

Retinal regeneration requires dynamic Notch signaling

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Abstract

Retinal damage in the adult zebrafish induces Müller glia reprogramming to produce neuronal progenitor cells that proliferate and differentiate into retinal neurons. Notch signaling, which is a fundamental mechanism known to drive cell-cell communication, is required to maintain Müller glia in a quiescent state in the undamaged retina, and repression of Notch signaling is necessary for Müller glia to reenter the cell cycle. The dynamic regulation of Notch signaling following retinal damage also directs proliferation and neurogenesis of the Müller glia-derived progenitor cells in a robust regeneration response. In contrast, mammalian Müller glia respond to retinal damage by entering a prolonged gliotic state that leads to additional neuronal death and permanent vision loss. Understanding the dynamic regulation of Notch signaling in the zebrafish retina may aid efforts to stimulate Müller glia reprogramming for regeneration of the diseased human retina. Recent findings identified DeltaB and Notch3 as the ligand-receptor pair that serves as the principal regulators of zebrafish Müller glia quiescence. In addition, multi-omics datasets and functional studies indicate that additional Notch receptors, ligands, and target genes regulate cell proliferation and neurogenesis during the regeneration time course. Still, our understanding of Notch signaling during retinal regeneration is limited. To fully appreciate the complex regulation of Notch signaling that is required for successful retinal regeneration, investigation of additional aspects of the pathway, such as post-translational modification of the receptors, ligand endocytosis, and interactions with other fundamental pathways is needed. Here we review various modes of Notch signaling regulation in the context of the vertebrate retina to put recent research in perspective and to identify open areas of inquiry.

Key Words: differentiation; gliosis; Müller glia; neuronal progenitor cell; Notch signaling; proliferation; quiescence; retinal development; retinal regeneration; zebrafish

Introduction

The vertebrate retina is a highly organized neural tissue consisting of six major types of neurons (rod and cone photoreceptors, horizontal cells, bipolar cells, amacrine cells, and ganglion cells) and three major classes of non-neuronal support cells (retinal pigment epithelial cells, Müller glia, and microglia). Retinal cell bodies are arranged in three distinct nuclear layers (outer nuclear layer, inner nuclear layer, and the ganglion cell layer) that are separated by two plexiform layers (outer plexiform layer and inner plexiform layer), which consist of the axonal and dendritic processes that form synaptic connections. This laminated structure results in efficient detection and pre-processing of sensory information that relies on the intimate cell-cell contacts between neurons, as well as between neurons and Müller glia, which represent the radial glia in the retina (Amini et al., 2017). Retinal damage leads to compromised neuronal function, cell death, disrupted cell-cell contacts, and impaired vision (Hoon et al., 2014). In response to damage, the mammalian retina enters a state of reactive gliosis, in which Müller glia hypertrophy disrupts retinal homeostasis. This leads to secondary

neuronal cell death, glial scarring, and vision loss (Bringmann and Wiedemann, 2012). Vision impairment, which affects an estimated 2.2 billion people worldwide (World Health Organization, 2019), often negatively affects quality of life beyond vision loss because of increased economic burden and mental health problems, including depression and loneliness, especially in the aging population (Gordois et al., 2012; Demmin and Silverstein, 2020). Therefore, there is great need for strategies that will heal the damaged retina and restore visual function.

The ultimate strategy is to replace the missing neurons in the damaged retina and restore a healthy, functioning retina. Zebrafish (*Danio rerio*) possess the ability to regenerate lost neurons from an endogenous source, the Müller glia (Figure 1A). Unlike in mammals, adult zebrafish Müller glia respond to retinal damage by transitioning to a transient gliotic state before entering a reprogrammed state (Thomas et al., 2016; Hoang et al., 2020). A reprogrammed Müller glial cell divides asymmetrically to maintain the Müller glia and to produce a neuronal progenitor cell (NPC) (Nagashima et al., 2013; Lahne et al., 2015). The NPC continues to proliferate to produce a

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cluster of multipotent progenitors that differentiate into all retinal cell types with a bias towards the cells lost to damage (Powell et al., 2016; Lahne et al., 2021). The regenerated neurons develop normal morphologies, form stereotypical connections, and restore circuitry for visual function (D’Orazi et al., 2016; McGinn et al., 2018, 2019).

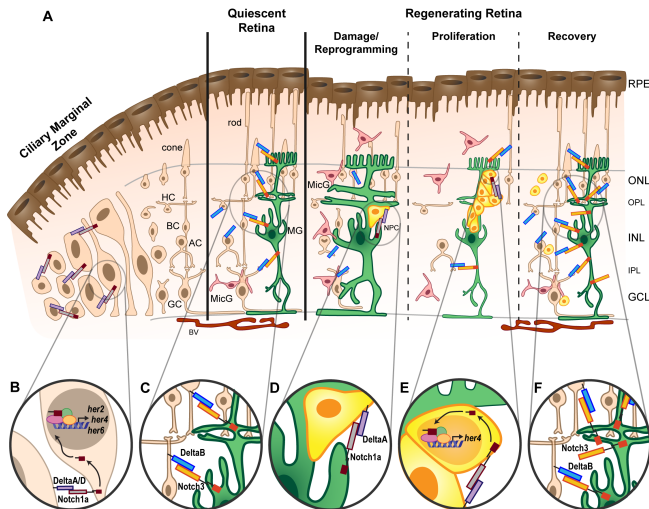


Figure 1 | Dynamic Notch signaling during retinal regeneration. (A) Schematic of the adult zebrafish retina. The retina is organized into three nuclear layers: ganglion cell layer (GCL), inner nuclear layer (INL), and outer nuclear layer (ONL), which are separated by the inner plexiform layer (IPL) and outer plexiform layer (OPL) where synaptic connections are located. Müller glia (MG) cell bodies reside in the INL, but their projections extend throughout all three nuclear layers. MG are mostly quiescent in the intact retina, except for occasional proliferation to give rise to rod photoreceptor progenitors. In the intact, quiescent retina, resting microglia (MicG, pink) reside in the plexiform layers with ramified morphology. The inner, basal surface of the retina is vascularized (blood vessels, BV), and the outer, apical surface is lined with the retinal pigment epithelium (RPE). The ciliary marginal zone (CMZ) at the periphery of the retina is an area of persistent neurogenesis with proliferative multipotent retinal progenitors that give rise to ganglion cells (GC), amacrine cells (AC), bipolar cells (BC), horizontal cells (HC), and cone photoreceptors. (B) The CMZ is marked by expression of *notch1a* and *notch1b* receptor genes, *deltaA* and *deltaD* ligand genes, and downstream target genes *her2*, *her4*, and *her6* (see text). (C) MG express Notch3, which when activated with the DeltaB ligand, maintains MG quiescence. Upon damage, neurons degenerate, and MG hypertrophy, followed by reprogramming and asymmetric cell division to retain a MG and generate a neuronal progenitor cell (NPC). MicG migrate to areas of damage and adopt an amoeboid morphology. (D) Notch3 expression is downregulated in the proliferating retina, but other Notch signaling components are necessary for continued proliferation of the NPCs, in particular Notch1a and DeltaA. (E) Continued NPC proliferation produces a cluster of NPCs in the regenerating retina. This process requires the Notch downstream target, *her4*. (F) The NPCs migrate to the areas of damage and differentiate into the lost neurons. Notch3 expression is reestablished in the quiescent MG.

A complex network of signaling pathways is necessary as retinal damage breaks cell-cell contacts and new contacts are formed through remodeling, proliferation, and differentiation. Notch signaling is an obvious candidate as a major regulator of retinal regeneration because of its well-characterized role in retinal progenitor cell maintenance and Müller glia specification during development (Furukawa et al., 2000; Jadhav et al., 2006a, b). Additionally, Notch signaling relies on contact-mediated interactions between neighboring cells, which means the loss of cell contacts during retinal damage and the generation of different cell contacts as NPCs are generated, proliferate, and commit to neuronal fates are opportunities for Notch involvement. Notch signaling is activated when the Notch receptor expressed on the signal-receiving cell binds a ligand expressed on the signal-sending

cell. A series of proteolytic cleavages are induced to release the Notch intracellular domain (NICD) for translocation into the nucleus of the signal-receiving cell where it effects gene expression changes (Henrique and Schweisguth, 2019). The outcomes of Notch signaling are highly context dependent with respect to specific tissue and to time, so despite the absence of signaling intermediates or second messenger amplification, there are abundant modes of Notch signaling regulation. These include post-translational modification during receptor production, ligand-receptor expression patterning, transendocytosis within the signal-sending cell, receptor cleavage events, NICD-dependent regulation of target gene transcription, and interactions with other signaling pathways (Figure 2).

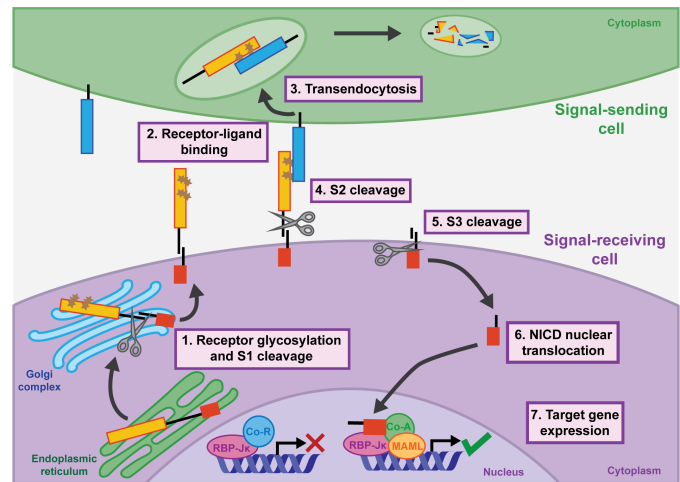


Figure 2 | Core components of the Notch signaling pathway. Schematic of the Notch signaling pathway highlighting points of possible regulation during retinal regeneration. 1: Notch receptors are post-translationally modified by glycosylation and Furin-dependent S1 cleavage. 2: Notch receptors on the signal-receiving cell bind ligands on the signal-sending cell. 3: Transendocytosis pulls the ligand-NECD complex into the signal-sending cell. 4: S2 site is available for metalloprotease-dependent cleavage. 5: γ -Secretase-dependent S3 cleavage releases NICD from the membrane. 6: NICD translocates into the nucleus of the signal-receiving cell and forms a complex with CSL transcription factor RBP-J κ , MAML, and co-activators. 7: Notch binding of the chromatin ultimately induces expression changes of NICD target genes. Co-A: Co-activator protein; Co-R: co-repressor protein; CSL: (CBF1/RBP-J κ , Su(H), LAG-1); MAML: Mastermind-like; NECD: Notch extracellular domain; NICD: Notch intracellular domain; S1: cleavage site 1; S2: cleavage site 2; S3: cleavage site 3.

