Development and Degeneration of Retinal Ganglion Cell Axons in *Xenopus tropicalis*

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Neurons make long-distance connections via their axons, and the accuracy and stability of these connections are crucial for brain function. Research using various animal models showed that the molecular and cellular mechanisms underlying the assembly and maintenance of neuronal circuitry are highly conserved in vertebrates. Therefore, to gain a deeper understanding of brain development and maintenance, an efficient vertebrate model is required, where the axons of a defined neuronal cell type can be genetically manipulated and selectively visualized in vivo. Placental mammals pose an experimental challenge, as time-consuming breeding of genetically modified animals is required due to their intrauterine development. *Xenopus laevis*, the most commonly used amphibian model, offers comparative advantages, since their embryos ex utero during which embryological manipulations can be performed. However, the tetraploidy of the *X. laevis* genome makes them not ideal for genetic studies. Here, we use *Xenopus tropicalis*, a diploid amphibian species, to visualize axonal pathfinding and degeneration of a single central nervous system neuronal cell type, the retinal ganglion cell (RGC). First, we show that RGC axons follow the developmental trajectory previously described in *X. laevis* with a slightly different timeline. Second, we demonstrate that co-electroporation of DNA and/or oligonucleotides enables the visualization of gene function-altered RGC axons in an intact brain. Finally, using this method, we show that the axon-autonomous, Sarm1-dependent axon destruction program operates in *X. tropicalis*. Taken together, the present study demonstrates that the visual system of *X. tropicalis* is a highly efficient model to identify new molecular mechanisms underlying axon guidance and survival.

Keywords: axon degeneration, axon guidance, development, *Xenopus tropicalis*

INTRODUCTION

The visual system has been a model for many discoveries of the molecular and cellular mechanisms underlying axon guidance (Varadarajan and Huberman, 2018). Retinal ganglion cells (RGCs) are the only projection neurons of the eye, which convey visual information processed in the retina to the visual centers of the brain. RGCs form the inner most layer of the retina, and their axons collect at the optic nerve head (also known as the optic disc) before exiting the eye. Outside the eye, RGC axons form the optic nerve, which enters the brain at the ventral midline of the diencephalon, where the two optic nerves originating from the opposite eyes cross and form the optic chiasm. In vertebrates, most RGC axons terminate at the two visual centers of the brain, the dorsal lateral geniculate nucleus of the thalamus, and the superior colliculus (also known as the optic tectum) of the midbrain. The retinotectal pathway, the collection of RGC axons terminating at the optic tectum, has been used as a key model to study axon guidance (Triplett, 2014), and its development in *Xen-
opus laevis, the classic amphibian model, has been described in detail with a particular focus on the temporal relationship between the growth of RGC axons and that of the rest of the body (Erdogan et al., 2016).

All but one species of the genus Xenopus are polypoid, and the X. laevis belongs to the tetraploid class, hence making it difficult to apply the modern genetic and genomic techniques (Graf and Kobel, 1991). Xenopus tropicalis is the only diploid species in the genus Xenopus and was recently adopted for research in developmental genetics and genomics (Hirsch et al., 2002). X. tropicalis shares the experimental advantages of X. laevis, such as the use of classical embryological techniques and the similarity of gross morphology and development to X. laevis, suggesting that the knowledge obtained in the studies using X. laevis might be applied to X. tropicalis (Kakebeen and Wills, 2019). However, whether this assumption holds true in the development of the retinotectal pathway has not been tested.

In the present study, we show the developmental trajectory of RGC axons in X. tropicalis. We use two techniques, a DiI-based labeling strategy to visualize all RGC axons navigating the brain, and an electroporation-based strategy to label a few RGC axons in mosaic while simultaneously altering the expression of a specific gene in labeled RGCs. Using these strategies, we report the detailed trajectory of RGC axon growth and guidance at specific developmental stages. Secondly, we present a detailed procedure of the electroporation technique designed specifically for X. tropicalis for sparse labeling and visualization of RGC axons in vivo. Finally, we use this technique to demonstrate that the axon-autonomous effect of a gene can be efficiently assessed using this system. Specifically, we use Wallerian degeneration, the stereotyped deterioration of a severed axonal fragment (Coleman and Hoke, 2020), as a model and demonstrate that Wlds gain-of-function or Sarm1 loss-of-function delays Wallerian degeneration in an axon-autonomous manner in X. tropicalis. Taken

![Fig. 1. Development of the retinotectal pathway in X. tropicalis.](image-url)
together, our study provides a useful framework for future studies to search for new molecular and cellular mechanisms underlying axon guidance and survival, using the embryologically accessible and genetics-friendly model of the retinal axons of X. tropicalis.

