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Review Article

# E3 ubiquitin ligase-mediated regulation of vertebrate ocular development; new insights into the function of SIAH enzymes

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Developmental regulation of the vertebrate visual system has been a focus of investigation for generations as understanding this critical time period has direct implications on our understanding of congenital blinding disease. The majority of studies to date have focused on transcriptional regulation mediated by morphogen gradients and signaling pathways. However, recent studies of post translational regulation during ocular development have shed light on the role of the ubiquitin proteasome system (UPS). This rather ubiquitous yet highly diverse system is well known for regulating protein function and localization as well as stability via targeting for degradation by the 26S proteasome. Work from many model organisms has recently identified UPS activity during various milestones of ocular development including retinal morphogenesis, retinal ganglion cell function as well as photoreceptor homeostasis. In particular work from flies and zebrafish has highlighted the role of the E3 ligase enzyme family, Seven in Absentia Homologue (Siah) during these events. In this review, we summarize the current understanding of UPS activity during *Drosophila* and vertebrate ocular development, with a major focus on recent findings correlating Siah E3 ligase activity with two major developmental stages of vertebrate ocular development, retinal morphogenesis and photoreceptor specification and survival.

## UPS system overview

The ubiquitin proteasomal system (UPS) is a highly selective post-translational mechanism which plays a role in a multitude of cellular processes including protein quality control, cell cycle control, proliferation, synaptic plasticity, transcriptional regulation, signal transduction and the development of several different tissues [1–9]. It plays an important role in maintaining cellular homeostasis and is directly responsible for protein quality control check of oxidized, mutated, misfolded, denatured or unnecessary proteins. The UPS regulates many different biological processes and responds to changing physiological conditions while mis-regulation is known to be associated with cancer, neurological disease as well as congenital disease, including visual impairment [10]. This review will concentrate on the UPS-mediated targeting of substrates for protein degradation during ocular system development with a particular focus on the Seven in Absentia Homologue (Siah) family of E3 ubiquitin ligases and their newly discovered roles during vertebrate ocular development.

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### Mechanisms of UPS-mediated protein targeting and proteasomal degradation

The UPS system involves a five-step process, starting with the specific identification of the substrate until its final degradation by the 26S proteasome [11]. Target proteins are modified by a covalent attachment of multiple ubiquitin molecules (highly conserved 76 amino acid polypeptides) and then

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degraded down to amino acids or small peptides by the 26S proteasome [10,11]. The mechanism is summarized in Figure 1. The first step in this process is the activation of the ubiquitin molecules by E1-ubiquitin activating enzymes [10,11]. Humans express 2 different isoforms of the E1-ubiquitin activating enzymes, UBA1 and UBA6 [12–16]. The activation process involves a thiol-ester bond between an internal cysteine residue of the E1 and the C-terminal glycine residue of the ubiquitin [10,11]. Subsequently, the E2 ubiquitin-conjugating enzymes transfer the activated ubiquitin to a protein substrate (target protein) bound to an E3 ubiquitin ligase enzyme. Humans express 38 different isoforms of E2 ubiquitin-conjugating enzymes [17,18], 36 in mouse and 32 in Drosophila [17]. Despite the fact that several studies have been performed to identify specific E2 ubiquitin conjugating enzymes in zebrafish, an exhaustive analysis is lacking. Thus, we estimated the number of E2 express in zebrafish by searching in its proteome the same E2 previously identified for human and/or mice, totaling a number of 38 similar E2s. Name of each enzyme and its presence in each species is summarized in Table 1. In contrast there are more than one thousand E3 ubiquitin-ligase enzymes [18–20]. Clearly, the specificity of this process is governed by the diversity and targeting of E3 ubiquitin-ligase enzymes. E3 enzymes recognize their substrates based on unique amino acid binding motifs [10,11]. E3 ubiquitin ligases are currently categorized into 4 families depending on molecular, structural and complex formation differences [10,11]. They include: (1) really interesting new gene (RING) finger, (2) homologous to E6AP carboxyl terminus (HECT), (3) Skp1-Cul1-F box (SCF), and (4) anaphase-promoting complex (APC) families [7,21,22].

