Developmental Neurobiology Unit

Professor Ichiro Masai

Abstract

Developmental neurobiology unit uses zebrafish as an animal model and will elucidate genetic program that regulate eye development. Specific research projects are to elucidate:

(1) Mechanisms that regulate retinal cell differentiation and neural circuit formation
(2) Mechanisms that regulate photoreceptor degeneration and regeneration
(3) Mechanisms that regulate lens fiber differentiation
(4) Mechanisms that regulate zebrafish behaviors linked to fear/anxiety response

During vertebrate development, the retina is originally derived from anterior neural plate. In this region, six major classes of retinal neurons differentiate and form neural circuits responsible for vision. Thus, the retina provides a good model for studying cell differentiation and neural circuit formation in the developing brain. First, we will investigate mechanisms that regulate cell differentiation and neural circuit formation. Second, we will investigate mechanisms that regulate photoreceptor degeneration. We will determine how photoreceptors monitor abnormalities in cellular functions and trigger apoptosis. When the zebrafish retina suffers from photoreceptor degeneration, one type of retinal glial cells, namely Müller cells, generate retinal neurons as neural stem cells. Recent studies revealed that brain-resident immune cells, namely microglia, play an important role in this regeneration program of Müller cells. We will investigate the role of microglia in photoreceptor regeneration. Third, we will investigate lens development, especially, mechanisms that regulate lens fiber differentiation. Through these projects, we will establish key concepts that govern development of multicellular organisms and contribute to our understanding of pathological processes of human retinal diseases. In addition to research project on ocular development above, we have collaborated with Greg Stephens unit to investigate behavioral mechanisms that regulate social interaction such as fish shoaling and fear/anxiety response using zebrafish as a model.

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2. Collaborations

2.1 In vivo functional analysis of hypoxia response genes using the zebrafish retina

- Description: In vivo functional analysis of hypoxia response genes using the zebrafish retina
- Type of collaboration: Joint research agreement
- Researchers: Dr. Ichiro Masai (Developmental neurobiology unit, OIST), Dr. Masayuki Matsushita (Department of Medicine, Ryukyu University)

2.2 Role of zebrafish BANP protein in tumor suppression of melanoma

- Description: Role of zebrafish BANP protein in tumor suppression of melanoma
- Type of collaboration: Joint research agreement
- Researchers: Dr. Ichiro Masai (Developmental neurobiology unit, OIST), Dr. Yutaka Kikuchi (Department of Biological Science, Hiroshima University)

3. Activities and Findings

3.1 Mechanism of neural circuit formation in zebrafish retinas

In the vertebrate retina, six major types of neurons and one glial cell type differentiate to form the neural circuit for vision (Fig. 1). Two types of photoreceptors, rods and cones form the outer nuclear layer (ONL). Three types of interneurons, horizontal cells, bipolar cells and amacrine cells, form the inner nuclear layer (INL). Retinal ganglion cells (RGCs) form RGC layer (RGCL). Finally, Müller glia have a nucleus positioned in the INL and extend apical and basal processes, whose end feet compose a part of the outer and inner limiting membranes, respectively. Two synaptic layers, called the outer plexiform layer (OPL) and the inner plexiform layer (IPL), are formed between ONL and INL and between INL and RGCL, respectively. The structure of these layers, called "retinal lamination", is one of the characteristic patterns along the apico-basal axis of the neural retina, and is essential for visual processing from photoreceptors to RGCs through modification by three types of interneurons. In addition, the retina is a two-dimensional (2D) sheet, in which different types of photoreceptors, especially cones, form a regular mosaic pattern called the "cone mosaic". This 2D planar pattern is essential to efficiently capture a topological visual image of the external world as well as to extract moving objects in static scene background. One of our objectives is to understand how this beautiful retinal neural circuit is constructed during development.

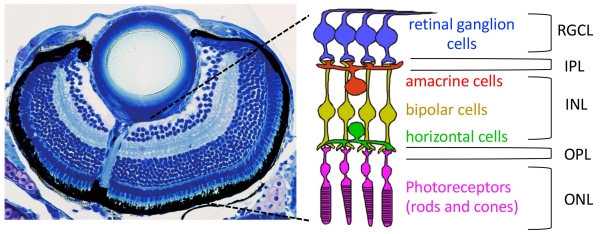

Figure 1. Zebrafish retina. 7dpf zebrafish retina. Six major classes of retinal neurons form neural circuits for vision. Retinal ganglion cells (RGCs) from RGC layer (RGCL); three interneurons, amacrine cells, bipolar cells and horizontal cells from inner nuclear layer (INL); two types of photoreceptors, rods and cones, from outer nuclear layer (ONL). Two synaptic layers, inner plexiform layer (IPL) and outer plexiform layer (OPL) form between RGCL and INL, and between INL and ONL, respectively.

To understand the mechanism of retinal lamination, we have investigated zebrafish *striatin interacting protein 1* (*strip1*) mutants. Strip1 is a recently identified protein with emerging functions in neuronal development. It was first described as one of the core components of the striatin-interacting phosphatase and kinase (STRIPAK) complex (Hwang and Pallas, 2014). The STRIPAK complex is an evolutionarily conserved supramolecular complex with diverse functions in cell proliferation, migration, vesicular transport, cardiac development, and cancer progression. In addition, several STRIPAK components participate in dendritic development, axonal transport, and synapse assembly. In *Drosophila*, Strip (a homolog of mammalian Strip1/2) is essential for axon elongation (Sakuma et al., 2014; Sakuma et al., 2015). In addition, Strip, together with other STRIPAK members, modulates synaptic bouton development and prevents ectopic retina formation (Sakuma et al., 2016). On the other hand, loss of mouse Strip1 causes early mesoderm migration defects leading to embryonic lethality (Bazzi et al., 2017). Thus, the role of Strip1 in the vertebrate nervous system is largely unknown.

