PORTLAND PRESS

Review Article

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

Warlen Pereira Piedade[1] and ![ORCID] Jakub K. Famulski[2]

[1]Department of Cardiology, Boston Children's Hospital, Harvard University, Boston, U.S.A.; [2]Department of Biology, University of Kentucky, Kentucky, U.S.A.

Correspondence: Jakub K. Famulski (jkfa226@uky.edu)

OPEN ACCESS

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.

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

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].
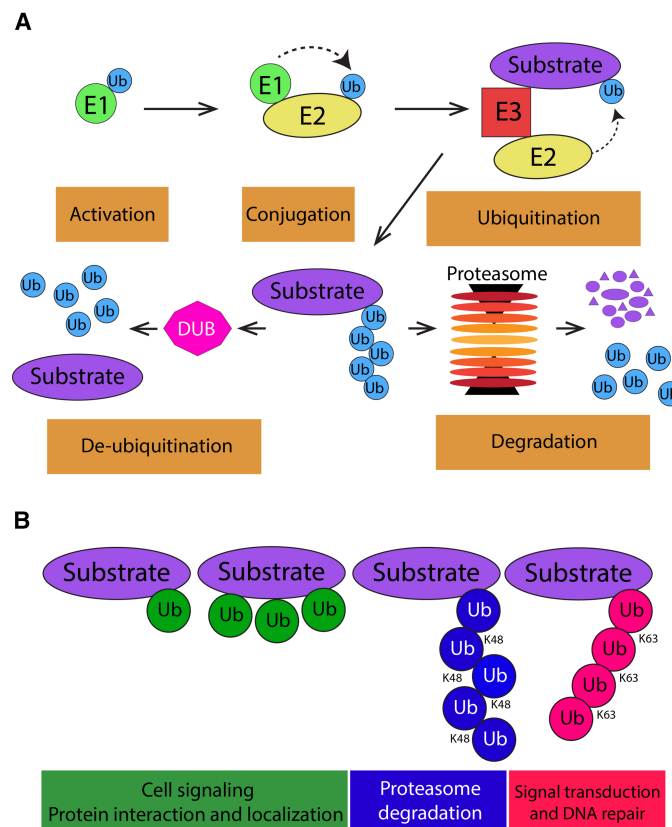
Figure 1. Mechanics of the Ubiquitin Proteasome System (UPS). Overview of the UPS system.
(**A**) Conjugation of ubiquitin molecules involves activation of ubiquitin (blue) by the E1 enzyme (green) and transfer of the charged ubiquitin to the E2 enzyme (yellow). The E2 enzyme binds to the E3 ligase (red) 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.

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/
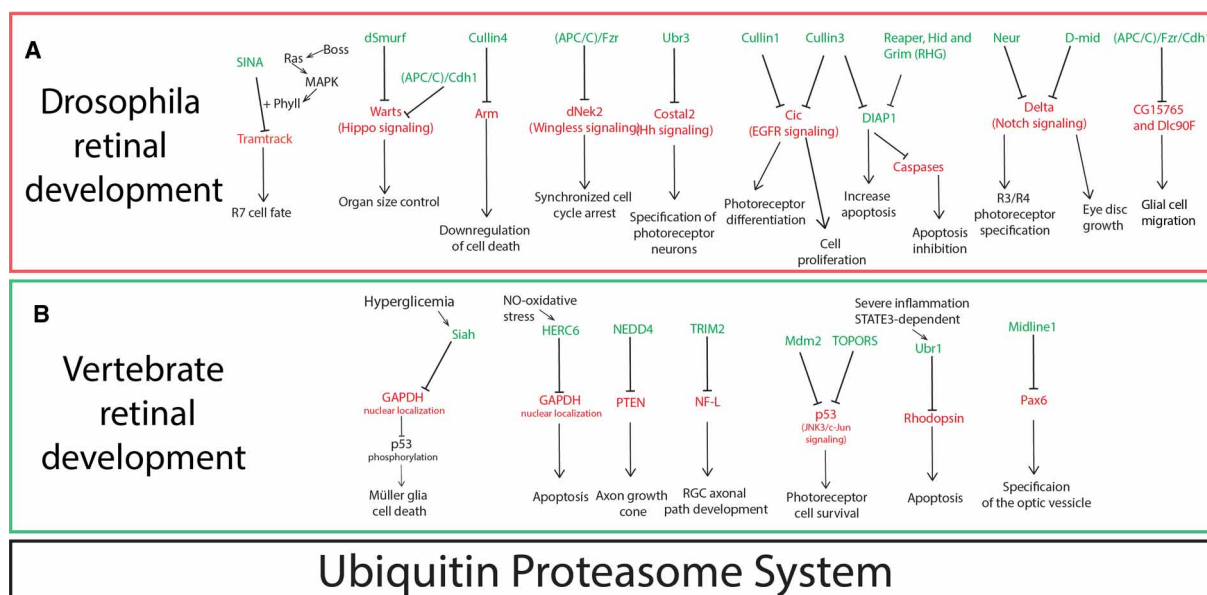
Figure 2. Summary of UPS mechanisms known to be involved in ocular development.
(**A**) Outline of UPS mechanisms involved in *Drosophila* ocular development. E3 ligase enzymes are indicated in green, their target substrates are indicated in red and the ocular development outcome under regulation of 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.