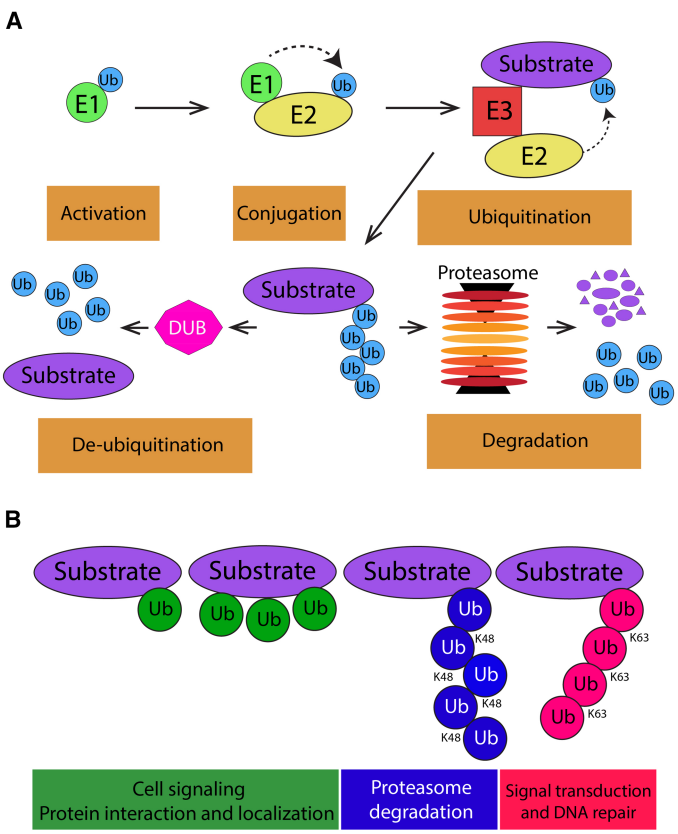


**Figure 1. Mechanics of the Ubiquitin Proteasome System (UPS). Overview of the UPS system.**
(**A**) Conjugation of ubiquitin molecules involves activation of ubiquitin (blue) by the E1 enzyme (green) and transfer of the charged ubiquitin to the E2 enzyme (yellow). The E2 enzyme binds to the E3 ligase (red) which in turn directly interacts with target substrates (purple) thus facilitating the transfer of ubiquitin molecules directly onto the target substrate. (**B**) Various levels of ubiquitination lead to differing biological outcomes. Monoubiquitination (green) leads to modulation of substrate protein interaction, localization and or activity. Polyubiquitination (blue) of the substrate via lysine 48 (K-48) linkages on ubiquitin leads to recognition by the 26S proteasome and subsequent degradation. Polyubiquitination via lysine 63 (K-63) linkages is unique to signaling DNA repair.

**Table 1 Species comparison of E1 activating and E2 conjugating enzymes**

| Name of enzyme | Zebrafish | Mouse | Human |
|---|---|---|---|
| **E1 ubiquitin activating** | | | |
| **Uba1** | | | |
| **Uba6** | | | |
| **E2 ubiquitin conjugating** | | | |
| **Ube2a** | | | |
| **Ube2b** | | | |
| **Ube2k** | | | |
| **Ube2ka** | | | |
| **Ube2kb** | | | |
| **Hr6bn** | | | |
| **Ube2g1** | | | |
| **Ube2g1a** | | | |
| **Ube2g1b** | | | |
| **Ube2g2** | | | |
| **Ube2r1** | | | |
| **Ube2r2** | | | |
| **Ube2d1** | | | |
| **Ube2d1a** | | | |
| **Ube2d1b** | | | |
| **Ube2d2** | | | |
| **Ube2d3** | | | |
| **Ube2d4** | | | |
| **Ube2e1** | | | |
| **Ube2e2** | | | |
| **Ube2e3** | | | |
| **MGC58426** | | | |
| **UBE2U** | | | |
| **Ube2j1** | | | |
| **Ube2j2** | | | |
| **Ube2h** | | | |
| **Ube2i** | | | |
| **Ube2f** | | | |
| **Ube2m** | | | |
| **Ube2n** | | | |
| **Ube2na** | | | |
| **Ube2nb** | | | |
| **Ube2nl** | | | |
| **Ube2t** | | | |
| **Ube2v1** | | | |
| **Ube2v2** | | | |
| **Ube2s** | | | |
| **Ube2c** | | | |
| **Ube2w** | | | |
| **Ube2wa** | | | |
| **Ube2wb** | | | |
| **Birc6** | | | |
| **Ube2o** | | | |
| **Ube2z** | | | |
| **Ube2l3** | | | |
| **Ube2l3a** | | | |
| **Ube2l3b** | | | |
| **Ube2l6** | | | |
| **Aktip** | | | |
| **Ube2q** | | | |
| **Ube2q1** | | | |
| **Ube2q2** | | | |
| **FTS** | | | |

***Color indicates the presence in the examined species.

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The efficiency for degradation of a targeted protein relies on the assembly of multiple ubiquitin molecules ultimately forming polyubiquitin chains [23–25]. For every newly added ubiquitin there is an exponential increase in the number of different positions that the subsequent ubiquitin ligation can occur [11]. The attachment of ubiquitin molecules involves either the N-terminus or one of seven internal lysine residues giving each subsequent ubiquitin molecule to be added up to eight potential attachment sites [11]. With each additional ubiquitin conjugation the possibilities grow therefore making the structure of the ubiquitin chain very diverse [11]. This diversity is often used by the cell for specific functions (Figure 1B). For example, lysine-48 (K-48) linked ubiquitin chains are often used for protein degradation while lysine-63 (K-63) chains are associated with signal transduction and DNA repair [26–29].