We identified zebrafish *strip1* mutants by zebrafish large-scale mutagenesis. In zebrafish *strip1* mutants, retinal lamination, especially IPL formation, is disrupted (Fig. 2A). Loss of Strip1 causes RGC death shortly after birth (Fig. 2B). Cells in the INL subsequently infiltrate the degenerating RGCL, leading to a disorganized IPL. Strip1 cell-autonomously promotes RGC survival; however, it is not required in INL cells for IPL formation. Therefore, Strip1-mediated RGC maintenance is required to establish the IPL. Mechanistically, we identified Striatin 3 (Strn3) as a Strip1-interacting partner. Both Strip1 and Strn3 show overlapping functions in RGC survival through suppression of the JNK/Jun-mediated apoptotic pathway (Fig. 2B, 2C). We also found that Strip1 is cell-autonomously required for RGC dendritic patterning, which likely promotes interaction between RGCs and ACs for IPL formation (Fig. 2D). We are now searching what regulatory factors are expressed in RGCs and promote the interaction between RGC dendrites and amacrine cells in a Strip1-dependent manner. In summary, we demonstrate that Strip1 is crucial for RGC survival during development and thereby coordinates proper wiring of the inner retina.

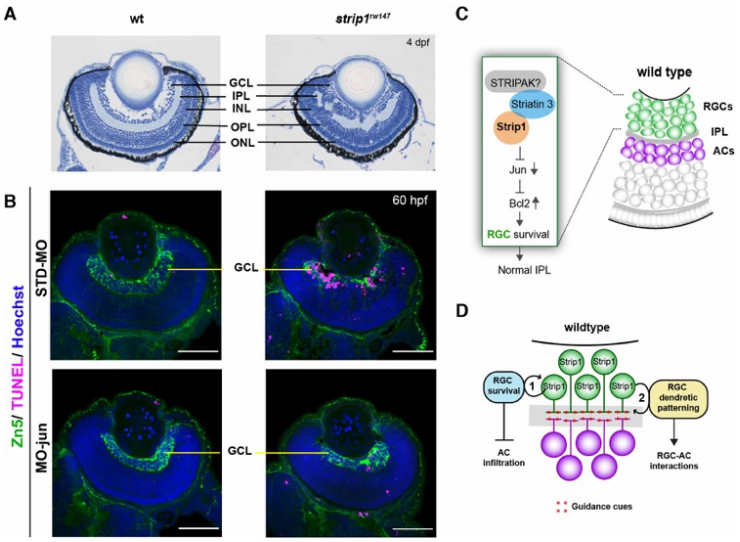

Figure 2. Strip1 regulates RGC survival and IPL formation.

(A) Wild-type and *strip1* mutant retinas at 4 dpf. IPL is disrupted in *strip1* mutants. (B) RGCs undergo apoptosis in *strip1* mutants, which depends on Jun. (C) Strip1 cooperates with Striatin 3 to suppress Jun-mediated RGC apoptosis. (D) Strip1 regulates RGC survival to prevent AC infiltration, and RGC dendritic patterning to promote RGC-AC interaction.

We discovered a novel neuroprotective mechanism governed by Strip1, probably through the STRIPAK complex, to suppress JNK/Jun-mediated proapoptotic signaling in RGCs during development. Since RGC death is associated with optic nerve damage, for example, human glaucoma, we compared RNA expression profiles between zebrafish *strip1* mutants and a zebrafish adult glaucoma model with optic nerve injury (McCurley and Callard, 2010; Veldman et al., 2007). Interestingly, Jun activation is also observed in the zebrafish glaucoma model. Thus, the mechanism underlying Strip1-mediated RGC apoptosis will provide important insight into therapeutic development of human glaucoma.

3.2 Mechanism of photoreceptor degeneration

Apoptosis is observed in developing tissues and is believed to remove abnormal cells. Although apoptosis is important for establishment of proper neural circuits by eliminating abnormally differentiated neurons, it is unclear how differentiating cells monitor their own abnormality, and how the threshold at which apoptosis is induced is determined. Photoreceptors provide a useful model for studying such a surveillance mechanism of neuronal development and homeostasis, because there are many hereditary retinal diseases in humans associated with photoreceptor degeneration. Although 281 genes linked to hereditary retinal diseases have already been identified, these genes encode diverse functions, including phototransduction, retinol metabolism, and intracellular protein transport (see the homepage of the Retinal Information Network at http://www.sph.uth.tmc.edu/Retnet/). To answer how photoreceptors monitor their differentiation status and homeostasis, and what kinds of molecular network determine the choice between cell survival and cell death in photoreceptors, we have investigated zebrafish photoreceptor degeneration mutants. Previous zebrafish mutagenesis identified zebrafish photoreceptor degeneration mutants (Nishiwaki et al., 2008). In FY2023, we especially focused on zebrafish *pday* mutants, in which ciliary transport is disrupted by a genetic mutation of male germ cell-associated kinase (MAK).

