor (HIF) and NF-κB, in the ARPE-19 RPE cell line. Consequences of impairment of the UPS include accumulation of HIF-1α and diminished NF-κB activation, which lead to enhanced expression and secretion of proangiogenic factors, such as the vascular endothelial growth factor (VEGF) and angiopoietin-2 (Ang-2) [161].

Heat shock proteins (HSPs), proteasomes, and autophagy regulate protein turnover in cells of the RPE [162]. In instances when HSP-linked protein folding fails, the misfolded proteins are initially tagged with a ubiquitin monomer and then transferred to proteasomes for their degradation [163, 164]. However, once the capacity of HSPs to rescue proteins becomes overwhelmed or if the UPS is suffering functional limitations, protein aggregates are formed at the cell periphery and delivered along the microtubule network via dynein-dependent retrograde transport to a perinuclear location. Such aggregates then form aggresomes that may be cleared through autophagic processes [163, 165, 166].

In a situation of oxidative stress, such as that generated upon treatment of RPE cells with diamide, which specifically oxidizes sulfhydryl groups, an elevation of the GSSG/GSH ratio has been reported, causing a dose-dependent inhibition of ubiquitination and thus of ubiquitin-dependent protein degradation [144]. It is also known that in RPE cells, the proteasome is more susceptible to oxidative inactivation than ubiquitinating enzymes [167]. Moreover, oxidative inactivation of the proteasome in the RPE is a molecular link between an oxidative stress status and overproduction of the proinflammatory cytokine interleukin-8 (IL-8) through activation of the p38 mitogen-activated protein kinase (MAPK) pathway [168]. The increase in IL-8 production upon prolonged inactivation of the proteasome is controlled by putative sequential events, as are activation of p38 MAPK, phosphorylation of the epidermal growth factor receptor (EGFR), and activation of the phosphatidylinositol 3-kinase (PI3K) pathway. The latter then activates interleukin 2-inducible T cell kinase (LTK) as well as other unidentified effectors, leading to upregulation of IL-8 in RPE cells [169].

Involvement of the Ubiquitin–Proteasome System in Retinal Disease

Given the numerous substrate proteins targeted to the proteasome and the multitude of processes involved, it is not surprising that anomalies in the UPS are implicated in the pathogenesis of many inherited and acquired human neurological pathologies in which the UPS can contribute directly or indirectly to the neurodegenerative process (Fig. 2) [28, 29]. Thus, in neurodegenerative diseases caused by the expression of mutant proteins, intracellular accumulation of these may overload the UPS, indirectly contributing to the disease process. However, mutations in genes encoding UPS components may directly cause pathological accumulation of proteins, and in instances in which they have a functional role in mitochondria, oxidative stress results from dysfunction of this organelle (Fig. 2). Many of the neurological pathologies not only affect the brain but also alter retinal function or they are otherwise eye-specific, as schematized in Fig. 3. The UPS involvement in these disorders, when known, is dealt with in this section.

The Ubiquitin–Proteasome System in Neurodegenerative Disorders Affecting the Retina

Alzheimer's disease is a neurodegenerative disorder that affects the retina in a fashion similar to chloroquine-induced retinal damage in experimental animals [170, 171]. Thus, in rat retinopathy elicited by this compound, a degeneration of ganglion cells occurs with colocalization of ubiquitin with apolipoprotein E, the lysosomal protease cathepsin D, amyloid precursor protein (APP), and amyloid β protein (Aβ) in the swollen ganglion cells and a few Müller supporting cells in the inner nuclear layer [172]. Their colocalization with ubiquitin suggests that the levels of these molecules may be regulated by similar or common mechanisms. In this context, the UPS system is activated in axonal dystrophy regardless of the cause leading to this pathology, as demonstrated by the increase taking place in APP and ubiquitin immunoreactivity [172].