After polyubiquitination, targeted proteins are degraded by the proteasome [10]. There are several proteasomal complexes including the 20S and 26S complexes as well as the immunoproteasome, the latter being involved in the processing of antigens to be presented by MHC class I molecules [10]. The 20S proteasome is responsible for degradation of monoubiquitinated targets without ATP consumption, while the 26S proteasome degrades polyubiquitinated proteins in an ATP-dependent manner [30,31]. These proteasomes are defined by the regulatory subunit (19S or 11S) that is associated with their catalytic core and the composition of their catalytic subunits [10]. Binding of the regulatory 19S subunit with the catalytic 20S subunit generates the 26S proteasome, while binding of 11S with 20S generates the 20S proteasome [10]. The best-studied proteasome is the 26S, a huge ∼2.5 MDa multicatalytic protease [10]. Its 20S catalytic core is made of four stacked rings: two outer α-rings and two inner β-rings, the inner β-rings are the proteolytic rings [32–34]. Each side of the 20S is capped by the two regulatory 19S, which is made of 19 different subunits organized into two domains. The base is responsible for interacting with the 20S and the peripheral lid which in turn is responsible for recognizing and biding to polyubiquitinated proteins [35,36]. While most cells contain different types of proteasomes, the relative ratio between them is cell-specific and provides enormous additional diversity [37,38]. Finally and importantly, ubiquitin modification via E3 ligases does not have to be permanent and can be removed by Ubiquitin Deubiquitinating enzymes (DUBs). Deconjugation of ubiquitin is mediated by a family of proteases found in six different families, C-terminal hyrolases (UCH), ubiquitin specific proteases (USP), Machado-Joseph disease protein domain proteases (MJD), ovarian tumor proteases (OUT), JAMM motif proteases and motif interacting with ubiquitin-containing novel DUB family (MINDY) [39,40]. The deconjugating mechanisms adds yet another layer of regulation and complexity to the highly versatile UPS system.

# UPS involvement during eye development

The UPS has been shown to play roles in various aspects of retinal function in several model systems, including *Drosophila*, zebrafish, *Xenopus* and mouse. However, a systematic and comprehensive study of the role UPS plays during early retinal development has yet to be undertaken [10]. Ubiquitin is found to be expressed throughout the retina, including cone and rod photoreceptors, retinal ganglion cells and the retinal pigmented epithelium, with majority of ubiquitin being covalently attached to a target protein [8,41–44]. Critical components of the UPS have also been detected in retina-derived cell culture, tissue sections and tissue homogenates. E1, E2 enzymes and proteasomal activity have all been observed in photoreceptor outer segments in addition to detection of ubiquitinated rhodopsin and transducin, both key components of rod and cone cells [42]. A previous review of UPS in retinal function has nicely outlined the association of UPS and degenerative ocular disease [10]. In addition, there have been several reports of DUBs also playing a role in retinal disease, in particular retintis pigmentosa [45]. The expression of DUBs in the mature murine retina has also been compiled [46]. Clearly, the UPS plays a role in the homeostasis of the many components of the retina, however, despite the discovery of many ubiquitinated proteins combined with an expanding catalogue of several different E3 ubiquitin ligases found in the retina, little is known about their function and their targets during retinal development. What we do know about the formation of the retina and its regulation by the UPS has been primarily from studies of the *Drosophila* compound eye.

## UPS regulation of compound eye formation in the fruit fly

Several studies have shown that UPS-mediated proteasomal degradation controls various aspects of *Drosophila* eye development, including eye size, photoreceptor (rhabdom) specification and differentiation, cell proliferation, glial cell migration as well as cell cycle arrest in mature retinal neurons. A summary of these events and the corresponding UPS regulatory pathways is presented in Figure 2A. In short, fly eye size is regulated by activity of two E3 ubiquitin ligases, dSmurf [47] and the anaphase-promoting complex/cyclosome (APC/