3.2.1 Role of MAK in ciliogenesis and photoreceptor degeneration

The outer segment (OS) is a specialized cilium of the photoreceptor, in which multiple photoreceptive membrane discs are regularly stacked to accommodate phototransduction molecules. The inner segment (IS) is a mitochondria-enriched region between the OS and the nucleus. The axoneme is anchored from the basal body in the IS of photoreceptors and extended apically through the connecting cilium. The connecting cilium bridges the IS and the OS, through which phototransduction molecules are transported from the ER, then to the Golgi and into the OS. Importantly, the connecting cilium is equivalent to the transition zone in other cell types and functions as a gating system, through which OS resident proteins are transported to the OS by the intraflagellar transport (IFT) complex and BBSome, composed of eight Bardet-Biedl syndrome (BBS) proteins. The process of ciliary development is called ciliogenesis, which consists primarily of three steps: centriole (basal body) docking to the apical plasma membrane, establishment of a transition zone, and ciliary axoneme extension. A number of factors are indispensable for ciliogenesis. Because cilia work as “rail tracks” in IFT-mediated trafficking, disruption of cilia affects IFT and further causes photoreceptor degeneration, suggesting that the structural and functional integrity of the connecting cilium is indispensable for photoreceptor functions and maintenance. However, mechanisms underlying ciliogenesis and ciliary regulation are not fully understood.

A zebrafish mutant, *payday*, was originally reported by Dr. Herwig Baier (Muto et al., 2005) (Fig. 3A). We recently cloned the *payday* mutant gene and found that a non-sense mutation occurs in the *male germ cell-associated kinase* (*mak*) gene in *payday* mutants. MAK belongs to the MAK/ICK/MOK serine/threonine kinase family. MAK is expressed in rod photoreceptors. In *Mak* knockout mice, photoreceptors show abnormally elongated axonemes and malformation of membrane discs in the OS, leading to photoreceptor degeneration. In humans, *MAK* mutations cause retinitis pigmentosa (RP), in which patients progressively lose their vision. However, human patients carrying *MAK* mutations do not show elongation of the photoreceptor layer. Thus, how *mak* mutations affect ciliary regulation in photoreceptors among vertebrate species is important.

We investigated photoreceptor degeneration phenotypes of zebrafish *mak* mutants (*payday*). In *mak* mutants, photoreceptors degenerate during embryonic development (Fig. 3B), and both rods and cones undergo apoptosis, although their degeneration processes differ (Fig. 3C). Interestingly, *mak* mutants fail to form axonemes in photoreceptor cilia (Fig. 3D), whereas basal bodies are specified, which is the opposite of mouse *Mak* knockout photoreceptors, with elongated ciliary axonemes. Furthermore, both cones and rods completely lack OSs in *mak* mutants (Fig. 3E), leading to ectopic distribution of opsins. These data suggest that MAK is essential to form axonemes

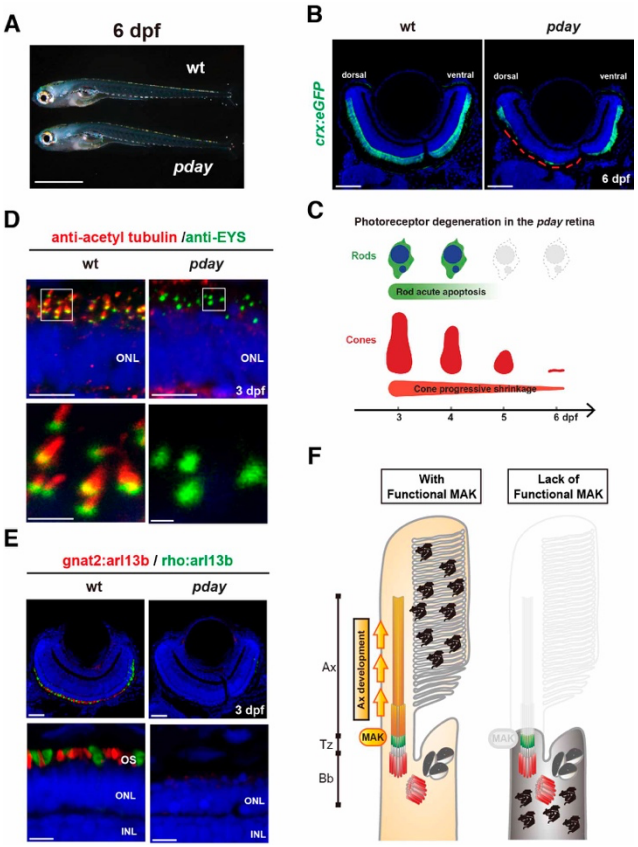

Figure 3. Mak promotes ciliogenesis and OS formation to ensure photoreceptor survival.

(A) Zebrafish *mak* mutants, *payday*. (B) Photoreceptors undergo degeneration in *mak* mutants. (C) In *mak* mutants, rods undergo apoptosis at 3-4 dpf, whereas cones show progressive shrinkage at 3-6 dpf. (D) Labeling of photoreceptor cilia with anti-acetylated tubulin and anti-Eys antibody, which visualize axoneme and transition zone, respectively. Axoneme is specifically missing in *mak* mutants. (E) The OS is absent in cones and rods of *mak* mutants. (F) A role of Mak in axoneme formation in zebrafish photoreceptors.

and OSs in photoreceptors. Finally, MAK kinase activity is critical for axoneme formation and photoreceptor survival. Thus, MAK is essential for ciliogenesis, OS formation, and photoreceptor survival (Fig. 3F).