PD is another neurodegenerative disorder in which retinal impairment is involved [173–175]. The mechanism by which retinal dopaminergic cells are lost in PD is thought to rely on an abnormal accumulation of α-synuclein protein bound to ubiquitin in such damaged cells [176]. The conformational changes derived from mutation or overexpression of the α-synuclein gene (*SNCA*), whose expression we have characterized in the mammalian retina [177], hamper its degradation by the UPS. As a consequence, the α-synuclein–ubiquitin complex triggers the formation of the proteinaceous, fibrillary cytoplasmic inclusions called Lewy bodies, commonly found in brains of patients with PD and related disorders. The Lewy body itself does not appear to be the direct cause of these symptoms. Rather, neuronal death could be due to metabolic disturbances related to α-synuclein accumulation, UPS dysfunction, and/or oxidative stress [96, 97, 176], as illustrated in Fig. 2 for neurodegenerative disease pathogenesis in general. Two proteins involved in the UPS and related to PD are the E3 ubiquitin ligase parkin and the DUB UCH-L1, which, as mentioned above, have been found by us to be expressed in the mammalian retina. In fact, loss-of-function mutations in the parkin gene (*PARK2*) cause autosomal recessive juvenile parkinsonism [178]. It has been recently shown that USP9X

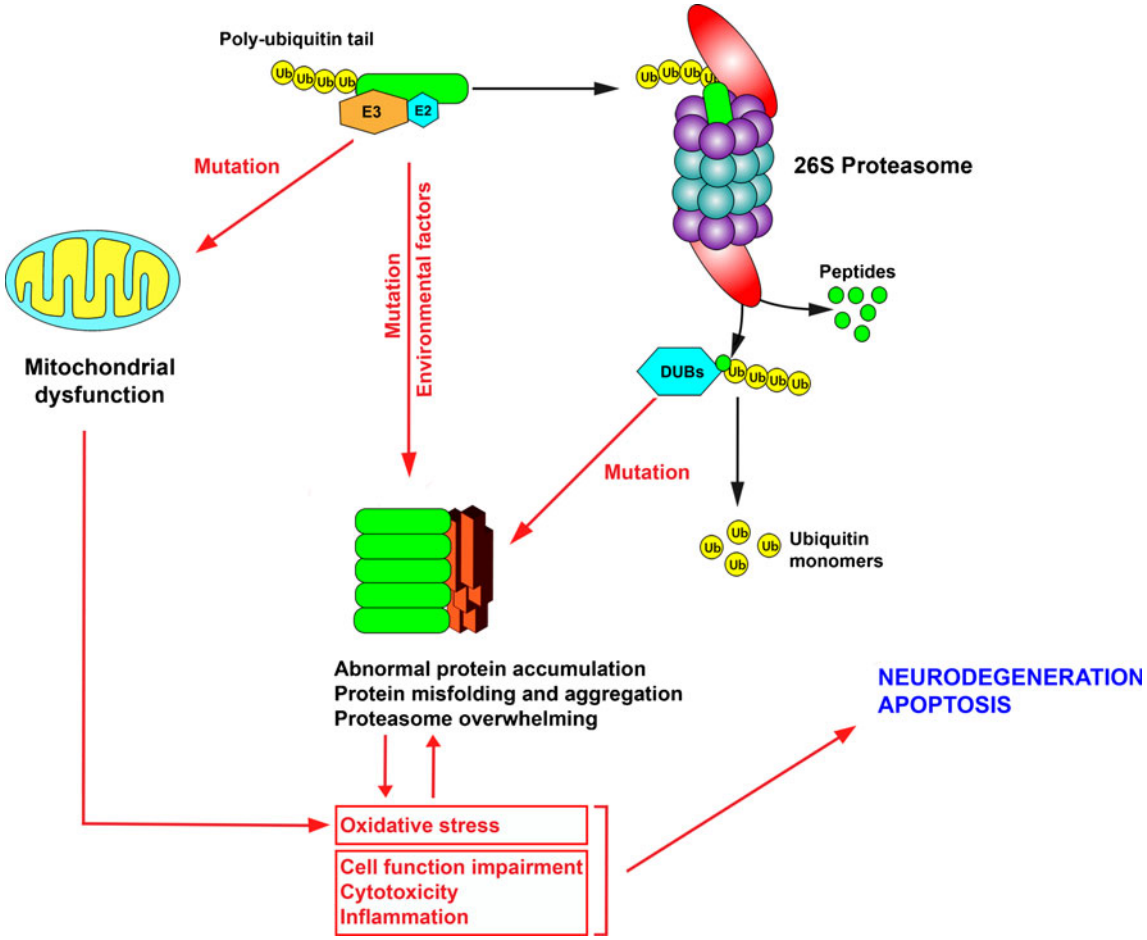

Fig. 2 The UPS and molecular mechanisms related to neuronal degeneration. A variety of environmental factors as well as mutations in genes encoding ubiquitin (Ub)-adding or deubiquitinating enzymes (DUBs) or UPS protein substrates can promote inactivation of the UPS. This leads to protein misfolding, mislocalization, and aggregation, which can result in the impairment of a series of cellular functions that eventually triggers cell degeneration and apoptotic death. Additionally, certain UPS components are necessary for proper mitochondrial homeostasis, and thus, mutations in their encoding genes can lead to mitochondrial dysfunction and consequent neuronal apoptotic death from elevated oxidative stress status and accumulation of noxious polypeptides