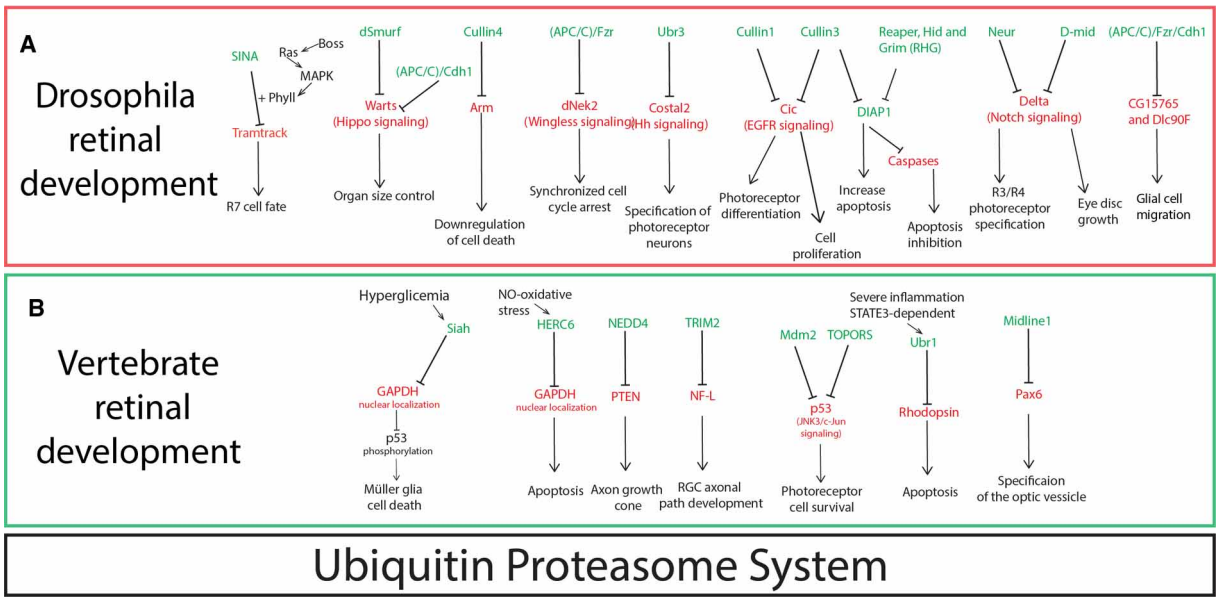


**Figure 2. Summary of UPS mechanisms known to be involved in ocular development.**

(**A**) Outline of UPS mechanisms involved in *Drosophila* ocular development. E3 ligase enzymes are indicated in green, their target substrates are indicated in red and the ocular development outcome under regulation of the system is indicated in black. (**B**) Outline of UPS mechanisms involved in vertebrate ocular development. E3 ligase enzymes are indicated in green, their target substrates are indicated in red and the ocular development outcome under regulation of the system is indicated in black.

C)/$^{Cdh1}$ [48] via modulation of Hippo signaling [49–51]. Synchronized G1 arrest prior to neuronal differentiation requires activity of the E3 ubiquitin ligase, Ubr3 [52] which modulates Hedgehog signaling (Hh) by targeting Costal2 (Cos2). Additionally, Hh signaling is also modulated by inhibition of Wingless (Wg) signaling via the E3 APC/C/$^{Fzr}$ complex targeting NimA-related kinase 2 (dNek2), a positive Wg modulator [53]. UPS-mediated specification of the eight photoreceptor cells (R1–R8) of the *Drosophila* compound eye ommatidia has been very well defined. The first to be specified is R8 [54,55] which subsequently induces the formation of R2, R5 and R3, R4 [55–57]. Next, photoreceptors R1 and R6 with R7 being the last to be specified. Specification of the R3/R4 pair involves Notch signaling via activation of Frizzled (fzd), only in R3 precursor cells [58]. This leads to activation of the Neuralized (Neur) E3 ubiquitin ligase which leads to proper internalization of Delta creating a directionality between R3 and R4 precursor cells [58,59]. Specification of R7 depends on the activity another E3 ubiquitin ligase, called Seven in Absentia (SINA) [60,61]. SINA forms a complex with Phyllopod (Phyl) and targets a transcription repressor, Tramtrack (Tram) for degradation [62–64]. *Phyl* expression is activated in R1/6/7 photoreceptors by lateral activation through a multi-pass transmembrane protein called bride of sevenless (boss) found in R8 [65,66]. In R1/6, EGFR activated proteins, such as Ro and Svp, inhibit Phyl activity to prevent R7's fate [67–69]. SINA loss of function results in R7s to become cone cells due to accumulation of Tram [63,70,71]. Finally, the DUB UCH-L1 has been shown to regulate eye development by modulating the MAPK pathway and that its overexpression induces a rough eye phenotype [72]. Owing to the major physiological differences between fly compound eye formation and that of vertebrate retina, it is unclear whether any of these pathways are conserved during vertebrate ocular development.