Within zebrafish retinal regeneration, we largely understand the roles of Notch signaling through the use of γ -secretase inhibitors that prevent NICD cleavage and nuclear translocation (Conner et al., 2014), a Notch reporter line that allows for visualization of NICD activity (Wan and Goldman, 2017), and a Notch1a NICD transgenic line that can overexpress NICD via a heat shock promoter (Wan et al., 2012). Recent results have identified DeltaB and Notch3 as the ligand-receptor pair that maintains Müller glia quiescence (Campbell et al., 2021) and have linked Notch signaling to the reprogramming factors *Ascl1a* and *Lin28a* (Elsaedi et al., 2018), the epigenetic factor *Hdac1* (Mitra et al., 2018), and the *Tgfb* signaling pathway (Lee et al., 2020; Sharma et al., 2020). Here we aim to review these recent findings in the context of Notch signaling regulation, but also review the potential modes of regulation that have not yet been explored in zebrafish retinal regeneration (Table 1). Understanding these regulatory mechanisms will guide future inquiry into the mechanism by which the zebrafish retina responds to disruptions in cell-cell contact to elicit the regenerative response.

Table 1 | Zebrafish and mammalian orthologs of the core components in Notch signaling

Component	Mammals	<i>Danio rerio</i> (Zebrafish)
Receptors	NOTCH1 NOTCH2 NOTCH3 NOTCH4	Notch1a, Notch1b Notch2 Notch3
Receptor proteases		
S1 cleavage (furin-dependent)	FURIN	Furina, Furinb
S2 cleavage (metalloprotease-dependent)	ADAM10	Adam10a, Adam10b
S3 cleavage (γ -secretase dependent)	Presenilin (PSEN1, PSEN2) Nicastrin (NCSTN) APH1 Presenilin enhancer (PSENEN)	Psen1, Psen2 Ncstn Aph1b Psenen
Glycosyltransferase modifiers		
O-glucosyl-transferase	POGLUT1 POGLUT2 POGLUT3	Poglut1 Poglut2 Poglut3
O-fucosyl-transferase	POFUT1	Pofut1
EGF domain-specific O-GlcNAc transferase	EOGT	Eogt
β 1,3-GlcNAc transferase	Lunatic Fringe (LFNG) Manic Fringe (MFNG) Radical Fringe (RFNG)	Lfng Mfng Rfng
Ligands		
	DLL1 DLL3 DLL4 Jagged1 (JAG1) Jagged2 (JAG2)	DeltaA, DeltaD DeltaB, DeltaC Dll4 Jag1a, Jag1b Jag2a, Jag2b
Endosomal sorting/membrane trafficking regulators		
Ring finger E3 ubiquitin ligase (ligand endocytosis)	Mindbomb (MIB1), Skeletrophin (MIB2)	Mindbomb (Mib1 and Mib2)
Ring finger E3 ubiquitin ligase (receptor endocytosis)	Deltex (DTX1, DTX2, DTX3, DTX4)	Dtx1, Dtx2, Dtx3, Dtx4a, Dtx4b
HECT domain E3 ubiquitin ligase (receptor endocytosis)	ITCH	Itcha, Itchb, Nedd4
Negative regulators	Numb, Numb-like	Numb, Numbl
Nuclear effectors		
CSL DNA-binding transcription factor	RBPJ	Rbpja, Rbpjb
Transcriptional coactivator	Mastermind-like (MAML1, MAML2, MAML3)	Maml1, Maml2, Maml3
Transcriptional corepressors	NCOR1, NCOR2 (SMRT) SPEN (SHARP/MINT)	Ncor1, Ncor2 Spn
Target genes		
bHLH repressors	<i>Hairy/Enhancer of Split and related genes (HES/HEY)</i> <i>Hes1</i> <i>Hes5</i>	<i>her and hey genes</i> <i>her6</i> <i>her2, her4</i>

Search Strategy and Selection Criteria

Literature cited in this review was published from 1997 to 2021 and was searched on PubMed and Google Scholar using combinations of keywords and citation tracking on PubMed. Search terms included: Notch signaling, receptor, ligand, target genes, retina, development, regeneration, zebrafish, Müller glia, and stem cells. Literature was also searched through ZFIN within each of the gene pages for the zebrafish factors listed in **Table 1**.

Elements of Notch Signaling in Context with Zebrafish Retinal Regeneration

Notch receptor synthesis and modification

Notch receptors are a highly conserved family of single-pass transmembrane proteins. The zebrafish genome contains four Notch receptor genes: *notch1a*, *notch1b*, *notch2*, and *notch3*. Each gene encodes a receptor that is processed into a heterodimer following ligand-independent, Furin-dependent S1 cleavage during transit through the Golgi network (Logeat et al., 1998). Zebrafish possess two Furin paralogs, *furina* and

furinb. Following S1 cleavage, the resulting Notch extracellular domain (NECD) dimerizes with the Notch transmembrane and intracellular domain (TMIC) through noncovalent interactions. The NECD is the ligand-binding domain and consists of 29–36 epidermal growth factor (EGF)-like repeats, a negative regulatory region, and cysteine-rich LIN-12/Notch repeats. The TMIC contains the NICD, which consists of a RAM domain, ankyrin repeats flanked by two nuclear localization signals, a transcriptional activation domain, and a C-terminal PEST domain (Kopan and Ilagan, 2009). The S1 cleavage event is necessary for biological activity of the Notch receptors (Lake et al., 2009), and therefore has the potential to serve as a regulatory point for Notch signaling in the signal-receiving cell. Following ligand binding, two additional proteolytic cleavage events must occur at the S2 and S3 cleavage sites in Notch to generate the NICD (Steinbuck and Winandy, 2018).

Notch receptor activity is highly regulated by glycosylation. Prior to the S1 cleavage event, O-linked glycan modifications can be added to each EGF-like repeat within the NECD (Saiki et al., 2021). O-glycosyltransferases add O-glucose to the serine residues within an EGF-like repeat. In mammals this is carried

out by POGGLUT1 (Rumi in flies) between the first two cysteines of an EGF-like repeat (consensus sequence C¹-X-S-X-(P/A)-C²) and by POGGLUT2 and POGGLUT3 within the ligand-binding domains of NOTCH1 and NOTCH3 (consensus sequence C³-X-N-T-X-G-S-F-X-C⁴; Takeuchi et al., 2018). O-fucosyltransferases add O-fucose to the serine or threonine residue between the second and third cysteines (consensus sequence C²-X-X-X-(S/T)-C³) (Moloney et al., 2000), and in mammals, this is achieved with POFUT1 (Shi and Stanley, 2003). O-linked-β-N-acetylglucosamine modification is added to the serine or threonine residue between the fifth and sixth cysteines (consensus sequence C⁵-X-X-G-X-(S/T)-G-X-X-C⁶) by the EGF domain-specific O-linked N-acetylglucosamine (GlcNAc) transferase EOGT in mammals (Sakaidani et al., 2012). While they do exist, little research has been reported on Notch signaling regulation with the orthologs of these enzymes in zebrafish.

Further fine-tuning of the O-fucose modifications leads to another layer of Notch regulation. This is achieved by the Fringe proteins, which catalyze the addition of GlcNAc to O-fucose (Rana and Haltiwanger, 2011). In mammals and zebrafish, this includes Lunatic fringe (Lfng), Manic fringe (Mfng), and Radical fringe (Rfng). In *Drosophila*, where there is a single Fringe protein, classic studies demonstrated that Fringe is restricted to the ventral half of the developing eye (Cho and Choi, 1998), where it inhibits Notch activity with the Serrate ligand and enhances Notch activity with the Delta ligand, thus creating a dorsoventral boundary that directs eye morphogenesis (Panin et al., 1997).

In the developing mouse retina, Lfng influences angiogenic sprouting by enhancing DLL4 ligand binding and reducing Jag1 ligand binding to Notch (Benedito et al., 2009). In zebrafish, Lfng maintains progenitor cells in the developing hindbrain by promoting lateral inhibition of neurogenesis through closely regulated Notch activation (Nikolaou et al., 2009), and in both heart and fin regeneration, Lfng is upregulated to increase Notch activation (Grotek et al., 2013; Münch et al., 2017). Recent single-cell RNA-seq data suggest that *lfng* is expressed in both activated and resting Müller glia in the zebrafish retina, while *mfng* is expressed in the retinal immune cells, the microglia (Hoang et al., 2020), which have been shown to play a role in regulating the Müller glia-derived regeneration response (Lahne et al., 2020; Iribarne, 2021). Investigations of the Fringe proteins in the zebrafish retina may help elucidate ligand-receptor relationships and the dynamic role of Notch signaling during regeneration.

Ligand-receptor interactions

The Notch ligands belong to the DSL (Delta/Serrate/Lag) family of transmembrane proteins (D'Souza et al., 2008). Zebrafish have five Delta-like ligands: DeltaA, DeltaB, DeltaC, DeltaD, and Delta-like4 (Dll4), as well as four Jagged ligands: Jag1a, Jag1b, Jag2a, and Jag2b. The ligands all have an extracellular domain composed of several EGF-like repeats and a cysteine-rich N-terminal DSL domain that is required for interaction with Notch. Similar to the NECD, the EGF-like repeats of DSL ligands can be glycosylated, however it is unclear if these types of modifications affect ligand activity (Panin et al., 2002). Instead, Notch ligands are regulated by expression pattern, ubiquitylation, and endocytosis.

