The Expression Pattern of the Xenopus laevis Rod-derived Cone Viability Factor1 (RdCVF1)

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Introduction

Background

In the United States alone, more than one million people are blind (1). More than 3.4 million people age forty and over are visually impaired, and over 11 million individuals age 12 and older suffer from visual impairments (2). More than 14 million people in the U.S. and 135 million worldwide have low vision (3).

The leading causes of these sight-threatening diseases are Diabetic Retinopathy, Age-related Muscular Degeneration (AMD), Retinitis Pigmentosa and Glaucoma (4). Diabetic Retinopathy is caused by damage of blood vessels of the retina and is the leading cause of blindness in working age Americans, affecting more than 5.3 million Americans above the age of 18 (4). Age- related Muscular Degeneration is a condition that primarily affects the part of the retina responsible for sharp central vision. AMD is the most common cause of legal blindness and vision impairments in older Americans, plaguing about 1.6 million Americans over the age of 60 (4). Glaucoma is a chronic condition marked by gradual damage to the optic nerve that carries visual information from the eye to the brain (4). Retinitis Pigmentosa results from the death of photoreceptor cells, which leads to the decline of vision (4). All of these diseases result from the death of one or more retinal cell type. Currently, there is no cure for blinding diseases caused by retina cell death.
The Vertebrate Retina

The vertebrate retina is the light sensitive tissue lining the inner surface of the eye. The retina receives image seen through the eye and it is responsible for transmitting this information through optical nerves to the brain (5). The retina is comprised of many cells, including the two types of photoreceptors, rods and cones. Rods are very sensitive to low light conditions and able to respond to a single photon of light; they are responsible for vision under low light conditions and also periphery vision. Rods are concentrated on the outer edges of the retina, and on average there are about 90 million rods in the retina (7). On the other hand, cones require more light for functioning and they provide information about color in very sharp detail. Cone cells are concentrated around the retina’s center. On average there are about 4.5 million cone cells in the retina. In humans, it is known that the loss of rod photoreceptors leads to degeneration of cones, and one of the commonly known disease in which this concept is manifested is Retinitis Pigmentosa. Approximately 100,000 people in the United States and 1.5 people worldwide are affected by Retinitis Pigmentosa, and there is no cure.

Retinitis pigmentosa (RP) is a heterogeneous group of inherited disorders characterized by the initial loss of rod photoreceptors (7). Since rod photoreceptors are finely tuned to detect low-light levels, rod cell death results in night-blindness, leaving daylight vision intact. Much more devastating to patients are the secondary degenerative changes resulting from rod loss, such as Müller glial cell hypertrophy, loss of synaptic layers, the death of interneurons and, most notably, subsequent death of the cone photoreceptors (Figure 1). Although cones
represent no more than 5% of all photoreceptors in the human eye, their role in vision is crucial. Cones are required for color vision, and their death leads to daylight vision loss and ultimately complete blindness (8). Uncoupling the cellular and molecular mechanisms that link rod cell loss to cone death would allow cones to survive and patients afflicted with Retinitis Pigmentosa to retain functional vision.
**Figure 1.**

**Model of retina degeneration.** This diagram shows the stages of retina degeneration. The normal retina has both rod and cone photoreceptors and Müller glial cells intact. **(Stage 1)** represents the death of rod photoreceptors, which is followed by secondary changes, including cone degeneration **(stage 2)**, reactive gliosis in the form of Müller cell hypertrophy **(stage 3)** and eventually the death of inner nuclear cells **(late stage 3)**.
Mammalian animal models that mimic aspects of Retinitis Pigmentosa have been critical for investigating the mechanisms of rod cell death, and the subsequent cone degeneration. *Xenopus laevis* has been used in the field of developmental biology for a long time. A wide variety of molecular biology techniques have been developed for this model organism, and many of the genes have been identified. Also, it is easy to obtain and maintain large numbers of *Xenopus* tadpoles in the laboratory. The Zuber lab recently developed a model of Retinitis Pigmentosa in the African Clawed Frog, *Xenopus laevis* (9) to examine whether *Xenopus laevis* will exhibit the same secondary cellular changes after rod ablation. To answer this question, Rene Choi generated a transgenic line of *Xenopus laevis*, driving the expression of *E. Coli* nitroreductase (NTR) under the control of the rod-specific rhodopsin promoter (XOP). Nitroreductase converts nitromidazole prodrugs, such as metronidazole (Mtz), into a cytotoxic DNA crosslinker. Using this drug, Choi and colleagues (9) demonstrated that apoptotic rods were detected at 24 hours and rod outer segment degeneration was extensive by five days in Mtz- treated XOPNTR transgenic tadpoles (Figure 2). Also, cone photoreceptor degeneration and death followed rod ablation (Figure 3).
Figure 2.