**MATERIALS AND METHODS**

**Xenopus experiments**

X. tropicalis embryos were generated by in vitro fertilization, raised in 0.1× modified Barth’s saline (MBS) (8.8 mM sodium chloride, 100 μM potassium chloride, 100 μM magnesium sulfate, 500 μM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 250 μM sodium bicarbonate, and 1 mM calcium chloride) at 21°C-24°C, and staged according to the tables provided in *Nieuwkoop and Faber* (1967) and Xenbase (http://www.xenbase.org/). All experiments complied with the protocols approved by the Yonsei University College of Medicine Institutional Animal Care and Use Committees (No. 2019-0039).

**Plasmids and morpholino oligonucleotides**

pCMV-tag2B FLAG-WldS was kindly gifted by Professor Michael Coleman (University of Cambridge). Sarm1 antisense morpholino (Sarm1 MO), 5’-GAAGAGTGAGAACCATGAATCCTTC-3’; and control MO, 5’-ATGGTTTCCAATCTCTCCATCCA-3’ conjugated to carboxyfluorescein at the 3’ end were purchased from Gene-Tools (USA).

**Dil labeling**

1,1’-Diocadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate (DiI; Sigma, USA) crystals were dissolved in chloroform. Embryos were fixed in 4% paraformaldehyde in 1× phosphate buffered saline (PBS) for 2 h at room temperature and immobilized in PBS on a dish containing solidified Sylgard (Sigma). The lens of one eye was removed, and Dil solution was filled into the cavity as shown in Fig. 1D. Dil was allowed to diffuse for approximately 16 h in a humidifying chamber to label RGC axons, after which the brain was dissected out and processed for imaging.

**Electroporation**

Electroporation was performed based on the method developed for X. laevis (Falk et al., 2007), with modifications to account for a smaller size of X. tropicalis embryos. Briefly, stage 27-28 embryos were anesthetized with ethyl 3-amino-benzoate methanesulfonate (MS222) in 1× MBS (300 μg/ml). Tadpoles were placed on a custom-made electroporation chamber that was designed to fit an X. tropicalis embryo, as shown in Fig. 2A, and two platinum electrodes were placed at the ends of the short axis of the chamber so that the electrical field is placed across the two eyes. Glass pipettes were prepared by pulling heated glass capillary tubes (1.0 mm × 0.78 mm, Harvard apparatus) using a micropipette puller (Sutter P-97). DNA and/or morpholino were dissolved in water to 1-2 μg/ml in total. Approximately 10 nl of solution was microinjected into the ventricular cavity of the optic vesicle near the cathode. The glass pipette was immediately pulled out of the optic vesicle and 1× MBS, after which rectangular

![Image](image_url)
pulses were delivered using an electroporator (Intracell TSS20 Ovodyne). Typically, eight consecutive 18 V, 50 ms steps were applied in 1-s intervals. The electroporated embryos were transferred to 0.1× MBS and raised at 21°C-24°C.

Axotomy
After unilateral retinal electroporation of enhanced green fluorescent protein (EGFP)-encoding plasmid, the stage 45 embryos were anesthetized with MS222 in 0.1× MBS, and the electroporated eye was removed. The embryos were transferred to 0.1× MBS and raised at 21°C-24°C for 1-3 days.