Notch receptor and ligand expression patterns influence the Notch regulation of cell fate by lateral specification (or lateral inhibition), which is the mechanism by which the fate of one cell influences the fate of its neighboring cells (Bray, 2016). This is accomplished through a negative feedback loop where the ligand-expressing cell induces the receptor-expressing cell to express Notch target genes, which are transcriptional repressors of the ligand genes, thereby repressing ligand expression in the signal-receiving cell. Within the damaged and

regenerating retina, the mechanism of lateral specification may indicate to surviving cells, in particular the Müller glia, the state of the neighboring cells, as well as direct the fate of Müller glia-derived progenitor cells. Notch3 protein, which is expressed in Müller glia of the undamaged retina, is downregulated as Müller glia proliferate, and is reestablished in Müller glia of the recovering retina (Campbell et al., 2021). Alternatively, *deltaB* mRNA is expressed throughout the undamaged retina and more highly in neurons than in the Müller glia, suggesting a complementary pattern to *notch3* expression (Campbell et al., 2021). DeltaB-Notch3 interaction is required to maintain the Müller glia in a quiescent, non-proliferative state in the intact retina (Figure 1C and F; Campbell, et al., 2021). In addition, expression of *dll4* is downregulated following injury and can be increased by forced expression of Fgf8a or Tgfb3, both of which inhibit injury-dependent proliferation, suggesting that Dll4 may also be a candidate ligand for Notch3 (Wan and Goldman, 2017; Lee et al., 2020). However, morpholino-mediated knockdown of Dll4 expression reduced Müller glia proliferation during light damage unlike the knockdowns of Notch3 and DeltaB, which both increased proliferation during light damage (Campbell et al., 2021).

Alternatively, the *deltaA* and *deltaD* ligands and the *notch1a* receptor are expressed in the developing zebrafish retina with expression gradually restricted over time to the ciliary marginal zone (Taylor et al., 2015), an area of persistent neurogenesis throughout the life of the fish (Figure 1B) (Hitchcock et al., 2004). Data from single-cell RNA-seq studies suggest that, similar to the developing retina, *deltaA*, *deltaD*, and *notch1a* are expressed in reprogrammed/activated Müller glia at the time of peak Müller glia proliferation (36 hours light treatment), with *deltaA*, *deltaC*, and *deltaD* also expressed in Müller glia-derived progenitors (Figure 1D; Hoang et al., 2020). Since Notch1 activity is known to maintain retinal progenitors in a proliferative state in the developing retina (Jadhav et al., 2006a), then the outcomes of Notch activation during regeneration may be dictated, in part, by specific ligand-receptor expression patterns.

Ubiquitylation and endocytic trafficking

In addition to receptor glycosylation and ligand-receptor expression patterns, Notch activity is also regulated by ubiquitylation and endocytosis. Ubiquitylation and endocytosis of the ligand serves two purposes: 1) to regulate the amount of Notch ligand at the cell surface and 2) to generate force on the ligand-receptor complex to expose the S2 cleavage site in the negative regulatory region of the Notch receptor (Weinmaster and Fischer, 2011). Mindbomb (Mib) and Neuralized (Neur) are E3 ubiquitin ligases that ubiquitylate the Notch ligands, although it appears that only Mib is required for vertebrate Notch signaling, while both Mib and Neur function in Notch signaling in *Drosophila* (Weinmaster and Fischer, 2011). The *mib1* mutant was first discovered in zebrafish and led to the description that Mib1 binds and ubiquitylates Delta ligands (Itoh et al., 2003). A functionally redundant Mib2 was identified in zebrafish and possesses an overlapping and distinct substrate specificity for the Notch ligands relative to Mib1 (Zhang et al., 2007a, b). During zebrafish development, the *mib1* mutant retina has decreased expression of the Notch target gene *her4* and increased expression of the neuronal differentiation marker *huC*, indicating that Mib is essential for increased Notch target gene expression, as well as neuronal differentiation during retinogenesis (Yoo et al., 2017). Furthermore, the Ran-binding protein family member RanBP9 was discovered to bind and antagonistically regulate Mib. Morpholino-mediated knockdown of RanBP9 increased *her4* and decreased *huC* expression and also decreased Müller glia differentiation (Yoo et al., 2017). The endocytic regulation of Notch ligands has significant impact on Notch signaling, so ligand gene expression data alone may not fully reflect Notch activity in the signal-receiving cell.

Endocytosis of the Notch receptor within the signal-receiving cell can also affect pathway activity by reducing Notch receptor levels at the cell surface or by allowing ligand-independent receptor activation (Schnute et al., 2018). Several E3 ubiquitin ligases have been implicated in this process, including Deltex, Itch, and Nedd4. In zebrafish, the developing nervous system expresses *deltex1*, which Notch signaling suppresses through the downstream targets Her2 and Her8a (Cheng et al., 2015). Overexpression of the complete *deltex1* coding sequence induced neuronal and glial cell differentiation, while expression of *deltex1* coding sequence lacking the essential RING finger motif reduced neuronal and glial cell differentiation (Cheng et al., 2015). Numb and Numb-like also negatively regulate Notch activity. Numb antagonizes Notch signaling by recruiting E3-ligases, such as Itch, to ubiquitylate the NICD, marking it for degradation and signaling for Numb endocytic activity (Steinbuck and Winandy, 2018). In the mouse retina, Numb antagonizes Notch signaling in late-stage retinal progenitor cells to drive the production of terminal asymmetric divisions (Kechad et al., 2012). Therefore, in the regenerating zebrafish retina, Numb may be required in the later stages of NPC amplification to transition the Müller glia-derived NPCs from proliferation to differentiation.

Metalloprotease-dependent cleavage

Prior to ligand-binding, the Notch receptor's S2 cleavage site is masked by the negative regulatory region. With ligand binding, the force of transendocytosis of the ligand-bound NECD into the signal-sending cell unfolds the negative regulatory region to allow cleavage of S2 by a metalloprotease (Gordon et al., 2007). Members of the ADAM (a disintegrin and metalloprotease) family of membrane-bound sheddases facilitate this event (Hsia et al., 2019). In multiple species, both ADAM10 and ADAM17 have been implicated in the S2 Notch cleavage, however only ADAM10 has been shown to be necessary for Notch function under physiological conditions (Alabi et al., 2021). In the developing retina, *Adam10* conditional knockout mice exhibit lamination defects and abnormal neurogenesis due to a reduction of proliferating progenitor cells, which is characteristic of a deficiency in Notch signaling. Constitutive activation of Notch signaling via the Notch1 ICD rescues the *Adam10* conditional knockout, confirming that Adam10 is essential for Notch signaling in the developing retina (Toonen et al., 2016).

Zebrafish have two Adam10 paralogs, Adam10a and Adam10b, and according to single-cell RNA-seq data, *adam10a* is expressed in all retinal cell types, including both resting and activated Müller glia following retinal damage (Hoang et al., 2020). Zebrafish *adam17a* is also expressed in all retinal cell types in response to damage (Hoang et al., 2020). While it may not play a significant role in Notch signaling, its expression is noted since a primary target of ADAM17 is tumor necrosis factor alpha (Tn α) (Hsia et al., 2019), a factor that is produced by dying neurons and microglia and required for Müller glia proliferation during retinal regeneration (Nelson et al., 2013; Iribarne et al., 2019; Hoang et al., 2020). In the undamaged zebrafish retina, the combination of Tn α and Notch repression is sufficient to activate a large percentage of Müller glia to proliferate and produce progenitor cells that commit to neuronal lineages in the absence of retinal damage (Conner et al., 2014). How Tn α and Notch signaling interact to induce this response is still an open area of inquiry.

In addition, secreted frizzled-related proteins (Sfrp), which are described as multifunctional regulators of Wnt signaling (Esteve et al., 2011b), can also regulate Notch signaling through ADAM10 during mouse retinal neurogenesis (Esteve et al., 2011a). While loss of Sfrp1/2 expression in the *Sfrp1^{-/-}*; *Sfrp2^{-/-}* mouse retina results in overactivation of Notch signaling, ADAM10 inhibition rescues the phenotype (Esteve et al., 2011a). Furthermore, Sfrp1 was shown to physically

interact with ADAM10 to inhibit processing of Notch (Esteve et al., 2011a). In zebrafish, *sfrp1a* and *sfrp5* are both expressed in the developing retina and are implicated in patterning along the dorsoventral axis (Holly et al., 2014). The convergence of Notch, Tn α , and Wnt signaling pathways on ADAM activity suggests that the regulation of Notch signaling for successful retinal regeneration may hinge, in part, on the regulation of Adam10 activity.

γ -Secretase-dependent cleavage

The S2 cleavage of ligand-activated Notch results in two products: the shed NECD polypeptide and a membrane-bound form called the Notch Extracellular Truncation, which is short-lived as it contains the S3 cleavage site and serves as the substrate for γ -secretase (Mumm et al., 2000). The cleavage by γ -secretase releases NICD from the membrane, allowing it to translocate directly to the nucleus (De Strooper et al., 1999). The γ -secretase complex is composed of four multi-pass membrane proteins that include the catalytic component Presenilin (Psen), Nicastrin, Anterior pharynx-defective 1, and Presenilin enhancer 2 (Wolfe, 2019). Many type-1 transmembrane proteins are γ -secretase substrates, although the most extensively studied are amyloid- β precursor protein and the Notch receptors (Hitzenberger et al., 2020). In zebrafish, morpholino-mediated knockdown of Psen1 results in abnormal eye morphology (Nery et al., 2017), and *psen2* is highly expressed in the developing eye (Groth et al., 2002).