**Rod Ablation in XOPNTR Tadpoles**

Transgenic (A-C) tadpoles were treated for 5 days with DMSO only (A) or for 5 or 10 days with Mtz (B and C). Retinal sections were stained with rod specific marker. In comparison to untreated transgenic tadpoles, a clear outer segment was observed in animals treated for as little as 5 days, and by 10 days of treatment the only observable rod outer segments was in the most peripheral retina.
Figure 3. Cone Degenerate after Rod Ablation.

Stage 52 XOPNTR tadpoles were treated with Mtz for 1 to 17 days. Rapid rod cell loss was observed. The number of cones reduced gradually but significantly, indicating cone degeneration and pending cone cell death.
The progressive loss of cones following the initial rapid period of rod photoreceptor death raises the possibility of rod-cone interactions playing a vital role in photoreceptor development and survival. Three possible mechanisms have been proposed to account for consequent cone loss (10). The first postulation indicates that rod breakdown adversely affects neighboring cones by exposing outer segments of cones to toxic chemicals that may be released from rod death. However, rods die by apoptosis, which normally avoids the release of toxic cellular metabolites that would poison cones, or cause inflammation, and lead to cone death. A second explanation proposes that oxidative stress is one of the causes of cone death. This mechanism states that the high metabolic rate of photoreceptors is an intrinsic risk factor for oxidative damage, especially for cones, which contain twice as many mitochondrial as rods. Therefore, when rods are damaged, it results in greater than 90% photoreceptor loss, leaving few photoreceptors (cones which represent 5% of photoreceptors) to consume the majority of the oxygen. It is said that this high oxygen concentration may be toxic for cones, leading to death (11). However in the *Xenopus* retina, there are equal numbers of rods and cones, making this mechanism the unlikely cause of cone death. Thirdly, it is hypothesized that rods produce some kind of signal that is essential for maintaining cone viability and the disappearance of rods deprives cones of this signal and triggers their degeneration. This third mechanism has gained significant traction with the discovery of Rod-derived Cone Viability Factor-1 (RdCVF1) in the laboratory of Dr. Thiery Léveillard (12).
RdCVF1 is a protein known to maintain the function and viability of cone photoreceptor cells in the retina. *Nucleoredoxin like1 (Nxnl1)* gene, a protein involved in the defense mechanism against oxidative stress, encodes RdCVF1 (13). In mice, alternative splicing of this gene generates two products. One is RdCVF1-L, a full-length protein composed of 217 amino acid sequences. This protein has a putative thioredoxin (enzyme involved in defense against oxidative stress) activity. The second product is C-terminal post-transcriptionally truncated protein, with 109 amino acid sequences. This latter form serves as a trophic factor. Chalmel and colleagues (14) identified RdCVF-2, a protein encoded by the *Nuclearedoxin-Like 2 (NXL2)* gene. Alternative splicing also produce a long form, RdCVF2-L, with putative thioredoxin enzyme activity, and short form, RdCVF2, with cone survival factors.

Both the RdCVF1 and RdCVF2 proteins have distinct features; there is a “cap” region, which is the C-terminal region present in RdCVF1/2-L but absent in RdCVF1/2-S, and this portion of the protein gives the long isoforms a putative thio-oxyreductase activity (14). There is also a conserved active site (CXXC) with two cysteine residues, but it is unknown if this domain is required for the proteins’ function. *RdCVF2* expression is not restricted to the retina but also in the brain, testis, placentas and other tissues types (14). However, in the mammalian retina, *RdCVF1* is fully restricted to the eye, where it is expressed prominently by photoreceptor cells in a rod-dependent manner and also by bipolar cells in the inner retina (15).
Our overall hypothesis is that RdCVF1 is expressed in the rods of the *Xenopus laevis* retina; rod ablation using the XOPNTR Mtz system results in the loss of RdCVF1, and, as a result, cone photoreceptors subsequently degenerate and die. If this hypothesis is true, a *Xenopus laevis* RdCVF1 ortholog must be present in the *Xenopus laevis* genome, and the protein’s functional domains must be conserved in *Xenopus* and other species. Also RdCVF1 must be expressed in the rod photoreceptors of the *Xenopus* retina.