deubiquitinates α-synuclein and that this process determines the partition of α-synuclein between the proteasomal and autophagic pathways [179]. Accordingly, monoubiquitinated α-synuclein is degraded by the proteasome, whereas deubiquitination of α-synuclein favors its degradation by autophagy. Given that USP9X levels and activity are decreased in the brain of patients with PD and related disorders, this DUB could represent a novel target for PD therapy.

Huntington's disease is an autosomal-dominant inherited pathology characterized by the expression of mutant forms of the protein huntingtin carrying an expanded polyglutamine repeat whose aggregation leads to the formation of intranuclear and cytoplasmic inclusions that effectively hinder the proteasome machinery, with subsequent cellular toxicity from overaccumulated abnormal huntingtin and/or other proteins [180]. An involvement of the retina has been inferred from impairments detected in visual tests made to patients with this disease [181]. Also, in the R6/2 transgenic mouse model, which carries in exon 1 of the human huntingtin gene a tract of 141–157 glutamine codon repeats [182], a strong punctate staining of mutant huntingtin aggregates containing ubiquitin can be observed in the three nuclear layers of the neural retina [183]. Deposits of elongated huntingtin are also visible in RPE cells, pointing out that this epithelium is involved as well in the retinal degeneration process in the R6/2 mouse [183]. Since the distribution patterns of huntingtin and ubiquitin in the R6/2 retina are almost identical, it is likely that most huntingtin aggregates are also ubiquitinated in this tissue, as it is the case for the brain of these transgenic mice [184]. Accordingly, in the *Drosophila melanogaster* model of Huntington's disease, BAG1, an antiapoptotic cochaperone of HSP70, reduces mutant huntingtin aggregation and increases its degradation by the proteasome, thereby reducing the rate of photoreceptor cell loss [185]. In contrast, in the mammalian retina, the proteasome would fail to efficiently degrade misfolded huntingtin, resulting inhibited upon disease progression. This, together with