## UPS-mediated regulation of vertebrate eye development

Despite the discovery of several E3 ubiquitin ligases involved in *Drosophila* eye development, in vertebrates the roles of UPS during retinal morphogenesis, retinal lamination and photoreceptor specification still remain open questions. This is largely due to the absence of a detailed examination of UPS regulation and activity during retinal development. In the following section and in Figure 2B we summarize the various aspects of vertebrate retinal development that are currently known to be associated with UPS regulation. Our primary focus moving forward will be to highlight known aspects of E3 ligase regulation of vertebrate ocular development.

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## E3 ligase regulation of vertebrate retinal morphogenesis

Morphogenesis of the vertebrate retina is a complex and highly dynamic event. Vertebrate retinal identity relies on the function of several major retinal fate determinants including pax2 and pax6, the latter of which needs to be excluded from the optic stalk in order to properly form the retina [73]. It was recently shown that the E3 ligase Midline1, a member of the RBCC/TRIM E3 ligase family can target pax6 for degradation precisely in regions where pax6 needs to be actively suppressed [74]. Absence of Midline1 function results in malformed and enlarged eyes. Interestingly, *midline1* expression was also shown to be regulated by sonic hedgehog (Shh), a morphogen that is key to proper retinal morphogenesis [74].

## E3 ligase regulation of vertebrate Retinal Ganglion Cell development

E3 ubiquitin ligases belonging to the HECT family, HERC6 and NEDD4, are known to be expressed in Retinal Ganglion Cells (RGC) [75,76] with NEDD4 shown to be involved in regulating RGC axon growth cones in *Xenopus* [76]. Disruptions of NEDD4 function with a dominant-negative mutant severely inhibits terminal branching [76]. In this case, NEDD4 regulates the protein stability of PTEN, a key regulator of axonal terminus arborization *in vivo* [76]. In mice, RGC axonal path development has been shown to be regulated by the RING family E3 ligase Tripartite motif-containing protein 2 (TRIM2) [77]. TRIM2 plays a crucial role by targeting the neurofilament light subunit (NF-L) [77]. Mouse TRIM2 mutants exhibit swollen axons in the retina and in the brain associated with axonopathy resulting from disorganization of the intermediate filaments and accumulation of NF-L [77]. Additionally, these mutants develop fewer retinal interneurons and RGCs, while the photoreceptor cells appear unaffected [77].

## UPS regulation of vertebrate photoreceptor cell development

Much effort has been spent on understanding UPS regulation of photoreceptor cell survival and homeostasis in correlation to ocular blinding diseases. The following is a brief outline of those mechanisms most likely also associated with photoreceptor development. While not specifically examined during development, mutations in a substrate adaptor of the Cul3-based E3 ligase complex, called KLHL7 was found to be the cause of retinitis pigmentosa in six independent families [78]. Other substrate adaptors of Cul-based E3 ligase complexes have been previously associated with the control of cell-cycle progression [79] and also with the degradation of dopamine D4 receptor in the brain [80]. Thus, KLHL7 mutations likely affect its ability to facilitate dopamine targeting leading to accumulation of toxic levels inside of metabolically demanding photoreceptor cells thus leading to their degeneration [78]. Another E3 ubiquitin ligase shown to be required for photoreceptor cell survival is the Mouse double minute 2 homolog (Mdm2) [10]. Mdm2 is responsible for regulating photoreceptor and retinal pigmented epithelium (RPE) survival by inhibition of the JNK3/c-Jun apoptotic pathway by targeting p53 [10,81]. Topoisomerase I-binding RS protein (TOPORS) is yet another E3 ubiquitin ligase known to be involved in the development of retinitis pigmentosa [45]. TOPORS is also involved in targeting the transcription factor p53 to proteasomal degradation [45]. Additionally, during severe inflammation in mice, photoreceptor cells down-regulate rhodopsin by a UPS-dependent mechanism involving the STAT3-dependent E3 ubiquitin ligase, Ubr1 [82]. Overactivation of inflammatory cytokines enhances STAT3 activity increasing Ubr1 degradation of rhodopsin to toxic levels and leading to tissue dysfunction [82]. Thus, these results indicate Ubr1 may be used as a therapeutic target for treating retinal inflammatory diseases [82]. Two relevant components of the UPS associated with a number of neurodegenerative disorders have been found to be highly expressed in the mammalian retina, Parkin and ubiquitin C-terminal hydrolase L1 (UCH-L1) [83]. Esteve-Rudd and collaborators characterized Parkin and UCH-L1 expression and protein localization in the retina of several mammalian models, such as mouse, rat, bovine and monkey as well as in human samples [83]. They found *parkin* to be expressed in photoreceptor cells, in inner nuclear layer (INL) cells, such as horizontal, bipolar and amacrine cells, as well as in the RGC. *UCH-L1* expression was not detected in rod photoreceptor cells, but was present in the INL and RGCs as well as cone cells [83]. Despite the fact that the authors did not identify potential targets or pathways that Parkin and UCH-L1 might be involved in, they pointed to their potential protective function against neuronal stress in the retina [83]. Lastly, the DUB USP45 has been shown to be critical in during eye development as its knockdown by morpholinos resulted in defective formation of retinal structures [84]. Interestingly, vertebrate DUB expression during development has recently been comprehensively catalogued and the results indicated specific DUB expression during photoreceptor specification and differentiation [85]. In particular, the authors highlighted the potential function of UPS48 in the cone differentiation pathway.