To determine if a *Xenopus laevis* RdCVF1 and RdCVF2 ortholog exists, we used the mouse RdCVF1 and RdCVF2 as a probe, to identify RdCVF1 and RdCVF2 DNA and protein sequences for homologs from other species. RdCVF1 and RdCVF2 protein sequences are present in the *Xenopus* genome. *Xenopus* RdCVF1 is 60.6% identical to mouse RdCVF1, and *Xenopus* RdCVF2 is 65.2% identical to mouse RdCVF2. The “cap” regions of both proteins and hydrophobic residues are conserved in *Xenopus* and other species. To determine if RdCVF1 is expressed in the *Xenopus* retina, I performed *in situ* hybridization using RdCVF1 RNA probe. RdCVF1 expression was detected in the retina outer nuclear layer, where rods and cones are located.

**Materials and Methods**

**RdCVF1 and RdCVF2 Sequence Comparison**

We used the mouse RdCVF1 and RdCVF2 protein sequences as probes to identify RdCVF1 and RdCVF2 DNA and protein sequences for homologs (presumably orthologs) from species using BLAST (Basic Local Alignment
Search). We then aligned full-length sequences from species using the megAlign (DNSTAR Inc. Madison WI, USA) and ClustalW algorithm (16). Protein sequences from thirteen species were aligned for RdCVF1, and protein sequences from sixteen species were aligned for RdCVF2. RdCVF1 and RdCVF2 protein sequences were analyzed, and conserved functional domains in *Xenopus laevis* and other species were denoted.

**Phylogenetic Analysis of RdCVF1 and RdCVF2**

*RdCVF1* and *RdCVF2* mRNA sequences orthologous to mouse *RdCVF1* and *RdCVF2* were identified using the BLASTn genomic database. The open reading frame (ORF) of *RdCVF1* mRNA sequences from thirteen species were aligned, and the ORF of sixteen *RdCVF2* mRNA sequences were aligned using MegAlign, (DNSTAR, Inc., Madison WI, USA) ClustalW algorithm (16). Using this software, a phylogenetic tree was constructed, with 2000 bootstrapping trials to reconstruct the evolutionary history of the RdCVF protein family.

**Generation of Xenopus laevis Embryos**

Wild-type females were injected one week and one day prior to egg collection with pregnant mare's serum gonadotropin (Sigma Aldrich. St. Louis, MO) and human chorionic Gonadotropin (Intervet, Millsboro, DE) respectively to induce egg laying, and the eggs were fertilized in vitro using wild-type sperm. Following fertilization, eggs were sorted through and the ones undergoing proper divisions were selected. For the first two days, the embryos were housed in Petri
dishes (Fisher Scientific Inc.) with 0.1XMMR and gentamicin. On the third day, embryos were moved to Petri dishes containing only 0.1XMMR until the embryos reached Stage 41/42. Their solutions were changed once a day. Nieuwkoop and Faber's *Normal Table for Xenopus Development* was consulted for embryo staging (17). For the first four days, embryos were kept at 16°C. After stage 42, when tadpoles began to swim, they were fed in the morning with clear nettle supernatant and their water was changed in the late afternoon. Two weeks post fertilization, the tadpoles were transferred to plastic tanks containing water supplemented with sea salt and phosphates. 25 tadpoles were housed in each small tank filled approximately halfway to the top with frog water. During this stage, tadpoles were fed alternate days with supernatant of nettle slurry, and water was changed three times a week.

**Fixing/ Mounting Tadpoles and Cryosectioning**

Stage 56 tadpoles were euthanatized in 1% methanesulfonate (Tricaine; Sigma Aldrich), which was made with 50µl tricaine and 5000µl frog water (deionized water supplemented with Sodium Phosphate and sea salt). Tadpoles were placed in 100% Ethanol, fixed in 4% paraformaldehyde for 1 hour, and immersed in 20% sucrose for 3 hours. Tissues were mounted in OCT and uncut blocks were stored at -80°C. Tadpole retina was cryostat sectioned (12µm) and slides were stored at -20°C.
Synthesis of DIG-labeled XlRdCVF1 RNA probe

*Xenopus laevis* RdCVF1 circular plasmid was linearized with different restriction enzymes, SmaI (New England BioLabs Inc.) and NotI (New England BioLabs Inc.). Restriction digest reaction with SmaI was set up using 64.97µl of Nuclease-Free water (QIAGEN Inc., USA), 10µl of NEBuffer 4 (New England BioLabs Inc.), 10µl of BSA (bovine serum albumin, New England BioLabs Inc.), 11.03µl of XIRdCVF1 DNA and 4µl of SmaI enzyme. Restriction digest reaction with NotI was set up using 64.97µl of Nuclease-Free water, 10µl of NEBuffer 3, 10µl of BSA, 11.03µl of XIRdCVF1 DNA and 4µl of NotI enzyme. Reactions were incubated for three hours at room temperature. 1µl of each sample was checked on 1% agarose gel (0.5g of agarose powder, 50mL of 1X TE buffer) for DNA linearization.