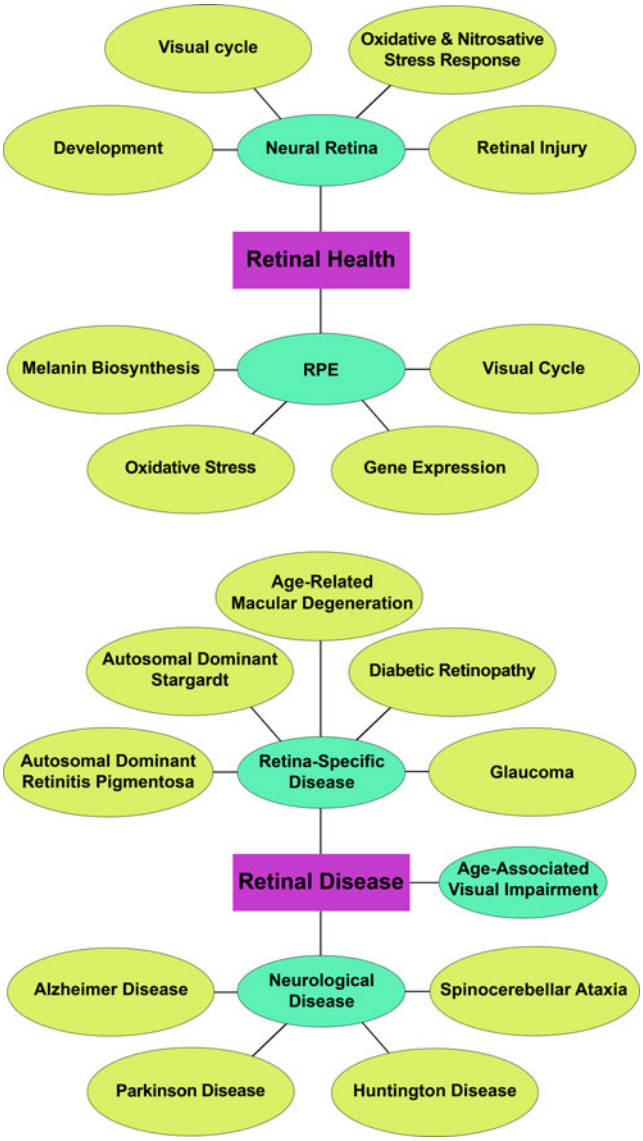

Fig. 3 Involvement of the UPS in retinal health and disease. The UPS is a crucial system for proper visual function, playing physiological roles related to retinal development, visual signal transduction, and response to many kinds of stresses, including oxidative and nitrosative stress and retinal injury (*upper graph*). The existence of a large number of retinal UPS elements and the need for a proper operation of each of them, together with their relevant functions in normal retinal physiology and in defense against pathogenesis, account for the fact that a significant number of visual impairments associated with neurological diseases as well as retina-proper illnesses (*lower graph*) are directly related to the dysfunction of particular or multiple UPS components

accumulation of mutant huntingtin and other proteasome substrates, would lead to neuronal toxicity.

Spinocerebellar ataxia type 7 is a neurodegenerative disease with accompanying macular degeneration caused by polyglutamine expansions in the ataxin-7 protein, encoded by the *SCA7* (=*ATXN7*) gene. Pathologically, the retina of patients with this disease is characterized by a loss of rods, cones, and ganglion cells and migration of RPE cells into the neural retinal layers [186]. This degenerative process is not limited to photoreceptors, since secondary alterations in their postsynaptic retinal neurons have also been reported [187]. In this regard, a transgenic mouse in which the human rhodopsin gene (*RHO*) promoter drives the overexpression of human ataxin-7 carrying 90 glutamine repeats (Q90) in rods develops progressive retinal degeneration accompanied by the formation of ubiquitin and proteasome immunoreactive nuclear inclusions composed of an N-terminal fragment of the ataxin-7(Q90) protein [187]. These ubiquitinated nuclear inclusions are, as in humans [188, 189], able to sequester chaperones and several proteasomal subunits [187], decreasing the amount of functional UPS machinery. This further prevents the cell from adequately processing the misfolded protein, leading to an accumulation of large, toxic aggregates. Mutant ataxin-7(Q90) is also known to misfold during translation and, as a defective ribosomal product, to be directly targeted to the UPS. In this light, proteasome inhibitors can favor mutant ataxin-7 protein aggregation [190–192].

The Ubiquitin–Proteasome System in Retinal Disease

Autosomal Dominant Retinitis Pigmentosa

Retinitis pigmentosa is a genetically heterogeneous group of progressive retinal dystrophies, characterized by the degeneration of rod and, subsequently, of cone photoreceptors. Patients experience poor night vision and peripheral visual field loss, eventually leading to impairment of central vision and complete blindness.

Mutations in the gene coding for the TOPORS E3 ubiquitin ligase have been detected in patients with autosomal dominant retinitis pigmentosa (adRP) [90]. Like other RING domain-containing E3 proteins, TOPORS bears specific E2 enzymes and is located in the nucleus of different cell types, including retinal cells [90]. The ubiquitous nature of TOPORS expression in healthy conditions and the lack of mutant protein in adRP patients are highly suggestive of haploinsufficiency, rather than a dominant-negative effect, as the molecular mechanism underlying this disease and makes rescue of the clinical phenotype amenable to somatic gene therapy.

Mutations affecting the BACK domain of KLHL7, a substrate adaptor for the CUL3-based E3 ligase, have also been found in patients with adRP [89, 193]. It is known that KLHL7 assembles with CUL3 through its BACK and BTB domains and that the *KLHL7* adRP-causative mutation A153V leads to the attenuation of E3 ligase activity of the CUL3–KLHL7 complex in a dominant-negative manner. This is thought to lead to an inappropriate accumulation in photoreceptors of substrates targeted for proteasomal degradation [194].