## Siah E3 ubiquitin ligase function during retinal development

As previously mentioned, the Siah family of ubiquitin E3 ligases are vertebrate homologs of *Drosophila* Seven In Absentia (SINA), known regulators of *Drosophila* R7 photoreceptor cell development. These E3 ubiquitin ligases have their catalytic activity based on a RING (Really Interesting New Gene) finger domain [86]. Additionally, their molecular structure also contains other evolutionarily conserved and functionally distinct domains. The SZF (SIAH-type zinc finger) with a dual zinc finger domains, the SBS (substrate binding site) which recognizes the target substrate and the DIMER (Dimerization) domain which facilitates homo/heterodimer formation between Siah proteins leading to self-degradation [86]. The RING domain is localized in the N-terminus and responsible for interacting with the E2-ubiquitin conjugating enzymes, activating the discharge of its ubiquitin cargo [7,87]. The SDB, SZF, SBS and DIMER domains are found in the C-terminus [86]. Most vertebrates encode three Siah paralogs in their genome (Siah1, Siah2 and Siah3) all equally orthologous of the invertebrate SINA [86]. Siah3 was the most recent discovered member of the Siah family [86,88,89]. It is present in most vertebrates excluding teleost fish and the squamate division of reptiles (including snakes and lizards) [86]. It was initially discovered to be a negative regulator of parkin [88]. Structurally, Siah1 and Siah2 are the most similar, showing high amino-acid identity in its RING, SZF, SBS and DIMER domains [86]. Their highest divergence occurs in regions of the Siah2 N-terminus [86]. Siah3, in another hand, lacks a catalytically active RING domain, therefore exhibiting a high degree of divergence when compared with Siah1 and Siah2 [86]. Additionally, Siah3 contains only a single zinc-finger motif compared with the double zinc-finger motif found in Siah1 and 2 [86]. Recent investigation of Siah paralog mRNA expression in different human epithelial cell lines, including cancer cell lines, found Siah1 and Siah2 mRNA in all cell lines analyzed, however, Siah3 mRNA was only found in a small group of human tumorigenic cell lines [86]. Thus, it was hypothesized that due to the lack of Siah3 RING domains, it might act as an endogenous negative regulator of Siah1 and Siah2, since RING-deleted Siah1 and Siah2 have been used in several studies (including ours) [86,90–95] as dominant-negative versions to functionally ablate endogenous Siah1 or Siah2 activities [86]. However, endogenous Siah3 function still remains largely understudied [86].

Since their discovery, the focus of investigation on Siah1 and Siah2 function has focused on their roles during hypoxia signaling, DNA damage signaling, oncogenesis, neuronal cell polarity and cellular senescence [87,96,97]. Their expression responds to several different environmental and intracellular cues such as oxygen deprivation (hypoxia) [98], glucose deprivation [99] or elevation [100], DNA damage and apoptosis [101,102]. Additionally, Siah activity can be modulated by post-translational modifications, such as phosphorylation. Siah2 phosphorylation by p38 MAPK [103] and the dual specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2) [104] both under hypoxia conditions results in increased Siah2 ubiquitination activity. In contrast, under normal oxygenation conditions, Siah2 phosphorylation at positions 26, 28 and 68 by homeodomain-interacting protein kinase 2 (HIPK2), decreases Siah2 activity by weakening its interactions with target proteins [105]. Siah2 has been classified as an oncogenic tumor-promoting gene, since it exhibits high gene expression in several types of human cancers including liver [106], pancreas [107], breast [108], lungs [109], prostate [110] and skin [93]. In contrast, Siah1 has been implicated it as a tumor suppressor [111–113]. However, Siah1's role in cancer suppression and other diseases is still poorly understood.