Digested XIRdCVF1 DNA was extracted with 100µl of Phenol: Chloroform and 100µl of Chloroform: Isoamyl. 10µl of 3M Sodium Acetate in Diethylpyrocarbonate water was then added. To precipitate the DNA, 250 µl of 100% Ethanol was added to each sample and the tubes were placed on ice for 5 minutes and then centrifuged at maximum speed in a 4°C microfuge (SIGMA labs Inc.) for 30 minutes. Extracted DNA samples were washed with 250µl of 75% Ethanol and vacuum dried at room temperature for 10 minutes. DNA was resuspended in 15µl of nuclease-free water and spectra analysis was performed using NanoDrop spectrophotometer (Fisher Thermo Scientific).

T7 RNA Polymerase-Plus (Roche no. 881 767) was used to synthesis antisense XIRdCVF1 probe in a reaction with the following components; 12µl
Nuclease-Free water, 2µl of 10x DIG-labeled mix (Roche no.1277073), 2µl 10X Transcription buffer, (Ambion no. AM8151G) and 1.9µl of XIRdCVF1 DNA digested with smaI enzyme. Sp6 RNA Polymerase -Plus (Roche no. 810274) was used to synthesis sense XIRdCVF1 probe in a reaction with the following components: 9.2µl of Nuclease-Free water, 2µl of 10x DIG-labeled mix, 2µl 10X Transcription buffer and 4.08µl of XIRdCVF1 DNA digested with NotI enzyme. Reaction was incubated for 3 hours at 37°C, then 1µl DNaseI (Thermo Scientific no. 89835) was added and incubated for 10 minutes at 37°C. 1µl 0.5M EDTA, pH 8.0 was added to stop reaction and 1µl of sample was removed to check on 1% agarose gel. 1.5µl LiCl, 66µl 100% Ethanol was added and tubes incubated at -20°C overnight for DNA precipitation. Precipitated RNA was centrifuged at 15,600 rcf in microfuge for 15 minutes at 4°C. Supernatant was poured off and 100 µl of RNF 75% Ethanol was added. Excess Ethanol was removed and RNA was resuspended in 20 µl Rnase Free water (QIAGEN). RNA spectrophotometer analysis was performed using NanoDrop spectrophotometer and XIRdCVF1 RNA sense and antisense probes were resuspended in hybridization buffer for a final concentration 20µg/mL.

**In Situ Hybridization**

*In situ* hybridization is a technique that relies on the hybridization of a specific labeled nucleic acid probe with an individual cell or tissue.

**Slide Preparation**

Slides were thawed out at room temperature for 30 minutes, washed in 1X
PBS (5 mL 10X PBS and 54 mL water), 100% Methanol, Ptw (50 mL of 10X PBS, 5mL of 10% Tween-20 and 445 mL water).

Pretreatment & Hybridization

PAP pen liquid blocker (Fisher Scientific no. NC972045) was used to label the edges of slides and *Xenopus* retina tissue sections were treated with Proteinase K for 30 seconds. Slides were washed in Glycine (100 mg Glycine and 50 mL of Ptw), and Ptw solutions and sections were fixed in 0.2% Glutaraldehyde solution (8 µl Glutaraldehyde and 1 mL of 4% PFA). They were then washed in Ptw, sodium borohydride and in hybridization buffer. Sections were pre-hybridized in hybridization buffer (25 mL Deionized formamide, 7.5 mL 5M NaCl, 5mL 10X PE, 0.5 mL 10mg/ml tRNA, 0.5mL 5% heparin, 2.5 20% SDS and 9 mL DEPC'd water) at 62°C for 1 hour. 100 µL of probe was added to slide coverslips and slides were inverted onto the coverslip with probe. XlRdCVF1 probe synthesized with T7 Polymerase-Plus was used as the antisense experimental group, and XlRdCVF sense probe synthesized with Sp6 Polymerase-Plus was used as the negative control. Slides were sealed with DPX (EMS no. 13514) and incubated overnight at 60°C.