Immunofluorescence and cell counting
Fixed embryos were saturated with 30% sucrose in 1× PBS, cryo-sectioned in the coronal plane at the 12-μm thickness, and mounted on a slide glass. Antigen retrieval was performed in 10 mM sodium citrate with 0.05% Tween-20 (pH 6.0) at 95°C for 20 min. The slides were then blocked in 5% normal donkey serum in 1× PBS-T for 30 min at room temperature and transferred to the blocking solution containing a rabbit anti-acetylated alpha tubulin antibody (1:300, ab125356; Abcam, UK). After incubating at 4°C for 16 h, the slides were washed three times with PBS-T, and the secondary antibody solution with an anti-rabbit IgG antibody conjugated to Alexa Fluor 555 (A-31572; Sigma) in PBS-T (1:1,000) was added. After incubating at room temperature for 1 h, the slides were washed three times with PBS-T, and nuclei were counterstained with Hoechst 33342 (Sigma) dissolved in 1× PBS (1:20,000). The slides were mounted in FluorSave (Sigma) with a cover glass. Five embryos were randomly chosen per group, and three coronal tissue sections around the longest axis of the eye were selected per embryo for cell counting in Fig. 3.

Imaging
For live imaging of in vivo RGC axons, unilaterally electroporated embryos were anesthetized in 300 μg/ml MS222 in 1× MBS, and the contralateral (un-electroporated) eye, skin, and meninges were removed to expose the contralateral optic tectum. The embryos were then immobilized on a glass-bottom dish in 1.5% low melting agarose dissolved in 300 μg/ml MS222 in 1× MBS. The dish was then filled with the same solution without agarose, and images were taken at 5-min intervals under an epifluorescence microscope (Eclipse Ti2; Nikon, Japan) equipped with a 20× objective. To image the retina and RGC axons before crossing the midline, the electroporated embryos were fixed in 4% paraformaldehyde dissolved in 1× PBS and cryo-sectioned in the coronal plane. To image RGC axons in the contralateral brain hemisphere, the brains were dissected out of the fixed embryos, the ventral midline was cut open, and the brain was flat-mounted on a slide glass containing 1× PBS with the superior side facing the cover glass, as shown in Fig. 1E. These preparations were imaged under a laser-scanning confocal microscope (LSM 700: Carl Zeiss, Germany) equipped with a 63× C-Apochromat (numerical aperture 1.2) objective.

Software
Images were analyzed using ImageJ (National Institute of Health, USA). Statistical analyses were performed using R.

RESULTS
Development of the retinotectal pathway in X. tropicalis
To systematically assess the developmental stage-dependent growth and pathfinding of RGC axons in vivo, we performed a series of time-course experiments. RGC axons exit the eye at the optic nerve head, form a bundle of the optic nerve, and enter the brain at the optic chiasm. In amphibian tadpoles and fish, which lack binocular vision, all RGC axons cross the midline, in contrast to mammals where the temporal RGCs project ipsilaterally. Upon entering the brain, RGC axons travel as a bundle near the superficial layer of the brain forming the optic tract. The RGC axons stay within the optic tract until entering the optic tectum, where they defasciculate and form topographically organized synapses with tectal neurons (Figs. 1A and 1B).

To map the temporal pattern of RGC axon development, we fixed the embryos at different developmental stages (Fig. 1C) and labeled the right retina with Dil (Fig. 1D). The RGC axons originating from the Dil-labeled eye and growing in or terminating at the contralateral (left) brain hemisphere were imaged in a “open-book preparation” (Fig. 1E). We found that the earliest born RGC axons cross the midline prior to stage 34, reach the tectum around stage 38, and enter and arborize in the tectum around stage 41 (Fig. 1F). Up until this stage, late-born RGC axons continue to follow, and the terminal arborization becomes more complex by stage 45. Based on these findings, we found that X. tropicalis RGC axon development occurs one or two stages behind when compared to X. laevis, and present an updated temporal map of retinotectal pathway development of X. tropicalis (Fig. 1G).

Mosaic labeling of retinal axons using unilateral electroporation
Next, we developed an electroporation method to RGC axons in mosaic. We modified the method developed for a much larger X. laevis embryo and built the electroporation chamber designed to fit a X. tropicalis embryo by making a cross-shaped indentation in a Sylgard dish, in the way that the electrical field can be applied across the two eyes (Fig. 2A). A stage 27 embryo was positioned in the long axis of the indentation on the ventral side up, so that the head and the eyes can be positioned between two electrodes in the short axis of the indentation (Fig. 2A). At this stage, the retina is in the shape of the optic vesicle, an evagination of the neural tube mainly comprised of neural stem cells, of which RGCs are the first-born cells. We injected several nanoliters of solution containing mCherry-encoding plasmids into the ventricle of the optic vesicle facing the cathode, so that the DNA solution diffuses from the center of the optic vesicle. Immediately following the injection, rectangular voltage pulses were delivered so that the negatively charged DNA or fluorescein-tagged morpholino (which by itself is not charged) moves towards the lateral neuroepithelial layer of the optic vesicle facing the cathode.