Inhibitors of the γ -secretase complex are often used to investigate Notch signaling during retinal regeneration. Two commonly used inhibitors are DAPT, which binds near the catalytic site on Presenilin, and RO4929097, which also binds and inhibits with a much stronger potency (Ran et al., 2017). Repression of Notch signaling with RO4929097 was sufficient to induce Müller glia proliferation in the absence of retinal damage and to increase proliferation during the light damage paradigm (Conner et al., 2014). When combined with TNF α exposure, Notch inhibition through RO4929097 induced a robust proliferative response in the undamaged zebrafish retina and produced Müller glia-derived progenitors that committed to the neuronal fate and differentiated into most types of retinal neurons (Conner et al., 2014). Similarly, DAPT exposure expanded the zone of proliferating Müller glia around the site of retinal poke injury where *ascl1a* expression was also increased (Wan et al., 2012). Expression of the Notch1a ICD with the *Tg(hsp70l:gal4)*; *Tg(UAS:myc-notch1a-intra)* transgenic line reversed the effect of γ -secretase inhibition, indicating that induced and increased Müller glia proliferation was regulated by the inhibition of Notch signaling (Wan et al., 2012; Conner et al., 2014).

In the poke-injured retina with DAPT-mediated Notch inhibition, the pool of Müller glia-derived progenitor cells was biased to adopt the Müller glia fate, and alternatively, the limited number of progenitors produced in the injured retina with Notch1a ICD overexpression tended to differentiate into photoreceptors (Wan et al., 2012). This was surprising given that the opposite effect is observed in the developing mouse retina, where Notch inhibition enhances photoreceptor production (Jadhav et al., 2006b), and Notch1 activity maintains the progenitor state and promotes the glial cell fate (Furukawa et al., 2000; Jadhav et al., 2006a). These results may indicate that Notch signaling acts differently in the injured adult retina where dedifferentiation and proliferation are restricted for the purpose of repair within the fully formed retina, rather than for the formation of the entire retinal tissue from a mass of progenitor cells as in development. Alternatively, the differences in these results may reflect the methods used to modulate Notch signaling activity. Notch repression with γ -secretase inhibitor broadly inhibits all Notch receptor activity and forced NICD expression induces NICD activity in all cells, regardless of endogenous Notch expression

or activity, presumably at a high level. Instead, targeting individual Notch signaling components in specific cell types may reveal unique roles for Notch signaling components that are hidden by broad inhibition or high levels of forced expression. For example, morpholino-mediated knockdowns of the Notch receptors in the light-damaged retina revealed that Müller glia quiescence is specifically maintained by Notch3, while Notch1a, Notch 1b, and Notch2 are each necessary for proliferation during regeneration (Campbell et al., 2021). While the available tools, whether pharmacological inhibition, knockdown techniques, or forced transgenic expression, are each useful in specific ways, we must consider how each tool influences Notch activity levels, as current modeling proposes that high and low Notch signaling activity influence different cell fate outcomes (Ivanov, 2019).

NICD-dependent transcriptional regulation

Following γ -secretase-dependent cleavage, the NICD translocates directly to the nucleus, where it forms a complex with the DNA-binding transcription factor CSL (CBF1/RBP-Jk in mammals, Su(H) in *Drosophila*, and LAG-1 in *C. elegans*) and the co-activating factor Mastermind (Zema et al., 2020). In zebrafish, orthologs of these factors include the RBP-Jk paralogs Rbpja and Rbpjb and the Mastermind-like homologs Maml1, Maml2, and Maml3.

The original model for transcriptional control of Notch target genes was that RBP-Jk binds to CSL-binding sites statically. In the absence of NICD, co-repressors form a complex with RBP-Jk to repress gene expression, and when Notch signaling is activated, the NICD displaces the co-repressors and activates transcription (Kao et al., 1998). However, this model was challenged by whole-genome binding profiles for RBP-Jk and NICD, which revealed that CSL-binding sites in the enhancers and promoters of Notch target genes are dynamically bound by RBP-Jk (Castel et al., 2013). In the absence of NICD, these sites are weakly bound by RBP-Jk, and Notch signaling activation recruits the RBP-Jk-NICD-MAML complex to these sites. Furthermore, for a set of binding sites, RBP-Jk does not recruit NICD, suggesting that RBP-Jk can function independently of NICD to directly regulate transcriptional targets (Castel et al., 2013). In the developing mouse retina, the proneural factor *Atoh7* is regulated, in part, by Notch signaling. Four CSL binding sites were identified within the *Atoh7* enhancer region that are regulated by RBP-Jk using both Notch-dependent and Notch-independent mechanisms. It is suggested that levels of RBP-Jk protein influence the RBP-Jk-bound state of the *Atoh7* locus (Miesfeld et al., 2018). In light of these and other data, it is suggested that instead of a switch model where NICD displaces co-repressors from RBP-Jk, a dynamic exchange of the entire complex occurs and levels of the components, including RBP-Jk and NICD, influence regulation of target transcription (Bray, 2016).

Further evidence that Notch signaling outcomes are dependent on protein levels comes from experiments where the Notch1 NICD was swapped with the Notch2 NICD and no abnormal phenotype was detected, indicating that the N1ICD and the N2ICD are functionally equivalent (Liu et al., 2015). Instead, Notch signaling outcomes appear to be dependent on the level of Notch signal. In the adult zebrafish telencephalic ventricular zone, the level of Notch signaling is suggested to influence radial glia oscillation between quiescent and neurogenic states (Chapouton et al., 2010). Cells with low levels of Notch activity express high levels of proliferation markers, whereas Notch1a ICD overexpression induced cell cycle exit and promoted a reversible quiescent state, such that cells could reenter the cell cycle after peak NICD overexpression. This led to the proposal that

endogenous oscillation of Notch levels promotes a quiescent radial glial state when Notch signaling is active and induces radial glia proliferation when Notch levels are low. In a lateral inhibition-like feedback mechanism, proliferating progenitors activate Notch in their neighboring cells to maintain the pool of quiescent and proliferating progenitors during adulthood (Chapouton et al., 2010). In the zebrafish retina, Notch3 maintains Müller glia quiescence, whereas the other Notch receptors are required for sufficient Müller glia and NPC proliferation following damage (Campbell et al., 2021). Therefore, it is surprising that the Notch1a ICD can reduce Müller glia proliferation following Notch repression with γ -secretase inhibitor (Conner et al., 2014). In light of the evidence for functional equivalence of NICDs and the proposal that Notch levels dictate progenitor states, the simple explanation for these results is that induced overexpression of Notch1a ICD does not recapitulate endogenous Notch1a expression, but instead increases Notch signaling activity to a level that rescues the γ -secretase inhibition and promotes a return to Müller glia quiescence. Based on this model, however, additional investigation is required to determine how a specific level of Notch signaling induces Müller glia quiescence, while another level promotes proliferation and yet another level induces neuronal cell commitment and differentiation.

A recent integrated model of retinal neurogenesis proposes that the Notch-mediated lateral specification mechanism induces an oscillation of *Hes1* expression, which drives expression of target gene products that not only oscillate, but also accumulate within a progenitor cell. The levels of these factors influence whether the cell differentiates to a neuronal phenotype, which is determined by the levels of transcription factors that have accumulated, or maintains a progenitor state and divides to give daughter cells that inherit the accumulated factors (Ivanov, 2019). This model further proposes that low Notch activity is required to promote the transition of progenitors into various retinal cell fates while high, sustained Notch activity promotes the Müller glia phenotype. Therefore, Notch1a ICD overexpression experiments must be interpreted carefully since the resulting reduction in Müller glia and NPC proliferation may be the result of high Notch signaling activity, which does not exclude the possibility that Notch1a (or Notch1b or Notch2) may be required for proliferation and progenitor transitions during regeneration.

Target gene expression

The principal transcriptional targets of Notch are members of the *hairy and enhancer of split (Hes)* and *Hes* related family of genes. The homologs in zebrafish are the *her* and *hey* genes, and they encode basic helix-loop-helix transcriptional repressors that play essential roles in maintaining progenitor cell populations and regulating cell fate decisions during tissue development (Kageyama et al., 2007). Two known Notch targets, *Hes1* and *Hes5*, are required for development of the mouse optic vesicles and play roles in retinal development (Hatakeyama and Kageyama, 2004). During retinogenesis, the *Dll1/Dll4-Notch1-Rbpj-Hes1* axis promotes retinal progenitor cell proliferation and inhibits retinal ganglion cell neurogenesis through suppression of *Atoh7* expression (Riesenberg et al., 2009; Maurer et al., 2014). *Hes5* is expressed in retinal progenitor cells once retinal neurogenesis begins, where it co-localizes with developing retinal ganglion cells. In addition, it is expressed in Müller glia of the adult mouse retina (Riesenberg et al., 2018).

The zebrafish ortholog of *Hes1* is *her6* and the orthologs of *Hes5* are *her2* and *her4* (including the five tandem repeats *her4.1-4.5*). In the zebrafish retina, *her6* is expressed in Müller glia and progenitor cells in the CMZ (Bernardos et al., 2005).

Zebrafish *her4* is also expressed in the CMZ (**Figure 1B**), as well as in slowly proliferating Müller glia in the adult retina, which is the source of newly generated rod photoreceptors throughout the life of the fish. In response to light damage, *her4* expression is upregulated in proliferating cells in the outer and inner nuclear layers, which likely corresponds to the Müller glia-derived NPCs (**Figure 1E**) (Wilson et al., 2016). Inhibition of Notch signaling in the undamaged retina via the γ -secretase inhibitor RO4929097 decreased *her4* expression but induced proliferation, giving further confirmation that *her4* is a target of Notch signaling but suggesting that *her4* is not required for Müller glia reprogramming (Conner et al., 2014). Instead, Her4 is suggested to regulate Müller glia reprogramming and proliferation through direct repression of *lin28a* since examination of the *lin28a* promoter revealed two Her4 binding sites, and morpholino-mediated knockdown of Her4.1 increased *lin28a* expression following poke injury (Mitra et al., 2018).