3.3 The role of microglia in neuronal degeneration and regeneration

Microglia are brain-resident immune cells, originally derived from mesoderm-derivative tissue or the hematopoietic stem cell-lineage, that migrate into brain, and patrol within the brain throughout life. Microglia are thought to eliminate dead or dying neurons to prevent inflammation. Interestingly, microglia-mediated inflammation is required for neuronal regeneration in response to traumatic brain injury in zebrafish, suggesting that microglia interact with neural stem cells and promote neural stem cells to initiate regeneration program in response to brain damage. Thus, it is important to understand the role of microglia in neuronal degeneration and regeneration.

3.3.1 Mechanism of microglia specification in zebrafish brain First, we considered it important to know how microglial precursors enter the neural retina in zebrafish and how microglial precursors differentiate into mature functional microglia. In zebrafish, early macrophage precursors are initially specified in lateral plate mesoderm. Then they move on the yolk from the 13 to 26 somite stage, and migrate into cephalic mesenchyme from 22 to 40 hpf (Herbomel et al., 1999), followed by further migration into the retina from 30 hpf and the optic tectum from 48 hpf (Herbomel et al., 2001). After early macrophage precursors enter the retina and brain, they start to express markers of functional mature microglia such as *apoeb* after 60 hpf. Since almost all the early macrophage precursors infiltrated into the brain eventually become functional mature microglia (Herbomel et al., 2001), so brain-resident early macrophage precursors are likely to acquire the fate of microglial precursors. However, it is unknown what kinds of guidance cues enable early macrophage precursors or possibly microglial precursors to migrate from yolk into developing brain and retina, and what kinds of mechanism promote microglial precursors to differentiate into functional mature microglia after they enter the brain and retina.

To answer the first question, we examined normal development of microglial precursors and their colonization mechanism in zebrafish retina. First, we conducted time-lapse imaging using zebrafish *Tg[mpeg1:GFP]* or *Tg[mfap4:tdTomato-CAAX]* transgenic lines, which specifically visualizes microglial precursors in zebrafish (Ellett et al., 2011; Walton et al., 2015). Microglial precursors progressively enter the optic cup from 24 to 54 hpf through the ventral optic fissure. Intraocular hyaloid blood vessels initially form from the ventral fissure and then expand near the lens, suggesting an overlap with the microglial precursors' colonization route. Indeed, microglial precursors use intraocular hyaloid blood vessels as a pathway to migrate into the optic cup via the choroid fissure, suggesting that blood vessels contribute to the guidance scaffold. Once retinal progenitor cells exit the cell cycle, microglial precursors associated with hyaloid blood vessels start to infiltrate the retina preferentially through

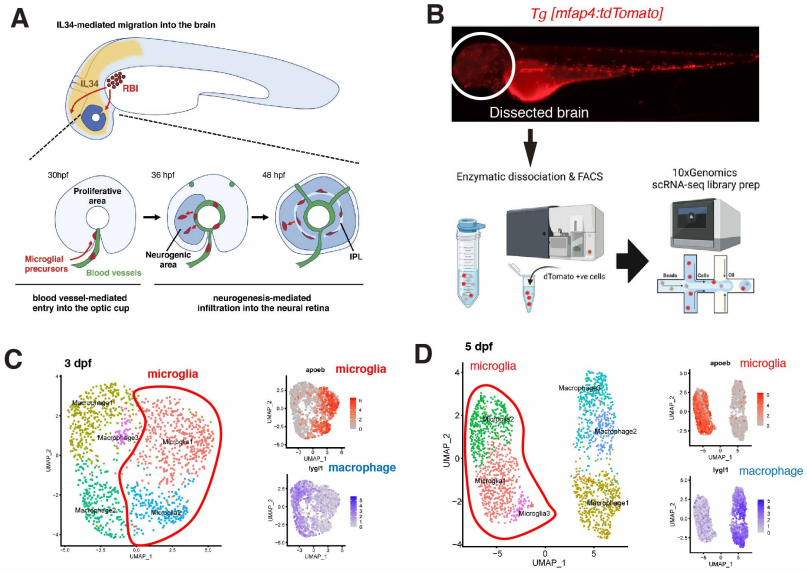

Figure 4. Mechanism of microglial colonization and maturation in zebrafish retinas.

(A) Mechanism underlying the colonization of microglial precursors into zebrafish retina. (B) Experimental design of scRNA-seq analysis. (C) UMAP of *mfap4:tdTomato-CAAX* cells in zebrafish retinas at 3 dpf. Two microglial clusters and three microphage clusters are identified. (D) UMAP of *mfap4:tdTomato-CAAX* cells in zebrafish retinas at 5 dpf. Three microglial clusters and three microphage clusters are identified and segregated each other, suggesting that maturation process of microglia proceed.

neurogenic regions. These data suggest that neurogenesis functions as a gate that allows microglial precursors to move into the neural retina. It was reported that brain-derived interleukin 34 (IL34) and its receptor, colony-stimulating factor 1 receptor a (csf1ra) regulate colonization of microglial precursors in zebrafish brain and eye (Wu et al., 2018). We confirmed that IL34 is required for colonization of microglial precursors into the retina. Thus, our findings suggest a step-by-step guidance mechanism by which microglial precursors move from yolk into the optic cup and colonize the neural retina (Fig. 4A).