First, we confirmed that this method targets DNA into the...
retina, by imaging the whole embryo (Fig. 2B) or the electroporated retina in tissue sections (Figs. 2C and 2D). mCherry expression was limited to the retina and not present the ipsilateral brain, indicating that this protocol selectively labels retinal neuroepithelium (Fig. 2C). Typically, 20%-50% of the retinal cells were targeted slightly biased toward the dorsal retina since the ventral retina forms later as the result of the continued evagination of the neuroepithelium after stage 27 (Holt, 1980), when the electroporation was performed. Using this labeling method, we visualized the axons of electroporated RGCs in the contralateral brain at different developmental stages, with the temporal map constructed in Fig. 1G in mind. We found that the RGC axons labeled in this way were several hours behind the earliest born RGC axons, which we visualized by DiI method (Figs. 2C-2H). For example, we could not see the electroporated RGC axons in the contralateral optic tract in stage 36 (Fig. 2E), whereas DiI-labeled RGC axons reached the diencephalon-mesencephalon border at this stage (Fig. 1F). Likewise, the electroporated RGC axons that crossed the midline could be visualized only at stage 38 (Fig. 2F), when the Dil-labeled axons already reached the optic tectum (Fig. 1F). This delay is inherent to the electroporation method, as it labels RGCs born at or after stage 27, which is after the birth of the first RGCs around stage 24. However, the developmental trajectory of the electroporated RGC axons were consistent and was of little difference to that of Dil-labeled axons at stage 41 or later, since RGC axons slow down prior to entering the optic tectum (Figs. 2G and 2H).

One advantage of the electroporation over the Dil-based method is that RGC axons are sparsely labeled. Sparse labeling is suitable for imaging individual axons, as evident in clear visualization of the growth cones, the transient structure at the tip of a growing axon, in the optic tract (Fig. 2F, inset), and the terminal axon branches at the optic tectum (Fig. 2G, inset [arrowhead]), which could not be appreciated when all axons were labeled by Dil (Fig. 1F). Second advantage is that it allows live imaging of RGC axons in an intact brain. We visualized the growth of a single RGC axon in vivo to see the...
rapid growth of a growth cone-tipped axon from the optic chiasm to the optic tectum (Figs. 2I and 2F, arrow), which took place in approximately 6 h, pausing of the growth cone at the tectal entry site, and breaking down of the growth cone and subsequent axon branching at the tectum (Fig. 2J, arrowhead) that occurs over days (Fig. 2I, Supplementary Movie S1). Therefore, the electroporation method described here can be used to label RGCs in mosaic and to visualize their axons growing in the contralateral brain.

**Co-electroporation strategy of gain- and loss-of-function approaches**

The final, and perhaps the most important, advantage of the electroporation over the Dil method is that gene expression of the RGC whose axons will be visualized can be selectively manipulated without altering the rest of the body by co-electroporating gene expression or function-altering reagent along with an axon tracer (Fig. 3A). For example, gain- or loss-of-function studies can be performed by co-electroporating a protein-coding plasmid or an anti-sense oligonucleotide with an mCherry-encoding plasmid as a tracer (Fig. 3B). Implicit in this approach is that mCherry-expressing axons always contain the co-electroporated molecules. We assessed this possibility by measuring the co-electroporation efficiency of mCherry and EGFP plasmids or mCherry and fluorescein-tagged antisense morpholino (MO), synthetic nucleotides that inhibit the translation of target mRNAs by steric hindrance of ribosome binding. After co-electroporation of a tracer plasmid (mCherry) together with a gain-of-function plasmid (EGFP) or a loss-of-function oligonucleotide (MO-fluorescein) at stage 27, we visualized the progeny of electroporated cells (i.e., mCherry-positive cells) in the retina at stage 37/38. We asked how many of mCherry-positive cells co-inherit EGFP or MO-fluorescein, by counting green and/or red cells in retinal sections. We found that over 99% and 84% of mCherry-positive cells were positive for EGFP (Figs. 3C and 3D) and MO-fluorescein (Figs. 3E and 3F), respectively. As fluorescence of the MO-fluorescein molecules does not amplify in contrast to the fluorescence originating from the EGFP-encoding plasmid, our co-electroporation efficacy of DNA-MO is likely to be an understimation. Therefore, imaging mCherry-positive axons in the contralateral optic tectum after co-electroporation gives a reasonably high chance of imaging retinal axons, in which the function and/or expression of a specific gene is altered, in an otherwise wild-type brain.