The functional investigation of downstream targets of Notch signaling during retinal regeneration has been limited to only a few target genes. However, it is likely that dynamic expression of combinations of *her* genes and other Notch targets contributes to the regulation of Müller glia quiescence, reprogramming, proliferation, and cell fate determination for successful regeneration. For example, the *Hes1* ortholog *her6*, which promotes retinal progenitor cell proliferation and inhibits retinal ganglion cell neurogenesis, is broadly expressed in resting and activated Müller glia following light damage and NMDA-induced damage (Hoang et al., 2020), suggesting that it is another Notch target that regulates reprogramming and proliferation during regeneration. Alternatively, *hey1* expression is transiently downregulated following retinal injury and can be upregulated with forced expression of quiescence factors Tgfb3 or Fgf8a, suggesting that *hey1* may be a target of Notch3 (Wan and Goldman, 2017; Lee et al., 2020). To fully understand the dynamics of Notch signaling, it will be essential to explore the contributions of multiple Notch targets.

Network interactions

Damage disrupts the retinal landscape and, in response, a complex network of signaling pathways must be employed for successful regeneration (Lahne et al., 2020; Gao et al., 2021). To fully understand the dynamic role of Notch signaling for successful retinal regeneration, we must understand the interactions of Notch within the broader network of signaling at each stage of regeneration.

Recent studies demonstrate that Notch signaling maintains Müller glia quiescence in concert with Tgfb signaling and Fgf8a signaling (Wan and Goldman, 2017; Lee et al., 2020). Quiescent Müller glia in the zebrafish retina express *tgfb3*, and *tgfb3* suppression is required for injury-dependent Müller glia proliferation. Furthermore, Tgfb3 suppresses reprogramming and proliferation and acts upstream of Notch (Lee et al., 2020). This result contrasts with another recent study that reports a pro-proliferative function of Tgfb signaling (Sharma et al., 2020), however the pro-proliferative function results from TGF- β 1 application, not Tgfb3. At later stages of regeneration, Tgfb signaling is required for terminating NPC proliferation through Notch (Sharma et al., 2020). Müller glia also express *fgf8a* in the uninjured retina, which is almost immediately downregulated following injury. Fgf8a suppresses Müller glia proliferation through Notch activation. However, suppression of Müller glia proliferation by Fgf8a only applies to the older Müller glia of the central retina where Fgf8a increases Notch signaling. In the peripheral retina, where persistent neurogenesis is constantly producing young retinal tissue, Fgf8a acts through Notch inhibition to

stimulate Müller glia proliferation (Wan and Goldman, 2017). Together, these studies suggest that additional signaling pathways act with Notch signaling to maintain quiescence in a context-dependent manner depending on age or on stage of regeneration.

Upon damage, rapid gene expression changes are induced in Müller glia as they enter a transient reactive state and progress into a reprogrammed/proliferative state (Hoang et al., 2020). The expression of *notch3* is rapidly and drastically downregulated within four hours of light treatment, while *notch1a* and *notch1b* expression increases by 20 hours (Campbell et al., 2021), but it is unclear what signals are upstream of damage-induced changes in *notch* expression. A proposed mechanism involves the neurotransmitter γ -aminobutyric acid (GABA). As photoreceptors die, the horizontal cells are no longer stimulated to release GABA, which is detected by Müller glia. Inhibition of GABA signaling induces Müller glia proliferation and activation of Notch target *her4* (Rao et al., 2017). Müller glia also respond to damaged-induced inflammatory signals such as TNF α , which is produced by dying neurons and enhances the Notch inhibition proliferative response (Nelson et al., 2013; Conner et al., 2014), and HB-EGF, which positively regulates *deltaA*, *notch1a*, and *her4* expression through the reprogramming factor *Ascl1a* (Wan et al., 2012).

The upregulation of Notch signaling components by *Ascl1a* appears contradictory given that Notch inhibition is required for proliferation. However, it supports a dual role for Notch signaling in both maintaining Müller glia quiescence through Notch3 as well as being necessary for Müller glia reprogramming and proliferation through a combination of Notch1a, Notch1b, and Notch2 (Campbell et al., 2021). This is similar to what is seen in the mouse and chick retinas. During retinal development in mice, Notch signaling regulates retinal progenitor cell proliferation (Jadhav et al., 2006a), but promotes gliogenesis at later stages (Nelson et al., 2011; de Melo et al., 2016). In the injured postnatal chick retina, Notch signaling is required for Müller glia dedifferentiation and proliferation but later inhibits differentiation of newly generated progenitor cells (Hayes et al., 2007; Ghai et al., 2010). Recent studies indicate that Notch signaling is regulated during the proliferative phase by multiple factors, including Hdac1, which is sequestered from NPCs and works with Mycb to inhibit *her4.1* in the cells that neighbor NPCs (Mitra et al., 2018, 2019).

The role of Notch signaling to maintain Müller glia quiescence in the undamaged retina is well established, due to multiple studies with γ -secretase inhibitors and with the identification of Notch3 as the specific receptor that maintains quiescence (Wan et al., 2012; Conner et al., 2014; Campbell et al., 2021). Additionally, reports of crosstalk between Notch signaling and other major pathways as well as Notch signaling studies during retinal development provide a solid framework for our understanding of the dynamic role of Notch signaling during retinal regeneration (**Table 2**). However, the mechanisms by which Notch3 maintains quiescence and by which another Notch receptor or combination of receptors function in reprogramming and NPC proliferation is unclear. Given what is known about Notch signaling, its regulation may occur at any point from the post-translational modifications that bias ligand-receptor binding to the cell-specific ligand-receptor expression patterning to the oscillations and combinations of target genes that are induced upon Notch signaling activation. Further investigation is needed to address these points of regulation so we can work towards inducing endogenous repair of retinal damage in mammals.

Table 2 | Key Notch signaling findings in retinal development, growth, and regeneration

Signaling component	Organism	Key finding	Reference
Development			
Notch1	Mouse	Maintains progenitor cell state and promotes cell proliferation Promotes MG formation Inhibits photoreceptor fate	Furukawa et al., 2000 Jadhav et al., 2006a Jadhav et al., 2006b
Dll4/Jagged1 and Fringe proteins	Mouse	Fringe glycosylation of Notch enhances Dll4 binding and weakens Jagged1 binding to regulate retinal angiogenesis	Benedito et al., 2009
Mindbomb	Zebrafish	RanBP9 antagonistically regulates Mindbomb, which influences <i>her4</i> expression	Yoo et al., 2017
Numb	Mouse	Promotes early-stage cell-cycle progression and late-stage terminal asymmetric cell divisions	Kechad et al., 2012
ADAM10	Mouse	Activates Notch signaling to promote progenitor cell proliferation Sfrp1 and Sfrp2 bind and downregulate ADAM10 activity	Toonen et al., 2016 Esteve et al., 2011a
Psen1	Zebrafish	Knockdown reduces eye size	Nery et al., 2017
Rbpj	Mouse	Interacts with four CSL binding sites to repress <i>Atoh7</i> expression in Notch-dependent and independent pathways	Miesfeld et al., 2018
Hes1	Mouse	Hes1 expressed in all progenitor cells Hes1 suppresses <i>Hes5</i> transcription	Riesenberg et al., 2018 Nelson et al., 2011
Hes5		Hes5 expressed in subsets of progenitors and in differentiating ganglion cells Hes5 expressed in adult MG	
Hes5	Mouse	Hes5-dependent gliogenesis requires Lhx2	de Melo et al., 2016
Dll1/Dll4-Notch1-Rbpj-Hes1	Mouse	Promotes retinal progenitor cell proliferation and inhibits retinal ganglion cell neurogenesis	Riesenberg et al., 2009 Maurer et al., 2014
Ciliary Marginal Zone (persistent neurogenesis)			
Notch1a, Notch1b, DeltaA, DeltaD, Her2, Her4, Her6	Zebrafish	Expression restricted to proliferative CMZ cells during retinal development and in the adult retina	Bernardos et al., 2005 Raymond et al., 2006 Taylor et al., 2015
Regeneration			
Notch3, DeltaB	Zebrafish	Notch3 expressed in MG, <i>deltaB</i> expressed in neurons; knockdown of either stimulates MG proliferation	Campbell et al., 2021
γ -Secretase	Zebrafish	γ -Secretase inhibition increased/induced MG proliferation, which was reversed by Notch1a ICD expression MG proliferation induced by γ -secretase inhibition was enhanced by TNF α	Wan et al., 2012 Conner et al., 2014
Notch1 and γ -secretase	Postnatal chick	Notch1 and signaling components upregulated after retinal damage γ -Secretase inhibition prohibited MG dedifferentiation and proliferation at early regeneration stages and increased newly generated neurons at late regeneration stages Notch1 expression is upregulated in proliferating MG in insulin/FGF2-treated retinas γ -Secretase inhibition blocked FGF2-induced proliferation	Hayes et al., 2007 Ghai et al., 2010
Notch signaling/Fgf8a	Zebrafish	Forced Fgf8a expression increases Notch signaling and suppresses MG proliferation in older tissue but inhibits Notch and stimulates MG proliferation in young tissue	Wan and Goldman, 2017
Dll4		<i>dll4</i> and <i>hey1</i> expression is suppressed after injury, but forced Fgf8a after injury increases expression of both	
Hey1			
Notch signaling/Tgfb signaling	Zebrafish	Tgfb3 acts upstream of Notch signaling to suppress injury-induced MG reprogramming and proliferation Forced Tgfb3 increases <i>dll4</i> and <i>hey1</i> expression	Lee et al., 2020
Dll4			
Hey1			
Her4		Pharmacological inhibition of Tgfb increases <i>her4.1</i> expression and decreases NPC proliferation at late stages	Sharma et al., 2020
Her4	Zebrafish	<i>her4</i> expressed in slowly proliferating MG of undamaged adult retina and in proliferating cells in ONL & INL following light damage GABA inhibition induces MG proliferation and <i>her4</i> :RFP expression Represses <i>lin28a</i> expression; two Her4 binding sites on <i>lin28a</i> promoter Late inhibition of <i>her4.1</i> increases NPC proliferation Mycb inhibits <i>her4.1</i> expression with Hdac1 in cells neighboring NPCs	Wilson et al., 2016 Rao et al., 2017 Mitra et al., 2018 Mitra et al., 2019
Stimulation of Regeneration in Mammals			
Notch1, Delta1, Hes1	Rat	Expression increased in neurotoxin-damaged retina	Das et al., 2006
γ -Secretase		γ -Secretase inhibition decreased cell cycle regulators <i>cyclinA</i> and <i>cyclinD1</i>	
Jag1	Mouse	Jag1-activation of Notch signaling with Wnt activation induced MG cell cycle reentry and rod photoreceptor differentiation in culture, retinal explant, and in S334ter rat <i>in vivo</i>	Del Debbio et al., 2010
Hes1	Rat	Notch activates MG by inhibiting p27 ^{Kip1} expression	Del Debbio et al., 2016
γ -Secretase	Mouse	γ -Secretase inhibition with forced <i>Ascl1a</i> and <i>Lin28</i> expression inefficiently stimulated MG proliferation in mouse retina	Elsaedi et al., 2018

ADAM: A disintegrin and metalloprotease 10; CMZ: ciliary marginal zone; Dll: delta-like; Her: hairy-related; Hes: hairy and enhancer of split; Hey: Hes-related; Jag: jagged; MG: Müller glia; Psen: presenilin.