Next, we examined what kinds of mechanism drive microglial precursors to differentiate into functional mature microglia after they enter the brain and retina. We used zebrafish transgenic line *Tg[mfap4:tdTomato-CAAX]*, which visualizes microglia/macrophage precursors, microglia and macrophages, and collected *mfap4:tdTomato-CAAX*-positive cells from zebrafish embryonic heads at 2 dpf and retinas at 3 and 5 dpf, using FACS. We applied single-cell RNA-seq analysis, and conducted clustering of those datasets separately, using Seurat (Fig. 4B). At 2 dpf, brain-derived *mfap4*-expressing cells form only one cluster. This single cluster expresses both microglial and macrophage markers *ccl34b.1* and *lygl1*, but importantly, it does not express a mature microglial marker, *apoeb*, indicating that this single cluster represents common precursors. At 3 dpf, *mfap4*-expressing cells are separated into 5 clusters, among which two clusters express *apoeb*, indicating mature microglia clusters (Fig. 4C). Importantly, in these *apoeb*-positive clusters, expression of the macrophage marker, *lygl1*, is very low. On the other hand, *lygl1* expression is maintained in other three *apoeb*-negative clusters. So, maturation of microglia and macrophages proceeded at 3 dpf. At 5 dpf, *mfap4*-expressing cells are more clearly segregated into mature microglia and mature macrophages (Fig. 4D). We generated the double transgenic line Tg[*mfap4:tdTomato-CAAX*; *ccl34b.1:EGFP*], by which we can distinct microglia and macrophage as *mfap4+; ccl34b+* cells and *mfap4+; ccl34b–* cells, respectively at 5 dpf. We confirmed that almost all *mfap4+; ccl34b+* cells are located in the brain; however, a majority of *mfap4+; ccl34b–* cells are located outside the brain. These data suggest that only *mfap4*-expressing cells that enter the brain can differentiate into mature microglia. This is consistent with a classic, pioneer work indicating that early macrophages outside the brain never express the microglial marker, Apoe (Herbomel et al., 2001). Our findings provide *in vivo* evidence on how microglial precursors enter the brain and retina and then differentiate into mature microglia in zebrafish.

3.4 Lens fiber differentiation

The lens is an intraocular organ that focuses visual images on retinal photoreceptors. It consists of two cell types: lens epithelial cells and lens fiber cells. Lens epithelium convers the anterior half of the lens fiber core (Lovicu and McAvoy, 2005; Mochizuki and Masai, 2014). At the lens equator, epithelial cells start to differentiate into lens fiber cells, which elongate and pile up to cover the old lens fiber core. Thus, the lens provides a good model for studying spatiotemporal coordination of cell differentiation and morphogenesis. In vertebrate lens, FGF is secreted from the retina and is believed to form a low-to-high gradient along the anterior-posterior axis of the lens. It was reported that FGF promotes cell proliferation of lens epithelial cells at low doses and lens fiber differentiation at high doses (McAvoy et al., 2017). These observations gave rise to the “FGF gradient hypothesis”, in which FGF regulates

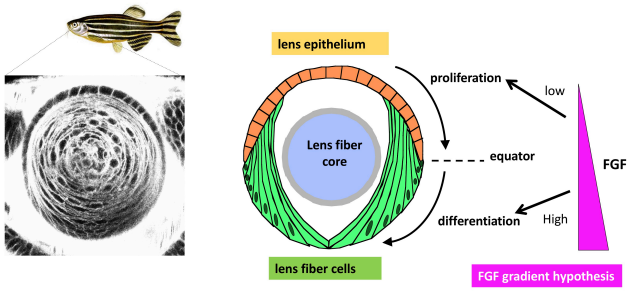

Figure 5. Mechanism of lens fiber differentiation

(Left) Zebrafish embryonic lens, which consists of two cell types: lens epithelial cells and lens fiber cells. (Right) Lens epithelial cells form a monolayer, which covers the anterior half of lens spherical lens fiber core. During development, lens epithelial cells proliferate and migrate toward the lens equator, where lens epithelial cells start to differentiate into lens fiber cells. Newly lens fiber cells elongate along the AP axis and cover the old lens fiber core, leading to growth of lens fiber core. It is generally accepted that FGF is secreted from the neural retina and form a gradient from low to high along the AP axis. Low dose of FGF promotes lens epithelial proliferation, whereas high dose of FGF promotes lens fiber differentiation.

multiple steps of lens fiber differentiation in a dose-dependent manner (Fig. 5). However, the mechanism that regulates lens epithelial proliferation and lens fiber differentiation is still not fully understood. FY2023, we investigated two research topics: (1) the identification of FGF ligands that regulate lens fiver differentiation in zebrafish, and (2) lens fiber morphogenesis in mice.

3.4.1 Identification of FGF ligands that regulate lens fiber differentiation in zebrafish

FGF signaling is unique, because more than 20 types of FGF ligands are identified in vertebrates (Ornitz and Itoh, 2015), which contribute to functional redundancy and robust developmental and morphogenetic processes (Leerberg et al., 2019). Although FGF is essential for lens development in zebrafish, it is still unknown what types of FGF ligands are involved in lens epithelial proliferation and lens fiber differentiation. To understand molecular mechanisms of lens fiber differentiation, it is necessary to identify FGFs that regulate lens development in zebrafish. We determined which FGF genes are expressed in zebrafish ocular tissue, using RT-PCR and *in situ* hybridization. Among the 27 FGF ligands, only Fgf3 and Fgf8a are predominantly expressed around developing lens in zebrafish. Fgf3 is expressed in the neural retina during lens placodal stage (from 15 somite stage to 18 hpf), whereas Fgf8a is expressed in differentiating RGCs adjacent to the lens equator after 36 hpf (Fig. 6A).