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
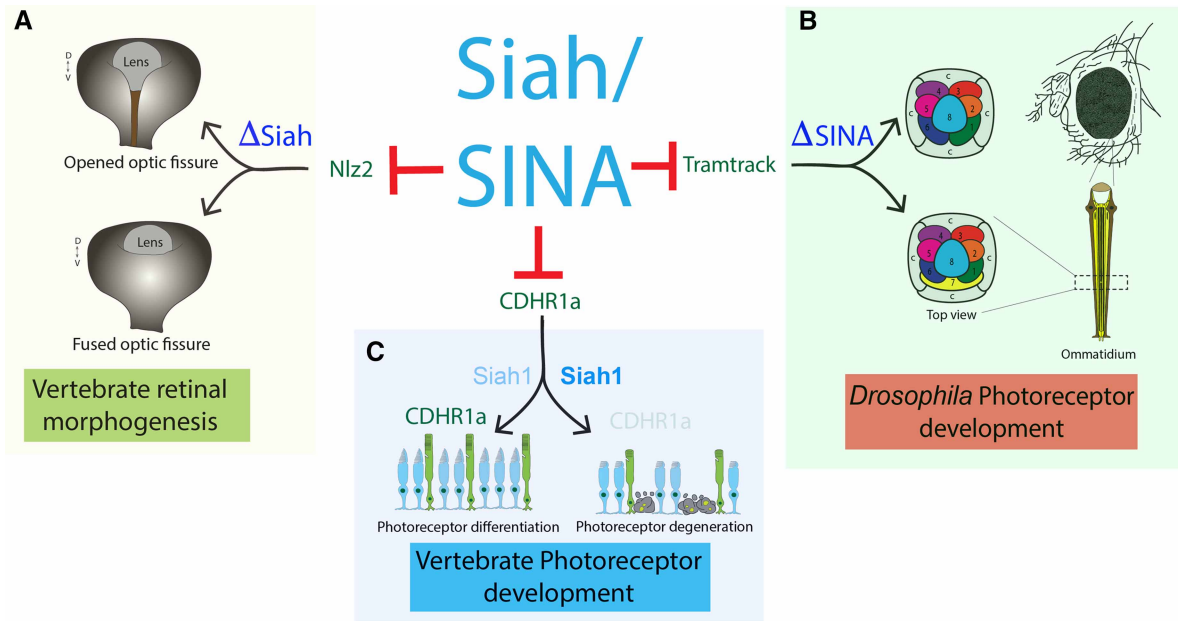
Figure 3. Siah/SINA mediated regulation of ocular development. Activities of the Siah/SINA family of E3 ubiquitin ligase enzymes during *Drosophila* and vertebrate ocular development.
(**A**) Siah activity is involved in vertebrate ocular morphogenesis by regulating optic fissure fusion by targeting 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.

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

PORTLAND PRESS

Open Access

Open access for this article was enabled by the participation of University of Kentucky in an all-inclusive *Read & Publish* pilot with Portland Press and the Biochemical Society.

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.

References

1   Geng, F., Wenzel, S. and Tansey, W.P. (2012) Ubiquitin and proteasomes in transcription. *Annu. Rev. Biochem.* **81**, 177–201 https://doi.org/10.1146/annurev-biochem-052110-120012
2   Haglund, K. and Dikic, I. (2012) The role of ubiquitylation in receptor endocytosis and endosomal sorting. *J. Cell Sci.* **125**, 265–275 https://doi.org/10.1242/jcs.091280
3   Mocciaro, A. and Rape, M. (2012) Emerging regulatory mechanisms in ubiquitin-dependent cell cycle control. *J. Cell Sci.* **125**, 255–263 https://doi.org/10.1242/jcs.091199
4   Fredrickson, E.K. and Gardner, R.G. (2012) Selective destruction of abnormal proteins by ubiquitin-mediated protein quality control degradation. *Semin. Cell Dev. Biol.* **23**, 530–537 https://doi.org/10.1016/j.semcdb.2011.12.006
5   Jiang, X. and Chen, Z.J. (2012) The role of ubiquitylation in immune defence and pathogen evasion. *Nat. Rev. Immunol.* **12**, 35–48 https://doi.org/10.1038/nri3111
6   Bendotti, C., Marino, M., Cheroni, C., Fontana, E., Crippa, V., Poletti, A. et al. (2012) Dysfunction of constitutive and inducible ubiquitin-proteasome system in amyotrophic lateral sclerosis: implication for protein aggregation and immune response. *Prog. Neurobiol.* **97**, 101–126 https://doi.org/10.1016/j.pneurobio.2011.10.001
7   Hershko, A. and Ciechanover, A. (1998) The ubiquitin system. *Annu. Rev. Biochem.* **67**, 425–479 https://doi.org/10.1146/annurev.biochem.67.1.425
8   Jahngen-Hodge, J., Obin, M.S., Gong, X., Shang, F., Nowell, T.R., Gong, J. et al. (1997) Regulation of ubiquitin-conjugating enzymes by glutathione following oxidative stress. *J. Biol. Chem.* **272**, 28218–28226 https://doi.org/10.1074/jbc.272.45.28218
9   Shang, F., Gong, X. and Taylor, A. (1997) Activity of ubiquitin-dependent pathway in response to oxidative stress. *J. Biol. Chem.* **272**, 23086–23093 https://doi.org/10.1074/jbc.272.37.23086
10 Campello, L., Esteve-Rudd, J., Cuenca, N. and Martín-Nieto, J. (2013) The ubiquitin-proteasome system in retinal health and disease. *Mol. Neurobiol.* **47**, 790–810 https://doi.org/10.1007/s12035-012-8391-5
11 Shang, F. and Taylor, A. (2012) Roles for the ubiquitin–proteasome pathway in protein quality control and signaling in the retina: implications in the pathogenesis of age-related macular degeneration. *Mol. Aspects Med.* **33**, 446–466 https://doi.org/10.1016/j.mam.2012.04.001
12 Handley-Gearhart, P., Stephen, A., Trausch-Azar, J., Ciechanover, A. and Schwartz, A. (1994) Human ubiquitin-activating enzyme, E1. indication of potential nuclear and cytoplasmic subpopulations using epitope-tagged cDNA constructs. *J. Biol. Chem.* **269**, 33171–8 https://doi.org/10.1016/S0021-9258(20)30113-7
13 Shang, F., Deng, G., Obin, M., Wu, C.C.N., Gong, X., Smith, D. et al. (2001) Ubiquitin-activating enzyme (E1) isoforms in lens epithelial cells: origin of translation, E2 specificity and cellular localization determined with novel site-specific antibodies. *Exp. Eye Res.* **73**, 827–836 https://doi.org/10.1006/exer.2001.1091
14 Jin, J., Li, X., Gygi, S.P. and Harper, J.W. (2007) Dual E1 activation systems for ubiquitin differentially regulate E2 enzyme charging. *Nature* **447**, 1135–1138 https://doi.org/10.1038/nature05902
15 Liu, X., Sun, L., Gursel, D.B., Cheng, C., Huang, S., Rademaker, A.W. et al. (2017) The non-canonical ubiquitin activating enzyme UBA6 suppresses epithelial-mesenchymal transition of mammary epithelial cells. *Oncotarget* **8**, 87480–87493 https://doi.org/10.18632/oncotarget.20900
16 Schulman, B.A. and Wade Harper, J. (2009) Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways. *Nat. Rev. Mol. Cell Biol.* **10**, 319–331 https://doi.org/10.1038/nrm2673
17 Michelle, C., Vourc'h, P., Mignon, L. and Andres, C.R. (2009) What Was the Set of ubiquitin and ubiquitin-Like conjugating enzymes in the eukaryote common ancestor? *J. Mol. Evol.* **68**, 616–628 https://doi.org/10.1007/s00239-009-9225-6
18 Ye, Y. and Rape, M. (2009) Building ubiquitin chains: E2 enzymes at work. *Nat. Rev. Mol. Cell Biol.* **10**, 755–764 https://doi.org/10.1038/nrm2780
19 Pickart, C.M. and Eddins, M.J. (2004) Ubiquitin: structures, functions, mechanisms. *Biochim. Biophys. Acta - Mol. Cell Res.* **1695**, 55–72 https://doi.org/10.1016/j.bbamcr.2004.09.019
20 Deshaies, R.J. and Joazeiro, C.A.P. (2009) RING domain E3 ubiquitin ligases. *Annu. Rev. Biochem.* **78**, 399–434 https://doi.org/10.1146/annurev.biochem.78.101807.093809
21 DeSalle, L.M. and Pagano, M. (2001) Regulation of the G1 to S transition by the ubiquitin pathway. *FEBS Lett.* **490**, 179–189 https://doi.org/10.1016/S0014-5793(01)02121-4
22 Peters, J.M. (2002) The anaphase-Promoting complex. *Mol. Cell* **9**, 931–943 https://doi.org/10.1016/S1097-2765(02)00540-3
23 Beal, R., Deveraux, Q., Xia, G., Rechsteiner, M. and Pickart, C. (1996) Surface hydrophobic residues of multiubiquitin chains essential for proteolytic targeting. *Proc. Natl Acad. Sci. U.S.A.* **93**, 861–866 https://doi.org/10.1073/pnas.93.2.861