In regard to potential developmental roles for Siah1 or Siah2, little is currently known. Since their discovery, *siah1* and *siah2* genes have been shown to be involved in vertebrate axis formation, hypoxia signaling, DNA damage, neuronal cell polarity and cellular senescence [87,96,97]. In neurons, Siah1 is known to be able to ubiquitinate the two major components of the Lewy bodies, synphilin-1 and a-synuclein [114], limiting their availability for the formation of these inclusion bodies [115,116]. The accumulation of this Lewy bodies is the hallmark of Parkinson's disease, a neurodegenerative disorder [87]. Additionally, Siah1 has also been implicated in the control of neuronal cell adhesion [97]. During cell cycle exit in in the developing granular layer of the cerebellum, Siah targets partitioning defective-3A family cell polarity regulator (PARD3A), a component of the PAR complex responsible for cell polarity formation, regulating its stability by UPS-mediated degradation [97]. Degradation limits the interaction of PARD3A with junctional adhesion molecule C ( JAM-C) reducing cell adhesion and preventing neuron progenitors from exiting the germinal zone [97]. During hypoxia, Siah2 is a crucial regulator of the HIF-1a activity and thus, the cellular response to hypoxia [98]. Siah2 knockout mice display mild phenotypes such as a slight increase in the number of hematopoietic progenitor cells [117]. In *Xenopus* embryos, Siah2 misexpression leads to the development of small eye phenotype [118], a clue to

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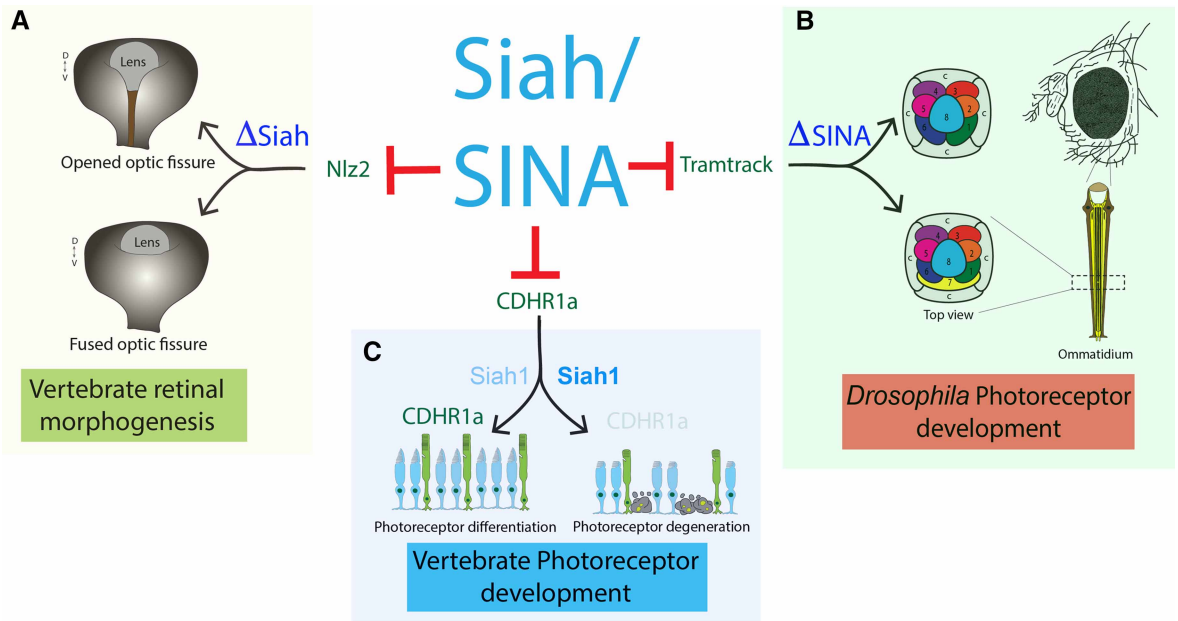


**Figure 3. Siah/SINA mediated regulation of ocular development. Activities of the Siah/SINA family of E3 ubiquitin ligase enzymes during *Drosophila* and vertebrate ocular development.**

(**A**) Siah activity is involved in vertebrate ocular morphogenesis by regulating optic fissure fusion by targeting nlz2 for proteasomal degradation. Failure of targeting nlz2 for degradation (ΔSiah) leads to failure of optic fissure fusion in zebrafish. (**B**) SINA activity regulates the development of the R7 photoreceptor in *Drosophila* by targeting tramtrack. Failure of targeting tramtrack (ΔSINA) leads to the loss of R7 photoreceptors. (**C**) Siah activity modulates vertebrate photoreceptor development by timely targeting the retina specific cadherin cdhr1a during zebrafish ocular development. Improper targeting of cdhr1a (Dark blue: increased Siah activity = less cdhr1a, Light blue: normal Siah activity = appropriate levels of cdhr1a) leads to improper photoreceptor development and decreased numbers of rod and cone photoreceptors.

potential function of these enzymes during ocular development. Additionally, as outlined previously, R7 photoreceptor development in *Drosophila* relies on the function of SINA, the fly homolog of Siah (Figure 3B). Finally, Siah1 and Siah2 mouse double KOs are embryonically lethal [117]. Thus, Siah function is essential in early development, functions during maintenance of cellular homeostasis in response to stress [87] and is also associated with ocular development.