Altered protein trafficking and accumulation was observed in retinal tissue due to the P23H mutation in the *RHO* gene. This change in rhodopsin amino acid sequence causes misfolding of the mutant protein, consequently getting retained within the endoplasmic reticulum instead of reaching the outer segments of rod photoreceptors. The misfolded rhodopsin variant is then tagged (by N-glycosylation) for retrotranslocation to the cytosol for its removal by the UPS, in most cases following polyubiquitination. However, the P23H rhodopsin variant does not appear to be efficiently degraded by the UPS and forms aggresomes spontaneously close to the cellular centrosome due to overwhelming of the proteasome machinery. These aggresomes further recruit normal rhodopsin protein as well, leading to its degradation. Furthermore, the formation of aggresomes close to the centrosome disrupts the intermediate filament network, altering intracellular protein and organelle transport with ensuing malfunction of a number of cellular pathways [195, 196].

Autosomal Dominant Stargardt's Macular Degeneration

A mouse model that mimics the human autosomal dominant Stargardt's macular dystrophy (adSTGD or STGD3) phenotype, exhibiting a null mutation in the *ELOVL4* gene, shows a significant increase in its protein product, the elongation of very long chain fatty acids-4 protein, in the outer plexiform layer of the retina and a high expression of ubiquitin in the photoreceptor inner segments [197]. This suggests that the UPS function is compromised in the adSTGD retina, in similarity to other retinal neurodegenerations caused by dominant-negative protein trafficking defects such as those provoked by the *RHO* P23H mutation mentioned above [195, 196].

Age-related Macular Degeneration

The AMD pathology is attributable to degenerative tissue alterations that occur at the interface between the macular retina (including cone photoreceptors and RPE cells) and the underlying choroid [198–200] and causes a progressive loss of central vision. It must be recalled in this context that the proteasome mediates pathways associated with oxidative stress and inflammation, two pathogenic events highly related to AMD.

Oxidative Stress in Age-related Macular Degeneration An increasing body of literature indicates that oxidative stress and dysfunction of the RPE are associated with the pathogenesis of AMD [201–203]. It is widely accepted that senescence is associated with detrimental events in cellular functions and increased oxidative damage, which leads to protein misfolding and aggregation and in certain circumstances even to cell death. In turn, upon aging, oxidative stress and protein misfolding promote the accumulation of HSPs and the rise of functional abnormalities in the RPE cells of AMD patients, processes that are strongly linked to AMD pathology [204–206]. The increase of HSP expression is closely related to the UPS, since inhibition of the proteasome machinery and the resulting accumulation of highly ubiquitinated proteins induce activation of heat shock factor 1 (HSF1), a transcription factor associated with the HSP response [207–209].

In addition, the UPS plays an important role in modulating the activities of HIF and NF-κB in the RPE, two transcription factors that accumulate in this epithelium as a consequence of UPS impairment. As stressed out above, the derived increase in the expression and secretion of proangiogenic factors, such as VEGF, Ang-1, and Ang-2, could favor the development of AMD-related phenotypes [161].

It has also been documented that upon age, there is an increase in the expression of the DUB enzyme UCH-L1 in the human ARPE-19 cell line [105]. Interestingly, this upregulation is also observed in the eye of aged people [105] and hence could be related to RPE dysfunction and the progression of human AMD.

Inflammation in Age-related Macular Degeneration Inflammation is an important pathological component of AMD [202, 210, 211], and oxidative stress in the RPE can trigger the activation of the complement system [212]. The proteasome is a target of oxidative damage in cultured RPE cells [167], and oxidative inactivation of the proteasome is a mechanistic link between oxidative stress and upregulation of production and secretion of IL-8 in this epithelium [168, 169]. This has led to the proposal that UPS impairment could play a relevant role in the pathogenesis of AMD [167, 169]. It has also been shown that advanced AMD is associated with switch from the standard proteasome to the immunoproteasome in the retina [110]. Hence, a dramatic upregulation of its inducible subunits LMP2/β1i and LMP7/β5i occurs in both the macular and peripheral neural retinas, together with an increase in the levels of the proteasome activator subunit PA28 (11S) in the macular region. However, the emerging hypothesis that the immunoproteasome plays a protective role against oxidative/nitrosative stress [213, 214] is supported by the upregulation of its inducible subunits elicited by NO donors [215] and by the increase of oxidized proteins found in a β1i knockout model [214] and the retina of AMD patients [198]. Therefore, the presence of the immunoproteasome at later stages of AMD could be an indicator of local inflammation or increased oxidative stress in the neural retina.