To determine the roles of Fgf3 and Fgf8a, we applied their morpholino antisense oligos. First, expression of an FGF target gene, *pea3*, in lens placode was suppressed in *fgf3* morphants, but not in *fgf8a* morphants. So, Fgf3 is the major FGF ligand that regulates lens placodal development. Indeed, both lens and retina become smaller in Fgf3 knockdown embryos (Fig. 6B). Fgf3 activates cell proliferation and cell survival in lens placodal cells through activation of ERK and AKT, respectively, whereas Fgf3 promotes retinal growth by activation of AKT for cell survival (Fig. 6D). Thus, Fgf3 regulates the growth rate of both lens and retina. On the other hand, after 36 hpf, *pea3* expression was suppressed in the lens equatorial region of *fgf8a* morphants, but not *fgf3* morphants, indicating that Fgf8s is the major FGF ligand that regulates equator-specific onset of lens fiber differentiation. Indeed, Fgf8a promotes lens fiber differentiation at the lens equator (Fig. 6C). Since Fgf8a is expressed in newly differentiating retinal ganglion cells, Fgf8a is likely to function as a mediator to couple retinal neurogenesis and lens fiber differentiation (Fig. 6E). In summary, Fgf3 and Fgf8a regulate lens development differently: Fgf3 secreted from the ventral retina regulates lens placodal cell proliferation in the early stages, whereas Fgf8a secreted from differentiating RGCs regulates lens fiber cell differentiation at the equator in the later stage. In addition, Fgf3 acts on the retina in an autocrine manner to promote retina growth through activation of AKT for cell survival, which ensures size matching between the retina and the lens. After 36 hpf, retinal neurogenesis generates RGCs, and

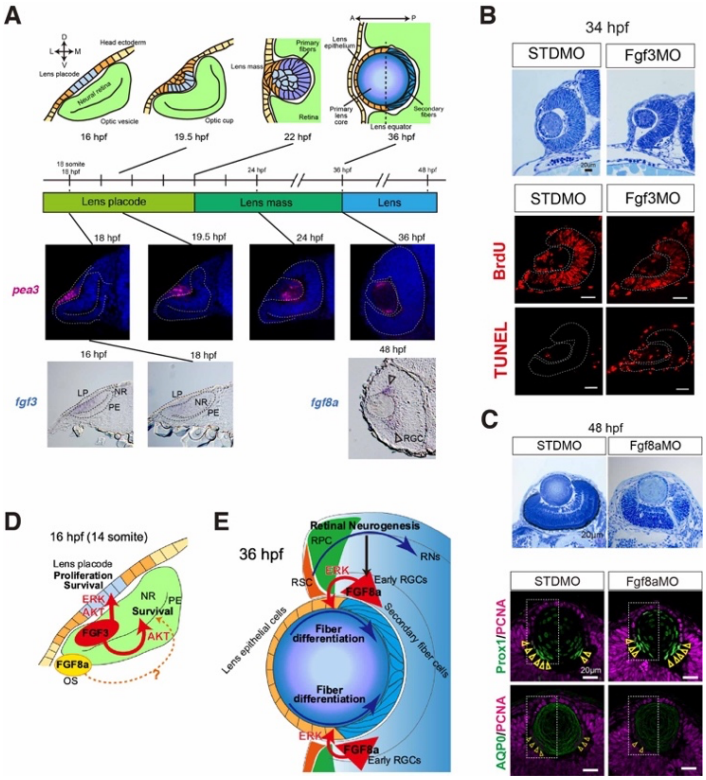

Figure 6. FGF3 and FGF8a differentially regulates lens development.

(A) Expression of a FGF signaling target, *pea3*, and two FGF ligands, *fgf3* and *fgf8a* in zebrafish eyes. (B) Morphology, TUNEL, and pH3 labeling of *fgf3* morphant eyes at 34 hpf. (C) Morphology and expression of lens fiber markers, Prox1 and AQP0, and a proliferative epithelial marker PCNA of *fgf8a* morphant eyes at 48 hpf. (D) Fgf3 roles in lens and retinal growth. (E) Fgf8a roles in lens fiber differentiation.

newly differentiating RGCs in turn become a new source of Fgf8a, which subsequently acts on lens epithelial cells to promote lens fiber differentiation. Thus, Fgf3 and Fgf8a coordinate both retina and lens development to ensure matching of organ size as well as differentiation rate between lens and retina during eye development.