**Wlds-protectable and Sarm1-dependent axon destruction program operates in X. tropicalis**

Wallerian degeneration is a sequence of stereotypical events leading to disintegration of the distal axonal fragment separated from the cell body (Coleman and Hoke, 2020). Recently, an exciting series of research demonstrated that Wallerian degeneration proceeds as a result of the active axon destruction program, whose components converge on the biochemical pathway that generates and consumes NAD⁺. For example, Nmnat2, an enzyme responsible for the rate-limiting step in the NAD⁺ biogenesis, is required for axon survival, and its gain-of-function by Wlds protects severed axons from Wallerian degeneration for weeks, a naturally occurring NMNAT mutant protein in mouse, which re-routes its enzymatic activity to ectopic subcellular localizations (Mack et al., 2001). Surprisingly, Wlds delays Wallerian degeneration even in fly, suggesting not only that an evolutionarily conserved mechanism regulates a programmed destruction of axonal fragments, but also that a forward genetic screening strategy can be applied to discover new genes that regulate axon destruction. In this approach, dSarm (Drosophila homolog of mammalian Sarm1), the gene with the exact opposite effect, was identified, and the loss-of-function in Sarm1 delayed Wallerian degeneration both in mouse and fly (Osterloh et al., 2012). Intriguingly, Sarm1 displayed an unexpected NADase activity that directly counteracts Nmnat2, positioning the NAD⁺ metabolism at the center of the axon destruction program. However, it remains unclear how the NAD⁺ metabolism regulates axon survival and destruction, and it is necessary to identify new genes and pathways that participate in the axon destruction program.

Based on the results of our co-electroporation study, we reasoned that the visual system of X. tropicalis is a good system to search for such genes, as the axon-autonomous effect of a gene can be directly visualized in vivo by co-electroporation. As a proof-of-concept, we constructed RGC-specific Wlds gain-of-function and Sarm1 loss-of-function models. We first established the time-course of Wallerian degeneration of wild-type retinal axons. After electroporation of EGFP-encoding plasmid at stage 27, the electroporated retina was separated from the optic nerve at stage 45 (Fig. 4A), when the most RGC axons form stably synapses with tectal neurons (Fig. 4B), and visualized distal axonal fragments following axotomy (Figs. 4C-4F). We could visualize highly reproducible morphological features of Wallerian degeneration in vivo, and fragmented axons were clearly visible in 24 h post-axotomy (Fig. 4D). Most axonal fragments were cleared in 48 h post-axotomy (Fig. 4E), and no traces of axons were visible in 72 h (Fig. 4F). The beaded morphology of degenerating axons, a key feature of Wallerian degeneration, became most evident in 48 h post-axotomy (Figs. 4G and 4H), and we used this time point for the remainder of our experiments.

Then, we co-electroporated a Wlds-encoding plasmid or a Sarm1 antisense MO with EGFP-encoding plasmid (as an axon tracer) and asked whether these manipulations protect axons from Wallerian degeneration. We imaged severed axons 48 h post-axotomy, when most wild-type axons degenerate (Figs. 5A and 5B). Strikingly, co-electroporating the Wlds-encoding plasmid prevented Wallerian degeneration in all animals tested (Figs. 5C and 5D), indicating that Wlds gain-of-function suppresses the axon destruction program in Xenopus. Sarm1 loss-of-function also protected axons, although we did not observe the protective effect in approximately 20% of the cases (Figs. 5E and 5F). Since MO electroporation-based loss-of-function approach inhibits the synthesis of Sarm1, without removing pre-excising Sarm1 proteins, the relatively weaker effect of the Sarm1 MO compared with the Wlds-plasmid may be ascribed to the function of Sarm1 proteins synthesized before MO delivery. It also might have resulted from a relatively lower (~84%) co-electroporation efficacy of DNA-MO compared to that of DNA-DNA co-electroporation (~99%) (Figs. 3D and 3F). Nonetheless, com-
pared to the wild-type axons, none of which survived axotomy, Sarm1 MO electroporation delayed Wallerian degeneration in nearly 80% of cases. Taken together, our results show that the axon destruction program operates in Xenopus, as described in mouse and fly, and suggest that the selective visualization of gene function-altered axons in wild-type brain is a promising strategy to search for new genes involved in the axon destruction program.