It is known that retinal inflammation, a process inherently linked to AMD, downregulates rhodopsin expression and impairs visual function. This decrease of rhodopsin protein in the outer segments is achieved in a post-translational

fashion, involving protein degradation through the UPS promoted by the STAT3-dependent E3 ubiquitin ligase, UBR1 [93]. On the other hand, the so-called suppressor of cytokine signaling 3 (SOCS3) minimizes and promotes recovery from this influence of inflammatory signaling by inhibiting STAT3 activation, thereby contributing to the preservation of rhodopsin expression and visual function [93]. It follows that SOCS3 could constitute an interesting potential therapeutic target for protecting photoreceptors during retinal inflammation and thereby counteract their degeneration associated with AMD.

Glaucoma

Glaucoma is an ocular pathology characterized by the progressive degeneration of retinal ganglion cells, and an increase of intraocular pressure is generally recognized as the main cause of the glaucomatous condition [216–218]. In rats injected with methylcellulose (MTC) in the anterior chamber, which mimics experimentally the overall condition of human acute glaucoma, an activation of the retinal UPS is observed, leading to ubiquitin accumulation in the retina, especially in ganglion cells [219]. In another glaucoma experimental system, prolonged ischemic retinal injury, a set of antiapoptotic proteins are found to become ubiquitinated, and as a consequence of their degradation, an apoptotic program is triggered [220] Thus, ubiquitin may promote survival or apoptosis depending upon the extent of damage [84, 221], and activation of the retinal UPS may be related to two different features of the cellular response to glaucoma-related induced stress: first, an antiapoptotic response occurs, aimed at the prevention of irreversible cell death and second, if this rescue fails, a proapoptotic signal becomes activated in order to enhance the removal of cells too damaged as to undergo repair.

Diabetic Retinopathy

The peptide hormone angiotensin II (Ang II), which becomes upregulated in diabetic retinopathy (DR), has long been known to have a role in this disease. Given that DR has been usually regarded as a vascular disorder, Ang II has been thought to act by elevating retinal blood pressure. Current knowledge indicates, however, that Ang II rather elicits the downregulation in a UPS-mediated fashion of the expression of synaptophysin (SYP), a protein essential for vision for being a main constituent of synaptic vesicles in the two plexiform layers of the retina, and whose levels become greatly reduced in murine models of DR [222, 223]. Through binding to its AT1R receptor, Ang II induces activation of ERK, a protein kinase of the MAPK family, resulting in excess levels of ubiquitin-conjugated SYP in diabetic mice, presumably involving the action of the SIAH E3 ubiquitin ligase [223]. Interestingly, since ROS levels are known to increase in the DR mouse retina and both AT1R and ROS promote ERK activation, a cross-talk between these two signaling pathways has been proposed [222, 223]. Subsequent proteasomal degradation of SYP impairs synaptic activity, adversely affecting neuronal cell survival and visual function [222]. Additionally, Müller glial cells result damaged by the oxidative stress condition generated in the diabetic rat retina [224].

In the diabetic retina, hyperglycemia leads to an elevated production of ROS by the vascular endothelium, especially superoxide, as a result of autooxidation of glucose and/or glycoxidation [225], which is considered an important mechanism contributing to retinal vascular dysfunction in DR [226]. In this oxidative stress context, a subcellular redistribution of glucose transporter 1 (GLUT1) occurs, decreasing its levels at the plasma membrane of retinal endothelial cells. Oxidative stress upregulates the UPS, and as a consequence, GLUT1 is mono- or diubiquitinated and thereby tagged for its endocytosis and subsequent lysosomal degradation, decreasing glucose transport into retinal endothelial cells [227].