Washing

Retina tissue sections were washed in hybridization buffer for 15 minutes at 60°C, in washing buffer -1 (3mL 5M NaCl, 5mL 10X PE, 2.5mL 20% SDS, 39.5mL water) at 60°C, in washing buffer-1.5 (500µl 5M NaCl, 5mL 10X PE, 250 µL 20% SDS, 44.5mL water) at 50°C, and rinsed briefly in NTE buffer (5mL 5M NaCl, 500 µL 1M Tris-Cl pH 8.0, 100 µl 0.5 EDTA and 44.4 mL water). Sections
were washed with 100 µg/ml RnaseA in NTE (5 µL of 20mg/mL RnaseA, 995 µl NTE) for 45 minutes at 37°C and rinsed briefly in NTE buffer. Sections were washed for 15 minutes with washing buffer -2 (15 mL 100% Formamide, 1.8mL 5M NaCl, 3 mL 10X PE, 1.5mL20% SDS and 8.7mL water) at 50°C water bath, in washing buffer-3 (15mL 100% Formamide, 900 µl 5M NaCl, 3mL10X PE, 300 µl 10%-Tween-20 and 10.8mL water) for 15 minute at 50°C water bath, and in washing buffer-4 (5mL 5M NaCl, 5mL 10X PE, 500 µl of 10%-Tween-20 and 39.5mL water) at room temperature for 2 minutes then at 70°C water bath for 10 minutes. Sections were treated with IXMABT, 2mM levamisole and 2% BMBR (100 µL 10X MAB, 10 µl 10%-Tween-20, 2µL 1M levamisole, 200 µL 10% BMBR, and 688 µL water) for 30 minutes at room temperature and then treated with (1/2000) anti-DIG AP in 1XMABT, 2mM Levamisole and 2% BMBR (100 µL 10X MAB, 10 µl 10%-Tween-20, 2µL 1M levamisole, 200 µL 10% BMBR, 0.5 µL anti-DIG AP and 687.5µL water) overnight at room temperature.

**Probe Detection**

Sections were washed with 2mM levamisole 1X MABT (300 µL 10X MAB, 30 µL 10%-Tween-20, 6 µL 1M levamisole and 2.664mL water) for 10 minutes, in AP Development Buffer (25µL 1M Levamisole, 500 µL 1M Tris-Cl pH 9.5, 100 µL 5M NaCl, 500 µL10%-Tween-20 and 3.825mLwater) for 2 minutes. BM purple AP Substrate solution with 2mm Levamisole (1 mL BM purple solution, 2 µL 1M Levamisole) was added to sections and slides were incubated at room temperature for 2 days. Sections were washed with PTw+EDTA and DAPI (300 µL 10X PBS, 30µL 10%-Tween-20, 6 µL of 0.5
EDTA, 1 µL and 6 µL water), and in Ptw and EDTA solution. FluroSave (VWR) with 4-Diazabicyclo [2.2.2] octane (DABCO, Sigma Aldrich) solution was added to slide coverslip, slides were inverted onto the coverslip and sealed slides were kept at -20°C.

**Microscopy**

Stained sections were visualized using a Leica DM600 B upright fluorescence light microscope with motorized Z-focusing (Leica Microsystems, Bannockburn, IL), fitted with a Retiga-SRV camera (Q-Imaging, Surrey, BC, Canada) for image capture. Images were processed using volocity software 5.0.3 (Improvison Inc.a PerkinElmer Company, Waltham, MA).

**Generation of XOPNTR Transgene and Transgenic Animals**

Rene Choi from the Zuber lab generated transgenic XOP-NTR animals. The transgene construct pXOP(-508/+41)-NTR was generated by replacing eGFP of pXOP(-508/+41)GFP with the *E. coli* nitroreductase gene. XOPNTR F₀ transgenic *Xenopus laevis* were generated using restriction enzyme-mediated integration, and four XOPNTR founders, two males and two females, were grown to adulthood. 21 progeny from the founder females, XOPNTR1 and XOPNTR2, were generated, and only XOPNTR2 tadpoles responded to metronidazole treatment.

**Generation of F1**

The transgenic female was injected 1 week and one day prior to egg collection with pregnant mare’s serum gonadotropin (Sigma Aldrich, St. Louis,
MO) and human chorionic gonadotropin (Intervet, Millsboro, DE), respectively, to induce egg laying, and the eggs were fertilized in vitro using wild-type sperm.