24   Pickart, C.M. (1997) Targeting of substrates to the 26S proteasome. *FASEB J.* **11**, 1055–1066 https://doi.org/10.1096/fasebj.11.13.9367341

25   Lam, Y.A., Lawson, T.G., Velayutham, M., Zweier, J.L. and Pickart, C.M. (2002) A proteasomal ATPase subunit recognizes the polyubiquitin degradation signal. *Nature* **416**, 763–767 https://doi.org/10.1038/416763a

26   Chau, V., Tobias, J., Bachmair, A., Marriott, D., Ecker, D., Gonda, D. et al. (1989) A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. *Science* **243**, 1576–1583 https://doi.org/10.1126/science.2538923

27   Thrower, J.S. (2000) Recognition of the polyubiquitin proteolytic signal. *EMBO J.* **19**, 94–102 https://doi.org/10.1093/emboj/19.1.94

28   Deng, L., Wang, C., Spencer, E., Yang, L., Braun, A., You, J. et al. (2000) Activation of the IκB kinase complex by TRAF6 requires a dimeric ubiquitin-Conjugating enzyme complex and a unique polyubiquitin chain. *Cell* **103**, 351–361 https://doi.org/10.1016/S0092-8674(00)00126-4

29   Chiu, RK., Brun, J., Ramaekers, C., Theys, J., Weng, L., Lambin, P. et al. (2006) Lysine 63-Polyubiquitination guards against translesion synthesis–induced mutations. *PLoS Genet.* **2**, e116 https://doi.org/10.1371/journal.pgen.0020116

30   Nandi, D., Tahiliani, P., Kumar, A. and Chandu, D. (2006) The ubiquitin-proteasome system. *J. Biosci.* **31**, 137–155 https://doi.org/10.1007/BF02705243

31   Glickman, M.H. and Ciechanover, A. (2002) The ubiquitin-Proteasome proteolytic pathway: destruction for the sake of construction. *Physiol. Rev.* **82**, 373–428 https://doi.org/10.1152/physrev.00027.2001

32   Bochtler, M., Ditzel, L., Groll, M., Hartmann, C. and Huber, R. (1999) The proteasome. *Annu. Rev. Biophys. Biomol. Struct.* **28**, 295–317 https://doi.org/10.1146/annurev.biophys.28.1.295

33   Groll, M., Ditzel, L., Löwe, J., Stock, D., Bochtler, M., Bartunik, H.D. et al. (1997) Structure of 20S proteasome from yeast at 2.4 Å resolution. *Nature* **386**, 463–471 https://doi.org/10.1038/386463a0