In an advanced stage of DR, overproliferation of capillary endothelial cells as a consequence of decreased oxygen from macular ischemia occurring at an earlier phase brings about neovascularization with abnormal formation of vessels in the retina and the vitreous, leading to severe vision loss [228]. The HIF-mediated signaling cascade plays a critical role in this process by activating the transcription of genes encoding angiogenic growth factors. Under hypoxia conditions, HIF-1α and HIF-1β subunits are rapidly protected from proteosomal degradation, allowing them to associate and form active HIF-1 transcription factor [229]. This binds to hypoxia-responsive target genes in endothelial cells and triggers the expression of a variety of angiogenic factors, including VEGF [230], erythropoietin [231], and endothelin-1 [232]. Conversely, under normoxia, proline hydroxylation in the oxygen-dependent degradation domain of HIF-1α mediates its proteasomal destruction promoted by an E3 ubiquitin ligase complex containing the von Hippel–Lindau tumor suppressor protein (pVHL) [233, 234]. YC-1, a synthetic HIF-1 inhibitor, suppresses vascular endothelial cell proliferation, migration, and vessel formation in human retinal microvascular endothelial cells while significantly increasing proteasome activity [235]. These data make YC-1 treatment a potential therapeutic approach to inhibit the formation and growth of new retinal vessels in the hypoxic retina.

Three connexin subtypes are expressed in the endothelium of blood vessels: Cx37, Cx40, and Cx43 [236], and the proteasome has been implicated in the turnover of these proteins. In this context, hyperphosphorylation of Cx43 in experimental hyperglycemia appears to be the triggering signal in bovine

retinal endothelial cells for degradation of this connexin by a proteasome-dependent mechanism. This contributes to a decrease in gap junction intercellular communication between retinal vascular endothelial cells, leading to the breakdown of the blood–retinal barrier characteristic of DR [237].

The Ubiquitin–Proteasome System in Aging-associated Visual Impairments

One of the hallmarks of aging is an increase in the levels of oxidized proteins, which has been reported in a variety of tissues including the retina [238–243]. Upon aging, a loss of proteasome function is found in many different tissues [244], which might account for the age-related accumulation of oxidized proteins. In this fashion, oxidative damage to cellular components is known to be largely involved in the pathogenesis of AMD [198, 205, 245]. It has even been suggested that the combined effect of an increase in oxidized proteins and oxidative inactivation of UPS proteases responsible for ridding the cell of oxidized proteins places the aged retina at greater risk for irreversible damage caused by oxidative stress [56]. In this context, in the retinal proteasome from old rats, a dramatic reduction in the rate of casein degradation takes place, concomitant with a high decrease of its chymotrypsin-like activity. This could be partially accounted for by a 50 % reduction in the expression of the C2α subunit, a constitutive component of the 20S core proteasome [56]. Additionally, cysteine oxidation appears to constitute a potential mechanism for age-related loss of retinal proteasome function, contributing to inhibition of its chymotrypsin-like activity. However, this phenomenon is not universal, as its effects appear to be tissue-specific. For instance, and in contrast with the neural retina, in the RPE no age-related change in proteasome activity is found [57]. Therefore, the loss in chymotrypsin-like activity in the aged neural retina could result in reduced tolerance to oxidative stress via several mechanisms, including the decreased clearance of oxidatively damaged proteins.

Conclusions and Future Directions

It is clear that multiple active UPS assemblies exist in retinal cells acting in a concerted fashion to allow normal homeostasis and function of the retina in mammals under physiological conditions. Among the roles of the UPS in this tissue are cell differentiation and development, modulation of visual signal transduction, and response to oxidative/nitrosative stress and injury, as described above, all of which constitute essential cellular pathways. Other, more general functions of the UPS also crucial in the retina include regulation of cell cycle, transcriptional regulation, and synaptic plasticity, in addition to the removal of damaged unfolded or obsolete proteins. For this reason, the altered expression of particular UPS components or an overall UPS dysfunction is involved in a number of neurodegenerative disorders affecting the retina, such as Alzheimer's, Parkinson's, and Hungtington's diseases and spinocerebellar ataxia as well as in retina-specific diseases, such as retinitis pigmentosa, macular degenerations, glaucoma, and DR. In this context, future investigations that focus on fundamental mechanisms involving the UPS, including identification of an ever-increasing number of substrates of the retinal UPS and their specific E2/E3 complexes, is warranted. As well, it should be of interest to screen the genes encoding UPS components for mutations or alterations in their expression levels in patients with the above-mentioned diseases. A better understanding of all the UPS elements present in retinal neurons, glia, and epithelial cells, together with the multiple targets and cellular processes in which they are involved, should provide valuable insight into the primary causes of all of these disorders. Needless to say, they should as well be useful to develop new and specific pharmacological modulators and specific therapies targeted to actors with a role at different levels of this system in a variety of retinal pathological states and conditions.

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