**Genotyping**

Stage 50-56 XOPNTR tadpoles were tail snipped and DNA was isolated using DNeasy Blood & Tissue Kit (Qiagen Incorporated, Valencia, CA). NanoDrop spectrophotometer was used to calculate DNA concentration. Polymerase Chain Reaction (PCR) was set up using NTR primers, 10Mm DNTP'S, dimethyl sulfoxide, Nuclease Free H20, 10X Econotaq buffer and Econotaq enzyme. Primers specific for the transgene

(5’ XOPNTR: 5’-CGCTAAATCCTTTTGTTGCTGACGC-3’

3’ XOPNTR: 5’-GTTGAACACGTAATTACCGGCAGC-3’) were used to identify transgenic tadpoles from their non-transgenic siblings. Gel electrophoresis was performed using 1% agarose gel, and transgenic animals were separated from their non-transgenic siblings.

**Metronidazole Treatment**

Metronidazole (Sigma Aldrich, no. M-1547) was dissolved in 0.1X MMR Containing 0.4% DMSO (Sigma Aldrich no. D8418) to a final concentration of 10mM immediately before use. 4 PCR positive XOPNTR animals between stages 50-54 were treated with Metronidazole and 4 PCR positive XOPNTR animals, serving as experimental control animals, were cultured in DMSO solution without metronidazole for 14 days. Preliminary experiments demonstrated that higher concentrations of metronidazole were toxic. Tadpoles were raised at 22˚C in
complete darkness for the indicated time because metronidazole is light sensitive. Solutions were changed and animals were fed alternate days.

**Immunohistochemistry**

Retina sections of Stage 56 wild type, transgenic treated and transgenic untreated tadpoles were washed in methanol, blocking solution containing 1X PBS, 0.1X Triton, 5% HIGS and water. Slides were incubated overnight in primary antibody, anti-Transducin polyclonal (1:100; no. Sc-389, Santa Cruz Biotechnology, Santa Cruz, CA) prepared in a solution containing 1X PBS, 0.1X Triton, 5% HIGS and water. The following day, slides were washed in 1X PBS solution and incubated in donkey anti-rabbit IgG Alexa 488 (1:500; no. A-21206) secondary antibody tagged with fluorescent molecules. The slides were mounted in a solution of FluorSave reagent (VWR), 2% 1,4-Diazabicyclo[2.2.2]octane (DABCO, Sigma Aldrich) and 10 mg/ml 4,6-Diamidino-2-phenyindole, dilactate (DAPI, Sigma Aldrich).

**Results**

As in the mammalian retina, cone cell degeneration and eventual death follows rod ablation in *Xenopus laevis*. Our hypothesis is that *RdCVF1* is expressed in the rods of the *Xenopus* retina, and cone cell degeneration and death result from the loss of *RdCVF1* following rod ablation. If this postulation is true, *RdCVF1* protein ortholog must be present in the *Xenopus* genome, *RdCVF1* functional domains must be conserved among species including *Xenopus*, and *RdCVF1* must be expressed in the rod photoreceptors of the *Xenopus* retina.
Figure 4 shows the multiple alignments of existing and predicted RdCVF1 full-length proteins. (GenBank Accession numbers for these sequences can be found in appendix A). Organism names of each protein sequence are listed on the left and predicted sequences are denoted (_P). Percent identity of species RdCVF1 to the mouse RdCVF1 and RdCVF2 is listed at the bottom panel.

Residues identical in all species are highlighted in yellow, hydrophobic residues (A,C,E, F,G,I,L,M,S,T,V,W,Y) are marked with black triangles. Catalytic (CXXC) sites are shown (red arrow) and the red box around the residues, blue arrow represents the beginning of the “cap” region.
Figure 5 shows the multiple alignments of existing and predicted RdCVF2 full-length proteins. (GenBank Accession numbers for these sequences can be found in the appendix B). Organism names of each protein sequence are listed on the left and predicted sequences are denoted (P). Percent identity of species RdCVF2 to the mouse RdCVF1 and RdCVF2 is listed at the bottom (right). Residues identical in all species are highlighted in yellow, and hydrophobic residues (A,C,E,F,G,I,L,M,S,T,V,W,Y) are marked with black triangles. Catalytic (CXXC) sites are shown (the red arrow) and the red box around the residues, and the blue arrow represents the beginning of the “cap” region.
Analysis of RdCVF1 and RdCVF2 Protein Sequence

Chamel and colleagues (14) identified RdCVF2, another bifunctional protein belonging to the Rod-derived Cone Viability Factor family. This isoform shares many similarities with RdCVF1 in terms of the gene structure. I asked whether RdCVF1 and RdCVF2, and in particular the domains of the proteins required for their function, are conserved among species, especially in *Xenopus*. I used the mouse RdCVF1 and RdCVF2 protein sequences as probes to identify RdCVF1 and RdCVF2 DNA and protein sequences for homologs (presumably orthologs) from species using BLAST. I then used the program megAlign (DNASTAR, Madison WI .USA) and ClustalW algorithm (16) to align the sequence and determine if functional domains are conserved in *Xenopus*. Only full-length sequences were used. Protein sequences from thirteen species were aligned for RdCVF1 and protein sequences from sixteen species were aligned for RdCVF2.