Concluding Remarks: Implications for Mammalian Retinal Regeneration

The goal of retinal regeneration research is to discover mechanisms to induce endogenous stem cells to produce neurons that are lost in human disease. In response to damage, Müller glia in the mouse retina enter a reactive state that is molecularly similar to Müller glia in the zebrafish and chick. However, while the zebrafish and chick Müller glia pass through this reactive phase to reprogram and proliferate, the mouse Müller glia pass through the reactive phase, arrest, and return to quiescence (Hoang et al., 2020). Forced expression of *Ascl1* together with HDAC inhibition can reprogram mouse Müller glia with limited neurogenic potential (Jorstad et al., 2017), and Müller glia-specific deletion of the NFI factors, *Nfia/b/x*, can induce proliferation and formation of retinal neurons (Hoang et al., 2020). Therefore, the mammalian retina possesses the potential to undergo a regenerative response following damage, but the dynamic network of endogenous signals is not fully employed as in the highly regenerative zebrafish retina or in the limited-regenerative postnatal chick retina.

How does the Notch pathway function in the damaged mammalian retina, and is it acting dynamically like in the zebrafish retina? Expression studies in mice confirm that Notch signaling remains active in postmitotic Müller glia after they begin to differentiate (Nelson et al., 2011) and that Notch pathway genes, such as *Notch1*, *Notch2*, *Hes1*, and *Hes5*, are expressed in resting and activated Müller glia in the adult (Hoang et al., 2020). In the damaged mouse retina, *Notch1*, *Delta1*, and *Hes1* expression increases (Das et al., 2006). Furthermore, activation of Notch and Wnt signaling together can induce limited Müller glia proliferation and differentiation into rod photoreceptors, both in mouse retinal explants and *in vivo* in the S334ter rat mutant that exhibits rod photoreceptor degeneration (Del Debbio et al., 2010, 2016). However, whereas forced expression of *ascl1a* and *lin28a* in combination with γ -secretase inhibition can stimulate widespread Müller glia proliferation and neuronal generation in the zebrafish retina, a similar forced expression and Notch inhibition in the mouse retina results in limited and inefficient Müller glia proliferation (Elsaedi et al., 2018). This suggests that additional endogenous molecular mechanisms limit Müller glia activation and robust proliferation in the mammalian retina. Identification of those mechanisms is a necessary first step towards inducing endogenous repair.

The Hippo pathway was recently identified as an essential molecular mechanism that restrains Müller glia reprogramming and proliferation (Hamon et al., 2019; Rueda et al., 2019). Following retinal damage in mice, the Hippo pathway effector Yes-associated protein (Yap) is upregulated, which signals the upregulation of cell-cycle genes for Müller glia activation. However, sustained activity of Yap in the damaged retina is prevented by active Hippo signaling that phosphorylates Yap, marking it for degradation and preventing its interaction with the TEAD transcription factor. Bypassing Hippo signaling through Yap overexpression or with a phosphodeficient form of Yap results in spontaneous and robust Müller glia reprogramming and proliferation, although neurogenic competence is not induced (Hamon et al., 2019; Rueda et al., 2019). In the zebrafish retina, *yap1* is upregulated in reactive Müller glia and knockdown of *Yap1* or pharmacological inhibition of the *Yap1/TEAD* interaction was shown to reduce Müller glia proliferation in the light-damaged retina, indicating a conserved role for Yap as an essential driver of Müller glia proliferation (Hoang et al., 2020). Recent studies have shown that the Hippo pathway interacts with other major signaling pathways through its role as a central mediator of cellular signals (Wang and Martin, 2017). Epidermal stem cell fate, for example, is promoted by Notch

inhibition through mechano-activation of YAP/TAZ (Totaro et al., 2017), however, the specific crosstalk between the Hippo and Notch pathways to regulate Müller glia activation requires further exploration.

Recent research describes a role for Notch signaling primarily as a positive regulator of Müller glia quiescence and inhibitor of proliferation, however knockdown of the individual Notch receptors in the regenerating zebrafish retina suggests that Notch signaling may be dynamic in its effects during regeneration. This leads to many outstanding questions about how Notch signaling is dynamically regulated during quiescence and in response to retinal damage, which include:

- What are the cell-specific expression patterns and functions of Notch receptors and ligands throughout the stages of retinal regeneration? How do specific ligand-receptor binding pairs elicit different responses during regeneration? What levels of Notch signaling activity are required for the progression through regenerative stages?
- What is upstream of Notch signaling that regulates ligand expression and promotes stability of cell-cell contact with regard to ligand-receptor binding in the intact retina? Does the death of specific cell types as a result of different damage methods influence the dynamics of Notch signaling?
- How do Notch regulatory factors such as the Fringe proteins, Mindbomb, Numb, and ADAM10, all which have identified roles in retinal development, influence Notch signaling at the different phases of zebrafish retinal regeneration?
- What are the transcriptional targets of Notch signaling in the retina and how do combinations of Notch receptors and targets interact with the network of signaling pathways during the regeneration time course?

Addressing these and other gaps in our understanding of dynamic Notch signaling in the regenerating retina will inform our efforts in employing potential Notch-based therapeutics to stimulate endogenous repair and restore vision in retinal damage and disease.

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References

- Alabi RO, Lora J, Celen AB, Marezky T, Blobel CP (2021) Analysis of the conditions that affect the selective processing of endogenous notch1 by ADAM10 and ADAM17. *Int J Mol Sci* 22:1846.
- Amini R, Rocha-Martins M, Norden C (2017) Neuronal migration and lamination in the vertebrate retina. *Front Neurosci* 11:742.
- Benedito R, Roca C, Sorensen I, Adams S, Gossler A, Fruttiger M, Adams RH (2009) The notch ligands Dll4 and Jagged1 have opposing effects on angiogenesis. *Cell* 137:1124-1135.