3.4.2 FGF promotes lens fiber morphogenesis by regulating rho and rac activity

At the lens equator, lens epithelial cells start to differentiate into lens fiber cells in an FGF-dependent manner. Newly differentiating lens fiber cells become flattened and elongated along the AP axis by extending their apical and basal tips towards the anterior and posterior poles of the lens sphere, respectively, and are piled up to cover the old lens fiber core. One of the important questions is how FGF regulates morphogenesis of lens fiber cells during development. First, we note that there are three distinct zones in the developing mouse lens (Sugiyama et al., 2024). After lens epithelial cells differentiate into lens fiber cells at the equator, their apical-basal axis is perpendicular to the AP axis of the lens. Then, newly differentiating lens fiber cells undergo a 90^{o} axis turn by anchoring their apical tips at the lens equator fulcrum, which makes lens fiber shape concave. Following this “axis-turning zone”, lens fiber cells convert from a concave to a convex configuration to form “curve conversion zone”. After that, convex lens fiber cells highly elongate along the AP axis of the lens by extending their apical and basal tip toward the anterior and posterior poles of the lens. Newly elongating fibers cover the old lens fiber core and are piled up to form the “convex layer formation zone”.

Rho family GTPase are key regulators of actin dynamics and promote various cellular changes, including cell shape and migration. Next, using mice, we examined how Rho and Rac regulate lens fiber morphogenesis. Previously, the absence of Rac activity compromises the posterior migration of basal tip of lens fiber cells at or near the equator of mouse lens, leading to failure in conversion from a concave to a convex configuration of lens fiber cells (Maddala et al., 2011). As Rac signaling drives cell migration by promoting formation of membrane protrusions or lamellipodia at migration front, so we examined basal membranes of lens fiber cells. Consistently, posterior-directed basal membrane protrusion was only observed in lens fiber cells in “curve conversion zone”. Rac inhibition suppresses extension of basal membrane protrusions of lens fiber cells. Interestingly, FGF application showed a similar suppression of basal membrane protrusions, suggesting that FGF negatively regulates Rac-mediated basal membrane protrusion of lens fiber cells. However, Rac inhibition does not affect the initial elongation of lens fiber cells in the “axis-turning zone”. We found that Inhibitors of ROCK, myosin, and actin inhibit the initial elongation of lens fiber cells in the “axis-turning zone”, suggesting that RhoA promotes initial elongation of lens fiber cells in response to FGF activation. Thus, our findings revealed that FGF differentially regulates Rho and Rac activity in a step-wise manner, to achieve lens fiber morphogenesis.

3.5 Zebrafish behavioral mechanism: The role of Mecp2 in fear/anxiety regulation in zebrafish brain

Rett syndrome is an autism spectrum disorder and one of the most common causes of mental retardation in women. Sporadic mutations on the transcription factor MeCP2 have been found as one of the most frequent causes for Rett syndrome in humans, but how MeCP2 mutations cause Rett syndrome is still largely unknown (Kyle et al. 2018). In recent years, multiple models of Rett syndrome have been developed in mice and zebrafish (Pietri et al. 2013). Mouse models show affected social behavior, but so far there are no good descriptions of how MeCP2 mutations affect social behavior adult zebrafish. To document the impact of MeCP2 on behavior, we examined behavioral phenotypes in zebrafish MeCP2 mutants. Interestingly, MeCP2 mutant adult zebrafish showed increased anxiety and fear responses, as well as reduced thigmotaxis, reminiscent of the Rett syndrome phenotype, specifically high anxiety. On the other hand, they showed normal social preferences. Next, we examined whether zebrafish MeCP2 mutants harbor defects in brain development. Our imaging analysis revealed

that zebrafish MeCP2 mutants showed increased size in the DM region of the telencephalon, a region homologous to the amygdala in mammals and responsible for fear behaviors, compared with wild-type siblings. These data suggest that MeCP2 plays a crucial role in neural circuit formation responsible for fear response in telencephalon.

4. Publications

4.1 Journals

(1) Sugiyama, Y*, Reed, D. A, Herrmann, D., Lovicu, F. J., Robinson, M. L., Timpson, P. and Masai, I. (2024) Fibroblast Growth Factor-induced lens fiber cell elongation is driven by the stepwise activity of Rho and Rac. ***Development*** 151, dev.202123. DOI: 10.1242/dev.202123.

(2) Chiang, H.-J., Nishiwaki, Y., Chiang, W.-C., and Masai, I.* (2024) Male germ cell-associated kinase is required for axoneme formation during ciliogenesis in zebrafish photoreceptors. ***Dis Model Mech.*** dmm.050618. DOI: 10.1242/dmm.050618.

(3) O'Shaughnessy, L., Izawa, T., Masai, I., Shaevitz, J. W., and Stephens, G. J.* (2023) Dynamics of dominance: maneuvers, contests, and assessment in the posture-scale movements of interacting zebrafish BioRxiv DOI: 10.1101/2023.11.21.567896

4.2 Books and other one-time publications

Nothing to report

4.3 Oral and Poster Presentations

(Oral, International conference)

(1) Nishiwaki, Y. and Masai, I. PDE6c dysfunctions induce structural defects and degeneration of cone photoreceptors through chronic elevation of intracellular Ca^{2+} concentration. 12th European Zebrafish Meeting (EZM 2023), 9-13 July 2023, Krakow, Poland.

(2) Ravishankar, D. Takeuchi, Y. and Masai, M. Roles for microglia in chronic photoreceptor degeneration and associated regeneration caused by *pde6c* dysfunction in zebrafish. 12th European Zebrafish Meeting (EZM 2023), 9-13 July 2023, Krakow, Poland.

(3) Chiang, W.-C., Takeuchi, Y., Nishiwaki, Y. and Masai, I. The role of ER membrane protein complex in ER proteostasis and proteotoxicity in retinal degeneration. The 29th East Asia Joint Symposium (EAJS), 24-27 October 2023, Cheonan, Korea

(4) Masai, I. Identification of FGF ligands that differentially control lens growth and lens fiber differentiation in zebrafish. The international conference of the lens 2023 (ICL2023), 10-15 Dec 2023 Kona, Hawaii.