RdCVF1 aligned protein sequence (Figure 4) showed conserved active sites (CXXC) with two distinct cysteine residues in all species except *Xenopus laevis, Xenopus Tropicalis* and Zebrafish. This site confers a thiol-reductase activity on RdCVF1 (14). In *Xenopus*, the first cysteine is replaced by a serine. A conserved “cap” region is present in all the species. The “cap” is the C-terminal region present in RdCVF1/2-L but absent in RdCVF1/2-S. This portion of the protein gives RdCVF1/2-L a putative thio-oxyreductase activity (14). Hydrophobic residues (A,C,F,G,I,L,M,S,T,V,W,Y) are conserved in all the species. This hydrophobic patch is more prevalent in the sequences before the
“cap” region and it is where RdCVF1 and RdCVF2 interact with other proteins or cell membrane structures (14). RdCVF2 aligned protein sequences (Figure 5) have conserved active sites (CXXC) with two distinct cysteine residues in all species. There is also a “cap” region in all species and hydrophobic residues are also conserved.

I conclude that RdCVF1 and RdCVF2 are present in the *Xenopus* genome. In *Xenopus* RdCVF1, the first cysteine residue at the catalytic site is not conserved. Instead of CXXC, serine has replaced the first cysteine making the active site SXXC. The “cap” region and hydrophobic residues are found in *Xenopus*.

**Conservation of RdCVF1 Gene Structure**

Cone viability is dependent on the production of RdCVF1-S form. In mouse, this isoform is generated by the presence of a stop codon at the end of the first exon of RdCVF1 and RdCVF2 (14). To evaluate if this stop codon is present, we aligned RdCVF1 gDNA, mRNA and protein of *Xenopus* and mouse. *Xenopus laevis* genome was provided by Richard Harlands lab (University of California, Berkeley), and Reyna Martinez from the Zuber lab blasted the genome to obtain *Xenopus RdCVF1* genomic DNA. The stop codon at the end of the first exon is strictly conserved in mouse and *Xenopus* (figure 6). This observation does not prove that RdCVF1-S exists in *Xenopus* but implies a possible existence.

GenBank Expressed Sequence Tag databases were searched for RdCVF1/2 short isoforms, and I was unable to find any EST’s. From this result, I conclude that RdCVF1-S isoform could be present in *Xenopus laevis*. 
Figure 6.

Conservation of Xenopus and Mouse RdCVF1 Stop Codon.

Mouse and Xenopus DNA, mRNA and amino acid sequences are aligned. Organism name is listed (left). The genomic region surrounding the stop codon at the end of the first coding exons of mouse and Xenopus RdCVF1 are aligned. Conserved stop codons are colored in red. (GenBank Accession numbers are listed on appendix D).
Figure 7.

Phylogenetic Tree of RdCVF Family.

Figure 7 shows the evolutionary relationship of *RdCVF1* and *RdCVF2*. Organisms are grouped in their respective classes. *RdCVF1* species are grouped together at the bottom and *RdCVF2* species are grouped together at the top. The arrow points to the *Xenopus laevis* *RdCVF1* I use in the lab. (GenBank Accession numbers are listed on appendices C and D).
Phylogenetic Analysis of RdCVF1 and RdCVF2

Chamel and colleagues identified a protein paralogous to RdCVF1 in mouse, RdCVF2. Both have similar protein sequences and gene structures are highly similar. It is shown that the degree of homology between RdCVF1 and RdCVF2 is 58.0% for the long isoforms and 53.5% for the short isoforms (14). It is important to reconstruct evolutionary history of the RdCVF family to determine if the RdCVF sequence used in our lab is RdCVF1. mRNA sequences orthologous to mouse RdCVF1 and RdCVF2 were identified using the BLASTn genomic database. The open reading frame (ORF) of RdCVF1 mRNA sequences from thirteen species were aligned and sixteen ORF of RdCVF2 mRNA were aligned using MegAlign (DNASTAR, Madison, WI USA), CrustalW algorithm (16) and the software was used to construct a phylogenetic tree.