34   Lowe, J., Stock, D., Jap, B., Zwickl, P., Baumeister, W. and Huber, R. (1995) Crystal structure of the 20S proteasome from the archaeon T. acidophilum at 3.4 A resolution. *Science* **268**, 533–539 https://doi.org/10.1126/science.7725097

35   Glickman, M.H., Rubin, D.M., Coux, O., Wefes, I., Pfeifer, G., Cjeka, Z. et al. (1998) A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-Signalosome and eIF3. *Cell* **94**, 615–623 https://doi.org/10.1016/S0092-8674(00)81603-7

36   Verma, R., Chen, S., Feldman, R., Schieltz, D., Yates, J., Dohmen, J. et al. (2000) Proteasomal proteomics: identification of nucleotide-sensitive proteasome-interacting proteins by mass spectrometric analysis of affinity-purified proteasomes. *Mol. Biol. Cell* **11**, 3425–3439 https://doi.org/10.1091/mbc.11.10.3425

37   Noda, C., Tanahashi, N., Shimbara, N., Hendil, K.B. and Tanaka, K. (2000) Tissue distribution of constitutive proteasomes, immunoproteasomes, and PA28 in rats. *Biochem. Biophys. Res. Commun.* **277**, 348–354 https://doi.org/10.1006/bbrc.2000.3676

38   Sixt, S.U., Alami, R., Hakenbeck, J., Adamzik, M., Kloß, A., Costabel, U. et al. (2012) Distinct proteasome subpopulations in the alveolar space of patients with the acute respiratory distress syndrome. *Mediators Inflamm.* **2012**, 1–9 https://doi.org/10.1155/2012/204250

39   Clague, M.J., Barsukov, I., Coulson, J.M., Liu, H., Rigden, D.J., Urbé, S. (2013) Deubiquitylases from genes to organism. *Physiol. Rev.* **93**, 1289–1315 https://doi.org/10.1152/physrev.00002.2013

40   Abdul. Rehman S.A., Kristariyanto, Y.A., Choi, S.-Y., Nkosi, P.J., Weidlich, S., Labib, K. et al. (2016) MINDY-1 Is a member of an evolutionarily conserved and structurally distinct New family of deubiquitinating enzymes. *Mol. Cell* **63**, 146–155 https://doi.org/10.1016/j.molcel.2016.05.009

41   Obin, M., Nowell, T. and Taylor, A. (1994) The photoreceptor G-Protein transducin (Gt) Is a substrate for ubiquitin-Dependent proteolysis. *Biochem. Biophys. Res. Commun.* **200**, 1169–1176 https://doi.org/10.1006/bbrc.1994.1574

42   Obin, M.S., Jahngen-Hodge, J., Nowell, T. and Taylor, A. (1996) Ubiquitinylation and ubiquitin-dependent proteolysis in vertebrate photoreceptors (Rod outer segments). *J. Biol. Chem.* **271**, 14473–14484 https://doi.org/10.1074/jbc.271.24.14473

43   Obin, M., Nowell, T. and Taylor, A. (1995) A comparison of ubiquitin-dependent proteolysis of rod outer segment proteins in reticulocyte lysate and a retinal pigment epithelial cell line. *Curr. Eye Res.* **14**, 751–760 https://doi.org/10.3109/02713689508995796

44   Naash, M.I., Al-Ubaidi, M.R. and Anderson, R.E. (1997) Light exposure induces ubiquitin conjugation and degradation activities in the Rat retina. *Invest Ophthalmol Vis Sci.* **38**, 2344–2354 PMID:9344358

45   Chakarova, C.F., Papaioannou, M.G., Khanna, H., Lopez, I., Waseem, N., Shah, A. et al. (2007) Mutations in TOPORS cause autosomal dominant retinitis pigmentosa with perivascular retinal pigment epithelium atrophy. *Am. J. Hum. Genet.* **81**, 1098–1103 https://doi.org/10.1086/521953

46   Esquerdo, M., Grau-Bové, X., Garanto, A., Toulis, V., Garcia-Monclús, S., Millo, E. et al. (2016) Expression atlas of the deubiquitinating enzymes in the adult mouse retina, their evolutionary diversification and phenotypic roles. *PLoS ONE* **11**, e0150364 https://doi.org/10.1371/journal.pone.0150364

47   Cao, L., Wang, P., Gao, Y., Lin, X., Wang, F. and Wu, S. (2014) Ubiquitin E3 ligase dSmurf is essential for Wts protein turnover and hippo signaling. *Biochem. Biophys. Res. Commun.* **454**, 167–171 https://doi.org/10.1016/j.bbrc.2014.10.058

48   Kim, W., Cho, Y.S., Wang, X., Park, O., Ma, X., Kim, H. et al. (2019) Hippo signaling is intrinsically regulated during cell cycle progression by APC/C Cdh1. *Proc. Natl Acad. Sci. U.S.A.* **116**, 9423–9432 https://doi.org/10.1073/pnas.1821370116

49   Udan, R.S., Kango-Singh, M., Nolo, R., Tao, C. and Halder, G. (2003) Hippo promotes proliferation arrest and apoptosis in the salvador/Warts pathway. *Nat. Cell Biol.* **5**, 914–920 https://doi.org/10.1038/ncb1050

50   Pantalacci, S., Tapon, N. and Léopold, P. (2003) The salvador partner hippo promotes apoptosis and cell-cycle exit in drosophila. *Nat. Cell Biol.* **5**, 921–927 https://doi.org/10.1038/ncb1051

51   Lai, Z.-C., Wei, X., Shimizu, T., Ramos, E., Rohrbaugh, M., Nikolaidis, N. et al. (2005) Control of cell proliferation and apoptosis by Mob as tumor suppressor, mats. *Cell* **120**, 675–685 https://doi.org/10.1016/j.cell.2004.12.036