- Bernardos RL, Lentz SI, Wolfe MS, Raymond PA (2005) Notch-Delta signaling is required for spatial patterning and Müller glia differentiation in the zebrafish retina. *Dev Biol* 278:381-395.
- Bray SJ (2016) Notch signalling in context. *Nat Rev Mol Cell Biol* 17:722-735.
- Bringmann A, Wiedemann P (2012) Müller glial cells in retinal disease. *Ophthalmologica* 227:1-19.
- Campbell LJ, Hobgood JS, Jia M, Boyd P, Hipp RI, Hyde DR (2021) Notch3 and DeltaB maintain Müller glia quiescence and act as negative regulators of regeneration in the light-damaged zebrafish retina. *Glia* 69:546-566.
- Castel D, Mourikis P, Bartels SJ, Brinkman AB, Tajbakhsh S, Stunnenberg HG (2013) Dynamic binding of RBPJ is determined by Notch signaling status. *Genes Dev* 27:1059-1071.
- Chapouton P, Skupien P, Hesel B, Coolen M, Moore JC, Madelaine R, Kremmer E, Faus-Kessler T, Blader P, Lawson ND, Bally-Cuif L (2010) Notch activity levels control the balance between quiescence and recruitment of adult neural stem cells. *J Neurosci* 30:7961-7974.
- Cheng YC, Huang YC, Yeh TH, Shih HY, Lin CY, Lin SJ, Chiu CC, Huang CW, Jiang YJ (2015) Deltex1 is inhibited by the Notch-Hairy/E(Spl) signaling pathway and induces neuronal and glial differentiation. *Neural Dev* 10:28.
- Cho KO, Choi KW (1998) Fringe is essential for mirror symmetry and morphogenesis in the Drosophila eye. *Nature* 396:272-276.
- Conner C, Ackerman KM, Lahne M, Hobgood JS, Hyde DR (2014) Repressing notch signaling and expressing TNF α are sufficient to mimic retinal regeneration by inducing Müller glial proliferation to generate committed progenitor cells. *J Neurosci* 34:14403-14419.
- D'Orazi FD, Zhao XF, Wong RO, Yoshimatsu T (2016) Mismatch of synaptic patterns between neurons produced in regeneration and during development of the vertebrate retina. *Curr Biol* 26:2268-2279.
- D'Souza B, Miyamoto A, Weinmaster G (2008) The many facets of Notch ligands. *Oncogene* 27:5148-5167.
- Das AV, Mallya KB, Zhao X, Ahmad F, Bhattacharya S, Thoreson WB, Hegde GV, Ahmad I (2006) Neural stem cell properties of Müller glia in the mammalian retina: regulation by Notch and Wnt signaling. *Dev Biol* 299:283-302.
- de Melo J, Zibetti C, Clark BS, Hwang W, Miranda-Angulo AL, Qian J, Blackshaw S (2016) Lhx2 is an essential factor for retinal gliogenesis and notch signaling. *J Neurosci* 36:2391-2405.
- De Strooper B, Annaert W, Cupers P, Saftig P, Craessaerts K, Mumm JS, Schroeter EH, Schrijvers V, Wolfe MS, Ray WJ, Goate A, Kopan R (1999) A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature* 398:518-522.
- Del Debbio CB, Balasubramanian S, Parameswaran S, Chaudhuri A, Qiu F, Ahmad I (2010) Notch and Wnt signaling mediated rod photoreceptor regeneration by Müller cells in adult mammalian retina. *PLoS One* 5:e12425.
- Del Debbio CB, Mir Q, Parameswaran S, Mathews S, Xia X, Zheng L, Neville AJ, Ahmad I (2016) Notch signaling activates stem cell properties of müller glia through transcriptional regulation and Skp2-mediated degradation of p27Kip1. *PLoS One* 11:e0152025.
- Demmin DL, Silverstein SM (2020) Visual impairment and mental health: unmet needs and treatment options. *Clin Ophthalmol* 14:4229-4251.
- Elsaedi F, Macpherson P, Mills EA, Jui J, Flannery JG, Goldman D (2018) Notch suppression collaborates with Ascl1 and Lin28 to unleash a regenerative response in fish retina, but not in mice. *J Neurosci* 38:2246-2261.
- Esteve P, Sandonis A, Cardozo M, Malapeira J, Ibanez C, Crespo I, Marcos S, Gonzalez-Garcia S, Toribio ML, Arribas J, Shimono A, Guerrero I, Bovolenta P (2011a) SFRPs act as negative modulators of ADAM10 to regulate retinal neurogenesis. *Nat Neurosci* 14:562-569.
- Esteve P, Sandonis A, Ibanez C, Shimono A, Guerrero I, Bovolenta P (2011b) Secreted frizzled-related proteins are required for Wnt/ β -catenin signalling activation in the vertebrate optic cup. *Development* 138:4179-4184.
- Furukawa T, Mukherjee S, Bao ZZ, Morrow EM, Cepko CL (2000) rax, Hes1, and notch1 promote the formation of müller glia by postnatal retinal progenitor cells. *Neuron* 26:383-394.
- Gao H, A L, Huang X, Chen X, Xu H (2021) Müller glia-mediated retinal regeneration. *Mol Neurobiol* 58:2342-2361.
- Ghai K, Zelinka C, Fischer AJ (2010) Notch signaling influences neuroprotective and proliferative properties of mature Müller glia. *J Neurosci* 30:3101-3112.
- Gordois A, Cutler H, Pezzullo L, Gordon K, Cruess A, Winyard S, Hamilton W, Chua K (2012) An estimation of the worldwide economic and health burden of visual impairment. *Glob Public Health* 7:465-481.
- Gordon WR, Vardar-Ulu D, Histen G, Sanchez-Irizarry C, Aster JC, Blacklow SC (2007) Structural basis for autoinhibition of Notch. *Nat Struct Mol Biol* 14:295-300.
- Grotek B, Wehner D, Weidinger G (2013) Notch signaling coordinates cellular proliferation with differentiation during zebrafish fin regeneration. *Development* 140:1412-1423.
- Groth C, Nornes S, McCarty R, Tamme R, Lardelli M (2002) Identification of a second presenilin gene in zebrafish with similarity to the human Alzheimer's disease gene presenilin2. *Dev Genes Evol* 212:486-490.
- Hamon A, Garcia-Garcia D, Ail D, Bitard J, Chesneau A, Dalkara D, Locker M, Roger JE, Perron M (2019) Linking YAP to müller glia quiescence exit in the degenerative retina. *Cell Rep* 27:1712-1725.
- Hatakeyama J, Kageyama R (2004) Retinal cell fate determination and bHLH factors. *Semin Cell Dev Biol* 15:83-89.
- Hayes S, Nelson BR, Buckingham B, Reh TA (2007) Notch signaling regulates regeneration in the avian retina. *Dev Biol* 312:300-311.
- Henrique D, Schweisguth F (2019) Mechanisms of Notch signaling: a simple logic deployed in time and space. *Development* 146:172148.
- Hitchcock P, Ochocinska M, Sieh A, Otteson D (2004) Persistent and injury-induced neurogenesis in the vertebrate retina. *Prog Retin Eye Res* 23:183-194.
- Hitzenberger M, Gotz A, Menig S, Brunschweiler B, Zacharias M, Scharnagl C (2020) The dynamics of γ -secretase and its substrates. *Semin Cell Dev Biol* 105:86-101.
- Hoang T, Wang J, Boyd P, Wang F, Santiago C, Jiang L, Yoo S, Lahne M, Todd LJ, Jia M, Saez C, Keuthan C, Palazzo I, Squires N, Campbell WA, Rajaii F, Parayil T, Trinh V, Kim DW, Wang G et al. (2020) Gene regulatory networks controlling vertebrate retinal regeneration. *Science* 370:8598.
- Holly VL, Widen SA, Famulski JK, Waskiewicz AJ (2014) Sfrp1a and Sfrp5 function as positive regulators of Wnt and BMP signaling during early retinal development. *Dev Biol* 388:192-204.
- Hoon M, Okawa H, Della Santina L, Wong RO (2014) Functional architecture of the retina: development and disease. *Prog Retin Eye Res* 42:44-84.
- Hsia HE, Tüshaus J, Brummer T, Zheng Y, Scilabra SD, Lichtenthaler SF (2019) Functions of 'A disintegrin and metalloproteases (ADAMs)' in the mammalian nervous system. *Cell Mol Life Sci* 76:3055-3081.
- Iribarne M (2021) Inflammation induces zebrafish regeneration. *Neural Regen Res* 16:1693-1701.
- Iribarne M, Hyde DR, Masai I (2019) TNF α induces müller glia to transition from non-proliferative gliosis to a regenerative response in mutant zebrafish presenting chronic photoreceptor degeneration. *Front Cell Dev Biol* 7:296.
- Itoh M, Kim CH, Palardy G, Oda T, Jiang YJ, Maust D, Yeo SY, Lorick K, Wright GJ, Ariza-McNaughton L, Weissman AM, Lewis J, Chandrasekharappa SC, Chitnis AB (2003) Mind bomb is a ubiquitin ligase that is essential for efficient activation of notch signaling by delta. *Dev Cell* 4:67-82.
- Ivanov D (2019) Notch signaling-induced oscillatory gene expression may drive neurogenesis in the developing retina. *Front Mol Neurosci* 12:226.
- Jadhav AP, Cho SH, Cepko CL (2006a) Notch activity permits retinal cells to progress through multiple progenitor states and acquire a stem cell property. *Proc Natl Acad Sci U S A* 103:18998-19003.
- Jadhav AP, Mason HA, Cepko CL (2006b) Notch 1 inhibits photoreceptor production in the developing mammalian retina. *Development* 133:913-923.
- Jorstad NL, Wilken MS, Grimes WN, Wohl SG, VandenBosch LS, Yoshimatsu T, Wong RO, Rieke F, Reh TA (2017) Stimulation of functional neuronal regeneration from Müller glia in adult mice. *Nature* 548:103-107.
- Kageyama R, Ohtsuka T, Kobayashi T (2007) The Hes gene family: repressors and oscillators that orchestrate embryogenesis. *Development* 134:1243-1251.
- Kao HY, Ordentlich P, Koyano-Nakagawa N, Tang Z, Downes M, Kintner CR, Evans RM, Kadesch T (1998) A histone deacetylase corepressor complex regulates the Notch signal transduction pathway. *Genes Dev* 12:2269-2277.
- Kechad A, Jolicoeur C, Tufford A, Mattar P, Chow RWY, Harris WA, Cayouette M (2012) Numb is required for the production of terminal asymmetric cell divisions in the developing mouse retina. *J Neurosci* 32:17197-17210.
- Kopan R, Ilagan MX (2009) The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell* 137:216-233.
- Lahne M, Brecker M, Jones SE, Hyde DR (2021) The Regenerating adult zebrafish retina recapitulates developmental fate specification programs. *Front Cell Dev Biol* 8:617923.
- Lahne M, Li J, Marton RM, Hyde DR (2015) Actin-cytoskeleton- and rock-mediated INM are required for photoreceptor regeneration in the adult zebrafish retina. *J Neurosci* 35:15612-15634.
- Lahne M, Nagashima M, Hyde DR, Hitchcock PF (2020) Reprogramming Müller glia to regenerate retinal neurons. *Annu Rev Vis Sci* 6:171-193.
- Lake RJ, Grimm LM, Veraksa A, Banos A, Artavanis-Tsakonas S (2009) In vivo analysis of the Notch receptor S1 cleavage. *PLoS One* 4:e6728.
- Lee MS, Wan J, Goldman D (2020) Tgfb3 collaborates with PP2A and notch signaling pathways to inhibit retina regeneration. *Elife* 9:e55137.