(Poster, international conferences)

(1) Hu, D, Nishiwaki, Y. and Masai, I. Investigation of the role of Nectin1a and Nectin3b in formation of zebrafish cone mosaic pattern. ARVO2023 (Annual meeting of the Association for Research in Vision and Ophthalmology 2023), 21-27 April 2023, New Orleans, USA.

(Invited talks)

(1) Masai, I. Mechanism underlying microglial colonization of the retina and their role in neural regeneration. Vision Science Forum 2023, 26-27 October 2023, OIST, Japan
(2) Rodriguez, L. C. Zebrafish MeCP2 mutation produce behavioral alterations reminiscent of Rett Syndrome. OIST RIKEN Brain Symposium, 21st –23rd, August 2023. z

(Oral, domestic conferences)

(1) Rodriguez, L. C., Izawa, T. and Masai, I. Zebrafish MeCP2 mutation produce behavioral alterations reminiscent of Rett Syndrome. The 56th Annual meeting of Japanese Society of Developmental Biologists, 22-25 July 2023, Sendai, Japan.
(2) Rodriguez, L. C., Izawa, T. and Masai, I. Zebrafish MeCP2 mutation produce behavioral alterations reminiscent of Rett Syndrome. The 46th Annual meeting of Japanese Society of Neuroscience, 1-4 August 2023, Sendai, Japan.
(3) Chiang, W.-C., Takeuchi, Y., Nishiwaki, Y. and Masai, I. The role of ER membrane protein complex in ER proteostasis and proteotoxicity in retinal degeneration. The 46th Annual meeting of Japanese Molecular Biology (MBSJ2023), 6-8 December 2023, Kobe, Japan.

(Poster, domestic conferences)

(1) Nishiwaki, Y. and Masai, I. PDE6c dysfunctions induce structural defects and degeneration of cone photoreceptors through chronic elevation of intracellular Ca^{2+} concentration. The 46th Annual meeting of Japanese Molecular Biology (MBSJ2023), 6-8 December 2023, Kobe, Japan.
(2) Takeuchi, Y. and Masai, I. Two distinct FGFs mediate coordination of stepwise developmental processes in zebrafish lens and retina. The 46th Annual meeting of Japanese Molecular Biology (MBSJ2023), 6-8 December 2023, Kobe, Japan.

5. Intellectual Property Rights and Other Specific Achievements

5.1 The Category or Type of Funding, like External Funding, Awards, etc.
Info on each item
Funding

KAKENHI (grants from the Ministry of Education, Science and Sport/JSPS)
PI name: Yuko Nishiwaki
Title: Mechanism of Drp1-mediated formation of endoplasmic reticulum-mitochondria contact site and its role in apoptosis induction
Category: Grant-in-Aid for Scientific Research (C)
Period: FY2021-FY2023

PI name: Wei-Chieh Chiang
Title: The mechanism of endoplasmic reticulum proteostasis and proteotoxicity in retinal degeneration.
Category: Grant-in-Aid for Scientific Research (C)

Period: FY2020-FY2023

PI name: Yuki Takeuchi
Title: Identification of FGF ligands involved in the regulation of lens fiber differentiation using zebrafish model.
Category: Grant-in-Aid for Scientific Research (C)
Period: FY2022-FY2024

PI name: Luis Carretero
Title: Role of amygdala in fear and anxiety behaviors.
Category: Grant-in-Aid for Scientific Research (C)
Period: FY2023-FY2025

PI name: Darshini Ravishankar
Title: Temporal profiling of photoreceptor degeneration in zebrafish PDE6c mutants and a potential role of microglia
Category: JSPS DC2
Period: FY2022-FY2023

6. Meetings and Events

([NOTE] You can include the following in "6. Meetings and Events":

6.1 Seminar

None

6.2 OIST course

- Title: Developing Neural Circuit Course 2023
- Co-organizer: Yoko Yazaki-Sugiyama (OIST), Sam Reiter (OIST), David L. van Vactor (Harvard Medical School), Shoi Shi (Tsukuba Univ)
- Dates: 11 – 23 July 2023
- Place: OIST Main Campus
- Lecturers: Tom Baden (Univ. Sussex), Mike Crickmore (Harvard Univ.), Dragana Rogulja (Harvard Univ.), Orkun Akin (UCLA), Shen-ju Chou (Academia Sinica), Michael Yartsev (UC Berkeley), Fumi Kubo (NIG), Todd Roberts (UT Southwestern), Yutaka Yoshida (OIST), Yukiko Goda (OIST), Takeshi Sakurai (IIIS, Tsukuba Univ.), Claire Wyart (Inst. Du Caerveau), Aakanksha Singhvi (Fred Hutchinson Cancer), Yukiko Goda (OIST), Paul Garrity (Brandeis Univ.)
- Tutors: Sarah Morson (OIST), Joanna Komorowska-Mueller (OIST), Yuichi Morohashi (OIST), Luis Carretero (OIST), Yuki Takeuchi (OIST), Yuko Nishiwaki (OIST), Makoto Hiroi (OIST), Olivier Fernandez (OIST), Haruna Fujioka (Tsukuba Univ), Chika Shimizu (Tsukuba Univ)

7. Other

Nothing to report.