52   Li, T., Fan, J., Blanco-Sánchez, B., Giagtzoglou, N., Lin, G., Yamamoto, S. et al. (2016) Ubr3, a novel modulator of Hh signaling affects the degradation of costal-2 and Kif7 through poly-ubiquitination. *PLoS Genet.* **12**, e1006054 https://doi.org/10.1371/journal.pgen.1006054

53   Martins, T., Meghini, F., Florio, F. and Kimata, Y. (2017) The APC/C coordinates retinal differentiation with G1 arrest through the Nek2-Dependent modulation of wingless signaling. *Dev. Cell* **40**, 67–80 https://doi.org/10.1016/j.devcel.2016.12.005

54   Wolff, T. and Ready, D. (1993) Pattern formation in the Drosophila retina. Dev. Drosoph. melanogaster 1277–1325

55   Fan, Y. and Bergmann, A. (2014) Multiple mechanisms modulate distinct cellular susceptibilities toward apoptosis in the developing drosophila Eye. *Dev. Cell* **30**, 48–60 https://doi.org/10.1016/j.devcel.2014.05.007

56   Freeman, M. (1996) Reiterative Use of the EGF receptor triggers differentiation of all cell types in the drosophila Eye. *Cell* **87**, 651–660 https://doi.org/10.1016/S0092-8674(00)81385-9

57 Yang, L. and Baker, N.E. (2001) Role of the EGFR/Ras/Raf pathway in specification of photoreceptor cells in the drosophila retina. *Development* **128**, 1183–1191 PMID:11245584

58 del Alamo, D. and Mlodzik, M. (2006) Frizzled/PCP-Dependent asymmetric neuralized expression determines R3/R4 fates in the drosophila eye. *Dev. Cell* **11**, 887–894 https://doi.org/10.1016/j.devcel.2006.09.016

59 Lai, E., Deblandre, G.A., Kintner, C. and Rubin, G.M. (2001) Drosophila neuralized Is a ubiquitin ligase that promotes the internalization and degradation of delta. *Dev. Cell* **1**, 783–794 https://doi.org/10.1016/S1534-5807(01)00092-2

60 Kumar, J.P. (2012) Building an ommatidium one cell at a time. *Dev. Dyn.* **241**, 136–149 https://doi.org/10.1002/dvdy.23707

61 Dickson, B.J. (1998) Photoreceptor development: breaking down the barriers. *Curr. Biol.* **8**, R90–R92 https://doi.org/10.1016/S0960-9822(98)70054-5

62 Tang, A.H., Neufeld, T.P., Kwan, E. and Rubin, G.M. (1997) PHYL acts to down-Regulate TTK88, a transcriptional repressor of neuronal cell fates, by a SINA-Dependent mechanism. *Cell* **90**, 459–467 https://doi.org/10.1016/S0092-8674(00)80506-1

63 Li, S., Li, Y., Carthew, R.W. and Lai, Z.-C. (1997) Photoreceptor cell differentiation requires regulated proteolysis of the transcriptional repressor tramtrack. *Cell* **90**, 469–478 https://doi.org/10.1016/S0092-8674(00)80507-3

64 Li, S., Xu, C. and Carthew, R.W. (2002) Phyllopod acts as an adaptor protein To link the sina ubiquitin ligase to the substrate protein tramtrack. *Mol. Cell. Biol.* **22**, 6854–6865 https://doi.org/10.1128/MCB.22.19.6854-6865.2002

65 Reinke, R. and Zipursky, S.L. (1988) Cell-cell interaction in the drosophila retina: the bride of sevenless gene is required in photoreceptor cell R8 for R7 cell development. *Cell* **55**, 321–330 https://doi.org/10.1016/0092-8674(88)90055-4

66 Hart, A.C., Kramer, H., Van Vactor, D.L., Paidhungat, M. and Zipursky, S.L. (1990) Induction of cell fate in the drosophila retina: the bride of sevenless protein is predicted to contain a large extracellular domain and seven transmembrane segments. *Genes Dev.* **4**, 1835–1847 https://doi.org/10.1101/gad.4.11.1835

67 Basler, K., Yen, D., Tomlinson, A. and Hafen, E. (1990) Reprogramming cell fate in the developing drosophila retina: transformation of R7 cells by ectopic expression of rough. *Genes Dev.* **4**, 728–739 https://doi.org/10.1101/gad.4.5.728

68 Kimmel, B.E., Heberlein, U. and Rubin, G.M. (1990) The homeo domain protein rough is expressed in a subset of cells in the developing drosophila eye where it can specify photoreceptor cell subtype. *Genes Dev.* **4**, 712–727 https://doi.org/10.1101/gad.4.5.712

69 Mlodzik, M., Hiromi, Y., Weber, U., Goodman, C.S. and Rubin, G.M. (1990) The drosophila seven-up gene, a member of the steroid receptor gene superfamily, controls photoreceptor cell fates. *Cell* **60**, 211–224 https://doi.org/10.1016/0092-8674(90)90737-Y

70 Xiong, W.C. and Montell, C. (1993) Tramtrack is a transcriptional repressor required for cell fate determination in the drosophila eye. *Genes Dev.* **7**, 1085–1096 https://doi.org/10.1101/gad.7.6.1085

71 Lai, Z.C., Harrison, S.D., Karim, F., Li, Y. and Rubin, G.M. (1996) Loss of tramtrack gene activity results in ectopic R7 cell formation, even in a sina mutant background. *Proc. Natl Acad. Sci. U.S.A.* **93**, 5025–5030 https://doi.org/10.1073/pnas.93.10.5025

72 Thao, D.T.P., An, P.N.T., Yamaguchi, M. and LinhThuoc, T. (2012) Overexpression of ubiquitin carboxyl terminal hydrolase impairs multiple pathways during eye development in drosophila melanogaster. *Cell Tissue Res.* **348**, 453–463 https://doi.org/10.1007/s00441-012-1404-x