- Liu Z, Brunskill E, Varnum-Finney B, Zhang C, Zhang A, Jay PY, Bernstein I, Morimoto M, Kopan R (2015) The intracellular domains of Notch1 and Notch2 are functionally equivalent during development and carcinogenesis. *Development* 142:2452-2463.
- Logeat F, Bessia C, Brou C, LeBail O, Jarriault S, Seidah NG, Israel A (1998) The Notch1 receptor is cleaved constitutively by a furin-like convertase. *Proc Natl Acad Sci U S A* 95:8108-8112.
- Maurer KA, Riesenberger AN, Brown NL (2014) Notch signaling differentially regulates Atoh7 and Neurog2 in the distal mouse retina. *Development* 141:3243-3254.
- McGinn TE, Galicia CA, Leoni DC, Partington N, Mitchell DM, Stenkamp DL (2019) Rewiring the regenerated zebrafish retina: reemergence of bipolar neurons and cone-bipolar circuitry following an inner retinal lesion. *Front Cell Dev Biol* 7:95.
- McGinn TE, Mitchell DM, Meighan PC, Partington N, Leoni DC, Jenkins CE, Varnum MD, Stenkamp DL (2018) Restoration of dendritic complexity, functional connectivity, and diversity of regenerated retinal bipolar neurons in adult zebrafish. *J Neurosci* 38:120-136.
- Miesfeld JB, Moon MS, Riesenberger AN, Contreras AN, Kovall RA, Brown NL (2018) Rbpj direct regulation of Atoh7 transcription in the embryonic mouse retina. *Sci Rep* 8:10195.
- Mitra S, Sharma P, Kaur S, Khurshed MA, Gupta S, Ahuja R, Kurup AJ, Chaudhary M, Ramachandran R (2018) Histone deacetylase-mediated müller glia reprogramming through Her4.1-Lin28a axis is essential for retina regeneration in zebrafish. *iScience* 7:68-84.
- Mitra S, Sharma P, Kaur S, Khurshed MA, Gupta S, Chaudhary M, Kurup AJ, Ramachandran R (2019) Dual regulation of lin28a by Myc is necessary during zebrafish retina regeneration. *J Cell Biol* 218:489-507.
- Moloney DJ, Shair LH, Lu FM, Xia J, Locke R, Matta KL, Haltiwanger RS (2000) Mammalian Notch1 is modified with two unusual forms of O-linked glycosylation found on epidermal growth factor-like modules. *J Biol Chem* 275:9604-9611.
- Mumm JS, Schroeter EH, Saxena MT, Griesemer A, Tian X, Pan DJ, Ray WJ, Kopan R (2000) A ligand-induced extracellular cleavage regulates γ -secretase-like proteolytic activation of Notch1. *Mol Cell* 5:197-206.
- Münch J, Grivas D, Gonzalez-Rajal A, Torregrosa-Carrion R, de la Pompa JL (2017) Notch signalling restricts inflammation and serpine1 expression in the dynamic endocardium of the regenerating zebrafish heart. *Development* 144:1425-1440.
- Nagashima M, Barthel LK, Raymond PA (2013) A self-renewing division of zebrafish Müller glial cells generates neuronal progenitors that require N-cadherin to regenerate retinal neurons. *Development* 140:4510-4521.
- Nelson BR, Ueki Y, Reardon S, Karl MO, Georgi S, Hartman BH, Lamba DA, Reh TA (2011) Genome-wide analysis of Müller glial differentiation reveals a requirement for Notch signaling in postmitotic cells to maintain the glial fate. *PLoS One* 6:e22817.
- Nelson CM, Ackerman KM, O'Hayer P, Bailey TJ, Gorsuch RA, Hyde DR (2013) Tumor necrosis factor- α is produced by dying retinal neurons and is required for Müller glia proliferation during zebrafish retina regeneration. *J Neurosci* 33:6524-6539.
- Nery LR, Silva NE, Fonseca R, Vianna MRM (2017) Presenilin-1 targeted morpholino induces cognitive deficits, increased brain Abeta1-42 and decreased synaptic marker PSD-95 in zebrafish larvae. *Neurochem Res* 42:2959-2967.
- Nikolaou N, Watanabe-Asaka T, Gerety S, Distel M, Koster RW, Wilkinson DG (2009) Lunatic fringe promotes the lateral inhibition of neurogenesis. *Development* 136:2523-2533.
- Panin VM, Papayannopoulos V, Wilson R, Irvine KD (1997) Fringe modulates Notch-ligand interactions. *Nature* 387:908-912.
- Panin VM, Shao L, Lei L, Moloney DJ, Irvine KD, Haltiwanger RS (2002) Notch ligands are substrates for protein O-fucosyltransferase-1 and Fringe. *J Biol Chem* 277:29945-29952.
- Powell C, Cornblath E, Elsaefi F, Wan J, Goldman D (2016) Zebrafish Müller glia-derived progenitors are multipotent, exhibit proliferative biases and regenerate excess neurons. *Sci Rep* 6:24851.
- Ran Y, Hossain F, Pannuti A, Lessard CB, Ladd GZ, Jung JI, Minter LM, Osborne BA, Miele L, Golde TE (2017) γ -Secretase inhibitors in cancer clinical trials are pharmacologically and functionally distinct. *EMBO Mol Med* 9:950-966.
- Rana NA, Haltiwanger RS (2011) Fringe benefits: functional and structural impacts of O-glycosylation on the extracellular domain of Notch receptors. *Curr Opin Struct Biol* 21:583-589.
- Rao MB, Didiano D, Patton JG (2017) Neurotransmitter-regulated regeneration in the zebrafish retina. *Stem Cell Reports* 8:831-842.
- Raymond P, Barthel L, Bernardos R, Perkowski J (2006) Molecular characterization of retinal stem cells and their niches in adult zebrafish. *BMC Dev Biol* 6:36.
- Riesenberger AN, Conley KW, Le TT, Brown NL (2018) Separate and coincident expression of Hes1 and Hes5 in the developing mouse eye. *Dev Dyn* 247:212-221.
- Riesenberger AN, Liu Z, Kopan R, Brown NL (2009) Rbpj cell autonomous regulation of retinal ganglion cell and cone photoreceptor fates in the mouse retina. *J Neurosci* 29:12865-12877.
- Rueda EM, Hall BM, Hill MC, Swinton PG, Tong X, Martin JF, Poché RA (2019) The hippo pathway blocks mammalian retinal müller glial cell reprogramming. *Cell Rep* 27:1637-1649.
- Saiki W, Ma C, Okajima T, Takeuchi H (2021) Current views on the roles of O-glycosylation in controlling notch-ligand interactions. *Biomolecules* 11:309.
- Sakaidani Y, Ichianagi N, Saito C, Nomura T, Ito M, Nishio Y, Nadano D, Matsuda T, Furukawa K, Okajima T (2012) O-linked-N-acetylglucosamine modification of mammalian Notch receptors by an atypical O-GlcNAc transferase Eogt1. *Biochem Biophys Res Commun* 419:14-19.
- Schnute B, Troost T, Klein T (2018) Endocytic trafficking of the notch receptor. *Adv Exp Med Biol* 1066:99-122.
- Sharma P, Gupta S, Chaudhary M, Mitra S, Chawla B, Khurshed MA, Saran NK, Ramachandran R (2020) Biphasic role of Tgf- β signaling during müller glia reprogramming and retina regeneration in zebrafish. *iScience* 23:100817.
- Shi S, Stanley P (2003) Protein O-fucosyltransferase 1 is an essential component of Notch signaling pathways. *Proc Natl Acad Sci U S A* 100:5234-5239.
- Steinbuck MP, Winandy S (2018) A review of notch processing with new insights into ligand-independent notch signaling in T-cells. *Front Immunol* 9:1230.
- Takeuchi H, Schneider M, Williamson DB, Ito A, Takeuchi M, Handford PA, Haltiwanger RS (2018) Two novel protein O-glycosyltransferases that modify sites distinct from POGlut1 and affect Notch trafficking and signaling. *Proc Natl Acad Sci U S A* 115:E8395-8402.
- Taylor SM, Alvarez-Delfin K, Saade CJ, Thomas JL, Thummel R, Fadool JM, Hitchcock PF (2015) The bHLH transcription factor NeuroD governs photoreceptor genesis and regeneration through delta-notch signaling. *Invest Ophthalmol Vis Sci* 56:7496-7515.
- Thomas JL, Ranski AH, Morgan GW, Thummel R (2016) Reactive gliosis in the adult zebrafish retina. *Exp Eye Res* 143:98-109.
- Toonen JA, Ronchetti A, Sidjanin DJ (2016) A disintegrin and metalloproteinase10 (ADAM10) regulates NOTCH signaling during early retinal development. *PLoS One* 11:e0156184.
- Totaro A, Castellan M, Battilana G, Zanconato F, Azzolin L, Giulitti S, Cordenonsi M, Piccolo S (2017) YAP/TAZ link cell mechanics to Notch signalling to control epidermal stem cell fate. *Nat Commun* 8:15206.
- Wan J, Goldman D (2017) Opposing actions of Fgf8a on Notch signaling distinguish two müller glial cell populations that contribute to retina growth and regeneration. *Cell Rep* 19:849-862.
- Wan J, Ramachandran R, Goldman D (2012) HB-EGF is necessary and sufficient for Müller glia dedifferentiation and retina regeneration. *Dev Cell* 22:334-347.
- Wang J, Martin JF (2017) Hippo pathway: an emerging regulator of craniofacial and dental development. *J Dent Res* 96:1229-1237.
- Weinmaster G, Fischer JA (2011) Notch ligand ubiquitylation: what is it good for? *Dev Cell* 21:134-144.
- Wilson SG, Wen W, Pillai-Kastoori L, Morris AC (2016) Tracking the fate of her4 expressing cells in the regenerating retina using her4:Kaede zebrafish. *Exp Eye Res* 145:75-87.
- Wolfe MS (2019) Structure and function of the γ -secretase complex. *Biochemistry* 58:2953-2966.
- World Health Organization (2019) World report on vision. www.who.int/publications-detail/world-report-on-vision.
- Yoo KW, Thiruvarangan M, Jeong YM, Lee MS, Maddirevula S, Rhee M, Bae YK, Kim HG, Kim CH (2017) Mind bomb-binding partner RanBP9 plays a contributory role in retinal development. *Mol Cells* 40:271-279.
- Zema S, Pelullo M, Nardoza F, Felli MP, Screpanti I, Bellavia D (2020) A Dynamic role of mastermind-like 1: A journey through the main (path)ways between development and cancer. *Front Cell Dev Biol* 8:613557.
- Zhang C, Li Q, Jiang YJ (2007a) Zebrafish Mib and Mib2 are mutual E3 ubiquitin ligases with common and specific delta substrates. *J Mol Biol* 366:1115-1128.
- Zhang C, Li Q, Lim CH, Qiu X, Jiang YJ (2007b) The characterization of zebrafish antimorphic mib alleles reveals that Mib and Mind bomb-2 (Mib2) function redundantly. *Dev Biol* 305:14-27.

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