DISCUSSION

The present study demonstrates the first detailed temporal map of retinotectal pathway development in X. tropicalis. Using this map, we developed a co-electroporation-based approach for mosaic visualization of retinal axons, in which the function of a specific gene can be manipulated, in a wild-type embryo. We also map the temporal pattern of retinal axon growth and arborization labeled in this way and show,
as a proof of concept, that the axon destruction pathway can be suppressed by Wlds gain-of-function and Sarm1 loss-of-function in an axon-autonomous manner.

The retinotectal pathway in *X. laevis* has been an invaluable model to study the molecular and cellular mechanisms underlying axon guidance (van Horck et al., 2004), topographic map formation (Mann et al., 2002) and axon survival (Yoon et al., 2012). However, the allotetraploid genome of *X. laevis* poses challenges in applying genetic approaches, and the diploid *X. tropicalis* has slowly been adopted to overcome this limitation (Kakebeen and Wills, 2019). Most genes in the *X. laevis* genome contain two homeologs, and both genes must be targeted, for example, by using a mixture of two guide RNAs in the Crispr/Cas9 system, to obtain a knockout embryo (Wang et al., 2015), whereas a single gene targeting can be used in *X. tropicalis* (Guo et al., 2014). The developmental processes of the retinotectal pathway in *X. tropicalis* and the experimental strategies to specifically visualize electroporated RGC axons in vivo described in the present study make it possible to utilize both classic knowledge gained in the studies of *X. laevis* and the advantage of the modern *X. tropicalis* model.

Recent studies have clearly shown that axon degeneration is run by the active axon destruction pathway, which is conceptually similar to but molecularly different from the programed cell death. The genes that promote or inhibit the axon destruction program has been identified, key molecules being Sarm1 and Nmnat2, respectively (Coleman and Hoke, 2020). Additional players of this pathway are being actively searched for, although the progress is not as rapid as hoped.

One potential difficulty might be gene redundancy of vertebrates, which may cause loss-of-function-based screenings fail to find a phenotype-altering gene. For example, *Drosophila axundead* (axed) is required for Wallerian degeneration to proceed downstream of Sarm1, suggesting that it is an executor of axon degeneration after injury (Neukomm et al., 2017) that is inhibited during developmental axon branching (Izadifar et al., 2021). However, it has not been determined whether this also operates in vertebrates, mainly since there are four putative vertebrate paralogs. Unlike genetic approaches that involve generation of quadruple knockout animals, the electroporation-based mosaic approach could provide a potentially efficient tool to simultaneously knock-out or knock-down multiple genes and examine the loss-of-function phenotype in F0 generation. Likewise, genetic screenings to search for the genes regulating axon guidance could also be performed efficiently, as a library of guide RNAs for Crispr/Cas9 can also electroporated into one eye, and the embryos with axon pathfinding defects can be screened in F0 generation, by comparing their axonal trajectories with the reference map presented here. Together, the information and techniques presented in this study provide a useful reference for future studies of axon development and degeneration.

**Note:** Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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AUTHOR CONTRIBUTIONS
B.C., H.K., J.J., and S.P. performed experiments. H.J. wrote the manuscript with the help of all authors.

CONFLICT OF INTEREST
The authors have no potential conflicts of interest to disclose.