73 Schwarz, M., Cecconi, F., Bernier, G., Andrejewski, N., Kammandel, B., Wagner, M. et al. (2000) Spatial specification of mammalian eye territories by reciprocal transcriptional repression of Pax2 and Pax6. *Development* **127**, 4325–4334 PMID:11003833

74 Pfirrmann, T., Jandt, E., Ranft, S., Lokapally, A., Neuhaus, H., Perron, M. et al. (2016) Hedgehog-dependent E3-ligase Midline1 regulates ubiquitin-mediated proteasomal degradation of Pax6 during visual system development. *Proc. Natl Acad. Sci. U.S.A.* **113**, 10103–10108 https://doi.org/10.1073/pnas.1600770113

75 Kim, J.-J., Kim, Y.-H. and Lee, M.-Y. (2009) Proteomic characterization of differentially expressed proteins associated with no stress in retinal ganglion cells. *BMB Rep.* **42**, 456–461 https://doi.org/10.5483/BMBRep.2009.42.7.456

76 Drinjakovic, J., Jung, H., Campbell, D.S., Strochlic, L., Dwivedy, A. and Holt, C.E. (2010) E3 ligase Nedd4 promotes axon branching by downregulating PTEN. *Neuron* **65**, 341–357 https://doi.org/10.1016/j.neuron.2010.01.017

77 Balastik, M., Ferraguti, F., Pires-da Silva, A., Lee, T.H., Alvarez-Bolado, G., Lu, K.P. et al. (2008) Deficiency in ubiquitin ligase TRIM2 causes accumulation of neurofilament light chain and neurodegeneration. *Proc. Natl Acad. Sci. U.S.A.* **105**, 12016–12021 https://doi.org/10.1073/pnas.0802261105

78 Friedman, J.S., Ray, J.W., Waseem, N., Johnson, K., Brooks, M.J., Hugosson, T. et al. (2009) Mutations in a BTB-Kelch protein, KLHL7, cause autosomal-Dominant retinitis pigmentosa. *Am. J. Hum. Genet.* **84**, 792–800 https://doi.org/10.1016/j.ajhg.2009.05.007

79 Sumara, I., Maerki, S. and Peter, M. (2008) E3 ubiquitin ligases and mitosis: embracing the complexity. *Trends Cell Biol.* **18**, 84–94 https://doi.org/10.1016/j.tcb.2007.12.001

80 Rondou, P., Haegeman, G., Vanhoenacker, P. and Van Craenenbroeck, K. (2008) BTB protein KLHL12 targets the dopamine D4 receptor for ubiquitination by a Cul3-based E3 ligase. *J. Biol. Chem.* **283**, 11083–11096 https://doi.org/10.1074/jbc.M708473200

81 Bhattacharya, S., Ray, R.M., Chaum, E., Johnson, D.A. and Johnson, L.R. (2011) Inhibition of Mdm2 sensitizes human retinal pigment epithelial cells to apoptosis. *Investig. Opthalmology Vis. Sci.* **52**, 3368 https://doi.org/10.1167/iovs.10-6991

82 Ozawa, Y., Nakao, K., Kurihara, T., Shimazaki, T., Shimmura, S., Ishida, S. et al. (2008) Roles of STAT3/SOCS3 pathway in regulating the visual function and ubiquitin-proteasome-dependent degradation of rhodopsin during retinal inflammation. *J. Biol. Chem.* **283**, 24561–24570 https://doi.org/10.1074/jbc.M802238200

83 Esteve-Rudd, J., Campello, L., Herrero, M.-T., Cuenca, N. and Martín-Nieto, J. (2010) Expression in the mammalian retina of parkin and UCH-L1, two components of the ubiquitin-proteasome system. *Brain Res.* **1352**, 70–82 https://doi.org/10.1016/j.brainres.2010.07.019

84 Toulis, V., Garanto, A. and Marfany, G. (2016) Combining zebrafish and mouse models to test the function of deubiquitinating enzyme (Dubs) genes in development: role of USP45 in the retina. *Methods Mol. Biol.* **1449**, 85–101 https://doi.org/10.1007/978-1-4939-3756-1_3

85 Esquerdo-Barragán, M., Brooks, M.J., Toulis, V., Swaroop, A. and Marfany, G. (2019) Expression of deubiquitinating enzyme genes in the developing mammal retina. *Mol. Vis.* **25**, 800–813 PMID:31819342

86 Pepper, I.J., Van Sciver, R.E. and Tang, A.H. (2017) Phylogenetic analysis of the SINA/SIAH ubiquitin E3 ligase family in metazoa. *BMC Evol. Biol.* **17**, 182 https://doi.org/10.1186/s12862-017-1024-x

87 Qi, J., Kim, H., Scortegagna, M. and Ronai, Z.A. (2013) Regulators and effectors of Siah ubiquitin ligases. *Cell Biochem. Biophys.* **67**, 15–24 https://doi.org/10.1007/s12013-013-9636-2

88   Hasson, S.A., Kane, L.A., Yamano, K., Huang, C.-H., Sliter, D.A., Buehler, E. et al. (2013) High-content genome-wide RNAi screens identify regulators of parkin upstream of mitophagy. *Nature* **504**, 291–295 https://doi.org/10.1038/nature12748

89   Robbins, C.M., Tembe, W.A., Baker, A., Sinari, S., Moses, T.Y., Beckstrom-Sternberg, S. et al. (2011) Copy number and targeted mutational analysis reveals novel somatic events in metastatic prostate tumors. *Genome Res.* **21**, 47–55 https://doi.org/10.1101/gr.107961.110