E3 ubiquitin ligases, like Siah, bind to substrates targeting them for proteasome-mediated degradation using a common and conserved binding motif that acts as a degradation signal or 'degron' [119]. The Siah degron has been elucidated to encode the P-[ARTE]-x-V-x-P, with the core V-x-P constituting residues with the highest conservation [120]. Interestingly, a recent screen of the zebrafish proteome identified 2 potential vertebrate targets related to eye development, nlz2 and cdhr1a. Subsequent investigation into the functional relationship between nlz2, cdhr1a and Siah revealed new and exciting links between the UPS and vertebrate ocular development.

## Siah-mediated regulation of vertebrate retinal morphogenesis and photoreceptor development

Nlz2, a zinc finger nuclease, has been shown to function as a negative regulator of *pax2a* expression [92,121]. In zebrafish, pax2a is a key driver of optic fissure fusion, a key morphogenetic event that ensures proper formation of the retina and the optic stalk. Failure in this process leads to a congenital blinding disorder coloboma (i.e. failure of the optic fissure to fuse). Recent work from our group determined that *siah1* and *nlz2* are co-expressed in the optic fissure and that nlz2 protein is a direct target of siah1 and the proteasome [87]. Furthermore, by modulating siah1 activity we show that regulation of nlz2 protein stability plays a role in maintaining proper levels of pax2a mRNA to ensure timely and proper fusion of the optic fissure (Figure 3A). It

was also determined that *siah1* expression is regulated by Shh activity, another major driver of optic fissure fusion. This was the first examination of UPS activity during retinal morphogenesis and specifically the regulation of optic fissure fusion.

The second, and most recently characterized Siah1 target associated with ocular development is cdhr1a, a retinal specific cadherin known to be associated with cone-rod dystrophy, a degenerative blindness disorder. Previous studies of Chdr1 in mice suggested it plays a role in regulating photoreceptor outer segment homeostasis but its role during development was unclear [122,123]. In our most recent study, we confirmed that during zebrafish retinal development *siah1* and *cdhr1a* are co-expressed in cone and rod photoreceptor cells, localize to the primary cilium and that siah1 can directly target cdhr1a protein for proteasomal degradation [92]. When modulating siah1 activity during early photoreceptor development we show that excessive degradation of cdhr1a can inhibit proper development and survival of both rod and cone cells while having little effect on other retinal neurons (Figure 3C). These findings indicate that siah1 activity plays a critical role in regulating steady state levels of cdhr1a to ensure proper and timely specification and survival of photoreceptors in zebrafish.

In conclusion, the UPS is a highly versatile mechanism for rapidly regulating biological processes including development of the visual system. While it has been studied in numerous contexts, in particular cancer biology, its function during visual system development has been primarily constrained to the study of the fruit fly eye. However, recent work from zebrafish and other vertebrate models highlights the importance of examining UPS function during retinal development. Approaches highlighted by our study of the Siah E3 ligase, in particular taking advantage of the known substrate degron motif to search for relevant and novel targets, outline nice and straightforward screening processes for potential targets and subsequent functional analysis which can lead to new and exciting future studies.

## Perspectives

- UPS regulation of biological processes has wide-ranging breadth and presents very tangible options for therapeutic intervention for various diseases including cancer and neurodegenerative disease. Ocular development and visual system function are potential new areas of focus for analysis of UPS function and subsequent options for therapeutic intervention.
- There is little doubt that UPS plays a role during ocular development, both invertebrate and vertebrate. The current roadblock to expanding our understanding of UPS function during ocular development stems from insufficient investment in molecular investigation of UPS targets and mechanisms in ocular tissues.
- Biochemical and proteomic identification of E3 ligase enzyme targets offers opportunities to examine targets of UPS associated with the development and function of the visual system. Correlation of UPS activity with ocular disease-associated genes may offer new avenues of investigation and possibly pharmacological therapeutic intervention.

### Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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### Author Contributions

J.K.F. and W.P.P. wrote the manuscript, W.P.P. generated the figures.

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## Abbreviations

APC, anaphase-promoting complex; DUBs, deubiquitinating enzymes; HECT, homologous to E6AP carboxyl terminus; INL, inner nuclear layer; RGC, retinal ganglion cells; RING, really interesting new gene; SBS, substrate binding site; Siah, Seven in Absentia Homologue; SINA, Seven in Absentia; SZF, SIAH-type zinc finger; TOPORS, Topoisomerase I-binding RS protein; TRIM2, Tripartite motif-containing protein 2; UCH-L1, ubiquitin C-terminal hydrolase L1; UPS, ubiquitin proteasome system.

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