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REFERENCES
Coleman, M.P. and Hoke, A. (2020). Programmed axon degeneration: from mouse to mechanism to medicine. Nat. Rev. Neurosci. 21, 183-196.
Erdogan, B., Ebbert, P.T., and Lowery, L.A. (2016). Using Xenopus laevis retinal and spinal neurons to study mechanisms of axon guidance in vivo and in vitro. Semin. Cell Dev. Biol. 51, 64-72.
Falk, J., Drinjakovic, J., Leung, K.M., Dwivedy, A., Regan, A.G., Piper, M., and Holt, C.E. (2007). Electroporation of cDNA/Morpholinos to targeted areas of embryonic CNS in Xenopus. BMC Dev. Biol. 7, 107.
Graf, J.D. and Kobel, H.R. (1991). Genetics of Xenopus laevis. Methods Cell Biol. 36, 19-34.
Guo, X., Zhang, T., Hu, Z., Zhang, Y., Shi, Z., Wang, Q., Cui, Y., Wang, F., Zhao, H., and Chen, Y. (2014). Efficient RNA/Cas9-mediated genome editing in Xenopus tropicalis. Development 141, 707-714.
Hirsch, N., Zimmerman, L.B., and Grainger, R.M. (2002). Xenopus, the next generation: X tropicalis genetics and genomics. Dev. Dyn. 225, 422-433.
Holt, C. (1980). Cell movements in Xenopus eye development. Nature 287, 850-852.
Izadifar, A., Courchet, J., Virga, D.M., Verreet, T., Hamilton, S., Ayaz, D., Misbaer, A., Vandenbogaerde, S., Monteiro, L., Petrovic, M., et al. (2021). Axon morphogenesis and maintenance require an evolutionary conserved safeguard function of Wnk kinases antagonizing Sarm and Axed. Neuron 109, 2864-2883.e8.
Kakebeen, A. and Wills, A. (2019). Advancing genetic and genomic technologies deepen the pool for discovery in Xenopus tropicalis. Dev. Dyn. 248, 620-625.
Mack, T.G., Reiner, M., Beirowski, B., Mi, W., Emanuelli, M., Wagner, D., Thomson, D., Gillingwater, T., Court, F., Conforti, L., et al. (2001). Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/ Nmnat chimeric gene. Nat. Neurosci. 4, 1199-1206.
Mann, F., Ray, S., Harris, W., and Holt, C. (2002). Topographic mapping in dorsoventral axis of the Xenopus retinotectal system depends on signaling through ephrin-B ligands. Neuron 35, 461-473.
Neukomm, L.J., Burdett, T.C., Seeds, A.M., Hampel, S., Coutinho-Budd, J.C., Farley, J.E., Wong, J., Karadeniz, Y.B., Osterloh, J.M., Sheehan, A.E., et al. (2017). Axon death pathways converge on Axundead to promote functional and structural axon disassembly. Neuron 95, 78-91.e5.
Nieuwkoop, P.D. and Faber, J. (1967). Normal Table of Xenopus Laevis (Daudin): A Systematical and Chronological Survey of the Development from the Fertilized Egg Till the End of Metamorphosis (2nd Edition) (Amsterdam: North-Holland Publishing Company).
Osterloh, J.M., Yang, J., Rooney, T.M., Fox, A.N., Adalbert, R., Powell, E.H., Sheehan, A.E., Avery, M.A., Hackett, R., Logan, M.A., et al. (2012). dSarm/ Sarm1 is required for activation of an injury-induced axon death pathway. Science 337, 481-484.
Triplett, J.W. (2014). Molecular guidance of retinotopic map development in the midbrain. Curr. Opin. Neurobiol. 24, 7-12.
vAn Horck, F.P., Weini, C., and Holt, C.E. (2004). Retinal axon guidance: novel mechanisms for steering. Curr. Opin. Neurobiol. 14, 61-66.
Varadarajan, S.G. and Huberman, A.D. (2018). Assembly and repair of eye-to-brain connections. Curr. Opin. Neurobiol. 53, 198-209.
Wang, F., Shi, Z., Cui, Y., Guo, X., Shi, Y.B., and Chen, Y. (2015). Targeted gene disruption in Xenopus laevis using CRISPR/Cas9. Cell Biosci. 5, 15.
Yoon, B.C., Jung, H., Dwivedy, A., O’Hare, C.M., Zivraj, K.H., and Holt, C.E. (2012). Local translation of extranuclear lamin B promotes axon maintenance. Cell 148, 752-764.