90   Stebbins, J.L., Santelli, E., Feng, Y., De, S.K., Purves, A., Motamedchaboki, K. et al. (2013) Structure-Based design of covalent siah inhibitors. *Chem. Biol.* **20**, 973–982 https://doi.org/10.1016/j.chembiol.2013.06.008

91   House, C.M., Moller, A. and Bowtell, D.D.L. (2009) Siah proteins: novel drug targets in the Ras and hypoxia pathways. *Cancer Res.* **69**, 8835–8838 https://doi.org/10.1158/0008-5472.CAN-09-1676

92   Piedade, W.P., Veith, S. and Famulski, J.K. (2019) Ubiquitin-mediated proteasome degradation regulates optic fissure fusion. *Biol. Open* **8**, bio044974 https://doi.org/10.1242/bio.044974

93   Qi, J., Nakayama, K., Gaitonde, S., Goydos, J.S., Krajewski, S., Eroshkin, A. et al. (2008) The ubiquitin ligase Siah2 regulates tumorigenesis and metastasis by HIF-dependent and -independent pathways. *Proc. Natl Acad. Sci. U.S.A.* **105**, 16713–8 https://doi.org/10.1073/pnas.0804063105

94   Möller, A., House, C.M., Wong, C.S.F., Scanlon, D.B., Liu, M.C.P., Ronai, Z. et al. (2009) Inhibition of siah ubiquitin ligase function. *Oncogene* **28**, 289–296 https://doi.org/10.1038/onc.2008.382

95   Zhang, Q., Wang, Z., Hou, F., Harding, R., Huang, X., Dong, A. et al. (2017) The substrate binding domains of human SIAH E3 ubiquitin ligases are now crystal clear. *Biochim. Biophys. Acta Gen. Subj.* **1861**, 3095–3105 https://doi.org/10.1016/j.bbagen.2016.10.019

96   Wong, K.S.Y., Rehn, K., Palencia-Desai, S., Kohli, V., Hunter, W., Uhl, J.D. et al. (2014) Siah ubiquitin ligases modulate nodal signaling during zebrafish embryonic development. *J. Biol. Chem.* **37**, 775–786 https://doi.org/10.14348/molcells.2014.0032

97   Famulski, J.K., Trivedi, N., Howell, D., Yang, Y., Tong, Y., Gilbertson, R. et al. (2010) Siah regulation of Pard3A controls neuronal cell adhesion during germinal zone exit. *Science* **330**, 1834–1838 https://doi.org/10.1126/science.1198480

98   Nakayama, K., Frew, I.J., Hagensen, M., Skals, M., Habelhah, H., Bhoumik, A. et al. (2004) Siah2 regulates stability of prolyl-hydroxylases, controls HIF1α abundance, and modulates physiological responses to hypoxia. *Cell* **117**, 941–952 https://doi.org/10.1016/j.cell.2004.06.001

99   Carlucci, A., Adornetto, A., Scorziello, A., Viggiano, D., Foca, M., Cuomo, O. et al. (2008) Proteolysis of AKAP121 regulates mitochondrial activity during cellular hypoxia and brain ischaemia. *EMBO J.* **27**, 1073–1084 https://doi.org/10.1038/emboj.2008.33

100 Yego, E.C.K. and Mohr, S. (2010) Siah-1 protein is necessary for high glucose-Induced glyceraldehyde-3- phosphate dehydrogenase nuclear accumulation and cell death in M??Ller cells. *J. Biol. Chem.* **285**, 3181–3190 https://doi.org/10.1074/jbc.M109.083907

101 Winter, M., Sombroek, D., Dauth, I., Moehlenbrink, J., Scheuermann, K., Crone, J. et al. (2008) Control of HIPK2 stability by ubiquitin ligase siah-1 and checkpoint kinases ATM and ATR. *Nat. Cell Biol.* **10**, 812–824 https://doi.org/10.1038/ncb1743

102 Fiucci, G., Beaucourt, S., Duflaut, D., Lespagnol, A., Stumptner-Cuvelette, P., Geant, A. et al. (2004) Siah-1b is a direct transcriptional target of p53: identification of the functional p53 responsive element in the siah-1b promoter. *Proc. Natl Acad. Sci. U.S.A.* **101**, 3510–3515 https://doi.org/10.1073/pnas.0400177101

103 Khurana, A., Nakayama, K., Williams, S., Davis, R.J., Mustelin, T. and Ronai, Z. (2006) Regulation of the ring finger E3 ligase Siah2 by p38 MAPK. *J. Biol. Chem.* **281**, 35316–35326 https://doi.org/10.1074/jbc.M606568200

104 Pérez, M., García-Limones, C., Zapico, I., Marina, A., Schmitz, M.L., Muñoz, E. et al. (2012) Mutual regulation between SIAH2 and DYRK2 controls hypoxic and genotoxic signaling pathways. *J. Mol. Cell Biol.* **4**, 316–330 https://doi.org/10.1093/jmcb/mjs047

105 Calzado, M.A., de la Vega, L., Möller, A., Bowtell, D.D.L. and Schmitz, M.L. (2009) An inducible autoregulatory loop between HIPK2 and Siah2 at the apex of the hypoxic response. *Nat. Cell Biol.* **11**, 85–91 https://doi.org/10.1038/ncb1816

106 Malz, M., Aulmann, A., Samarin, J., Bissinger, M., Longerich, T., Schmitt, S. et al. (2012) Nuclear accumulation of seven in absentia homologue-2 supports motility and proliferation of liver cancer cells. *Int. J. Cancer* **131**, 2016–2026 https://doi.org/10.1002/ijc.27473

107 Schmidt, R.L., Park, C.H., Ahmed, A.U., Gundelach, J.H., Reed, N.R., Cheng, S. et al. (2007) Inhibition of RAS-Mediated transformation and tumorigenesis by targeting the downstream E3 ubiquitin ligase seven in absentia homologue. *Cancer Res.* **67**, 11798–11810 https://doi.org/10.1158/0008-5472.CAN-06-4471

108 Chan, P., Möller, A., Liu, M.C., Sceneay, J.E., Wong, C.S., Waddell, N. et al. (2011) The expression of the ubiquitin ligase SIAH2 (seven in absentia homolog 2) is mediated through gene copy number in breast cancer and is associated with a basal-like phenotype and p53 expression. *Breast Cancer Res.* **13**, R19 https://doi.org/10.1186/bcr2828

109 Ahmed, A.U., Schmidt, R.L., Park, C.H., Reed, N.R., Hesse, S.E., Thomas, C.F. et al. (2008) Effect of disrupting seven-in-absentia homolog 2 function on lung cancer cell growth. *JNCI J. Natl. Cancer Inst.* **100**, 1606–1629 https://doi.org/10.1093/jnci/djn365

110 Qi, J., Nakayama, K., Cardiff, R.D., Borowsky, A.D., Kaul, K., Williams, R. et al. (2010) Siah2-Dependent concerted activity of HIF and FoxA2 regulates formation of neuroendocrine phenotype and neuroendocrine prostate tumors. *Cancer Cell* **18**, 23–38 https://doi.org/10.1016/j.ccr.2010.05.024

111 Brauckhoff, A., Malz, M., Tschaharganeh, D., Malek, N., Weber, A., Riener, M.-O. et al. (2011) Nuclear expression of the ubiquitin ligase seven in absentia homolog (SIAH)-1 induces proliferation and migration of liver cancer cells. *J. Hepatol.* **55**, 1049–1057 https://doi.org/10.1016/j.jhep.2011.02.019

112 Matsuo, K., Satoh, S., Okabe, H., Nomura, A., Maeda, T., Yamaoka, Y. et al. (2003) SIAH1 inactivation correlates with tumor progression in hepatocellular carcinomas. *Genes Chromosome Cancer* **36**, 283–291 https://doi.org/10.1002/gcc.10170

113 Kim, C.J., Cho, Y.G., Park, C.H., Jeong, S.W., Nam, S.W., Kim, S.Y. et al. (2004) Inactivating mutations of the Siah-1 gene in gastric cancer. *Oncogene* **23**, 8591–8596 https://doi.org/10.1038/sj.onc.1208113

114 Engelender, S., Kaminsky, Z., Guo, X., Sharp, A.H., Amaravi, R.K., Kleiderlein, J.J. et al. (1999) Synphilin-1 associates with α-synuclein and promotes the formation of cytosolic inclusions. *Nat. Genet.* **22**, 110–114 https://doi.org/10.1038/8820

115 Nagano, Y., Yamashita, H., Takahashi, T., Kishida, S., Nakamura, T., Iseki, E. et al. (2003) Siah-1 facilitates ubiquitination and degradation of synphilin-1. *J. Biol. Chem.* **278**, 51504–51514 https://doi.org/10.1074/jbc.M306347200

116 Liani, E., Eyal, A., Avraham, E., Shemer, R., Szargel, R., Berg, D. et al. (2004) Ubiquitylation of synphilin-1 and -synuclein by SIAH and its presence in cellular inclusions and Lewy bodies imply a role in Parkinson's disease. *Proc. Natl Acad. Sci. U.S.A.* **101**, 5500–5505 https://doi.org/10.1073/pnas.0401081101

117 Frew, I.J., Hammond, V.E., Dickins, R.A., Quinn, J.M.W., Walkley, C.R., Sims, N.A. et al. (2003) Generation and analysis of Siah2 mutant mice. *Mol. Cell. Biol.* **23**, 9150–9161 https://doi.org/10.1128/MCB.23.24.9150-9161.2003

118 Bogdan, S., Senkel, S., Esser, F., Ryffel, GU., Pogge, V. and Strandmann, E. (2001) Misexpression of Xsiah-2 induces a small eye phenotype in xenopus. *Mech. Dev.* **103**, 61–69 https://doi.org/10.1016/S0925-4773(01)00332-X

119 House, C.M., Frew, I.J., Huang, H.-L., Wiche, G., Traficante, N., Nice, E. et al. (2003) A binding motif for Siah ubiquitin ligase. *Proc. Natl Acad. Sci. U. S.A.* **100**, 3101–3106 https://doi.org/10.1073/pnas.0534783100

120 House, C.M., Hancock, N.C., Möller, A., Cromer, B.A., Fedorov, V., Bowtell, D.D.L. et al. (2006) Elucidation of the substrate binding site of Siah ubiquitin ligase. *Structure* **14**, 695–701 https://doi.org/10.1016/j.str.2005.12.013

121 Brown, J.D., Dutta, S., Bharti, K., Bonner, R.F., Munson, P.J., Dawid, I.B. et al. (2009) Expression profiling during ocular development identifies 2 Nlz genes with a critical role in optic fissure closure. *Proc. Natl Acad. Sci. U.S.A.* **106**, 1462–1467 https://doi.org/10.1073/pnas.0812017106

122 Rattner, A., Smallwood, P.M., Williams, J., Cooke, C., Savchenko, A., Lyubarsky, A. et al. (2001) A photoreceptor-specific cadherin is essential for the structural integrity of the outer segment and for photoreceptor survival. *Neuron* **32**, 775–786 https://doi.org/10.1016/S0896-6273(01)00531-1

123 Burgoyne, T., Meschede, I.P., Burden, J.J., Bailly, M., Seabra, M.C., Futter, C.E. (2015) Rod disc renewal occurs by evagination of the ciliary plasma membrane that makes cadherin-based contacts with the inner segment. *Proc. Natl Acad. Sci. U.S.A.* **112**, 15922–15927 https://doi.org/10.1073/pnas.1509285113