RdCVF1 sequences grouped together on the phylogenetic tree away from RdCVF2 group (Figure 7). For all the species aligned, RdCVF1 sequence similarity as compared to the mouse RdCVF1 (Figure 4) ranged from 45.6% (cichlid fish) to 82.4% (elephant). The sequence similarity of all species aligned RdCVF1 sequence compared to the mouse RdCVF2 ranged from 27.6% (Xenopus laevis) to 34.0% (wild-turkey). The *Xenopus laevis* RdCVF that we have in the lab is 53.5% similar to mouse RdCVF1 and only 27.6% similar to mouse RdCVF2. From these results, I conclude that the *Xenopus laevis* RdCVF we use in the lab is most likely the *Xenopus laevis* ortholog.
Expression Pattern of *Xenopus laevis* RdCVF1

In the mouse Retinitis Pigmentosa model, RdCVF1 protein expression is rod dependent and mainly expressed in the Outer Nuclear Layer (ONL) of the retina. Also, Reichman and colleagues (18) demonstrated that RdCVF1 is expressed slightly in the Inner Nuclear Layer (INL), specifically in bipolar cells. If RdCVF1 is present in our *Xenopus* model, then it should be expressed similarly. I prepared RdCVF1 probe and performed *in situ* hybridization to examine the expression pattern.
Figure 8a.

Circular plasmid of *Xenopus laevis RdCVF1* (XlRdCVF1). This figure represents the plasmid that was linearized to synthesize XlRdCVF1 probe. NotI and SmaI restriction sites are indicated. The open reading frame of the DNA is marked (blue arrow). The Sp6 forward promoter and T7 reverse promoter are also shown.
Figure 8b.

Gel Electrophoresis Analysis of Linearized XIRdCVF.

Lane 1, 10 kb ladder; lane 2, XIRdCVF digested with NotI enzyme; lane 3, XIRdCVF1 digested with SmaI enzyme; lane 4, undigested XIRdCVF1.

Undigested plasmid size is about 5 kb.
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Figure 8c.

**Spectra Analysis of Xenopus RdCVF1 Probe.** Probe concentration was (ng/µl) calculated using NanoDrop spectrophotometer. RNA has absorption at 260 nm. The ratio of absorbance at 260 nm, and 280 nm is used to assess the purity of RNA.
Figure 8d.

**Gel Electrophoresis Analysis of XLRdCVF1 Probe.** Lane 1, 10 kb ladder; lane 2, anti-sense XLRdCVF1 probe synthesized with T7 RNA Polymerase-Plus; Lane 3, sense XLRdCVF1 probe synthesized with Sp6 RNA Polymerase-Plus.
**Probe Preparation**

*Xenopus laevis* RdCVF1 circular plasmid (Figure 8a) was linearized by restriction enzyme digest using SmaI and NotI restriction enzymes (Figure 8b). I extracted the linearized DNA by Phenol: Chloroform extraction. XlRdCVF-NotI DNA, transcription buffer, DIG-labeling mix and Sp6 RNA Polymerase -Plus were used to synthesize RdCVF1 sense probe. XlRdCVF1-SmaI DNA and T7 RNA Polymerase- Plus were used to synthesize RdCVF1 anti-sense probe. The probe concentration and purity was analyzed (Figure 8c), using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc. USA ), and I also checked the purity of the probe on agarose gel (Figure 9d). 2.02 and 2.03 260/280 indicated that the probe didn’t have any protein contamination. The probes were resuspended in hybridization buffer.

**In Situ Hybridization**

*In situ* hybridization uses a labeled a complementary DNA or RNA strand such as a probe to localize a specific DNA or RNA sequence in a tissue. I used a DIG-labeled anti-sense XLRdCVF probe to localize the expression of RdCVF1 in the *Xenopus* retina. A DIG-labeled sense XLRdCVF probe was used as a control. In retina tissue stained with the anti-sense probe, RdCVF1 expression was localized in the outer nuclear layer of the *Xenopus* retina where rod and cone photoreceptors are located. I also observed a slight expression in the Inner nuclear
layer (Figure 10, panels A and A”). Retina stained with sense probe (Figure 10, panels C and C”), RdCVF expression was not detected in the retina. The nucleus of all retina sections was stained with DAPI. Results from the In situ hybridization experiment conclude that RdCVF1 is expressed in the outer nuclear layer and also possibly in the inner nuclear layer of the Xenopus retina.
Figure 9.

*In situ* Localization of *Xenopus* RdCVF1: Antisense Probe.
Figure 9 shows *In Situ* hybridization results using XLRdCVF anti-sense probe (Panels A-A’’). *RdCVF1* expression is the purple staining (arrow on panel A’’) on panels (A) and (A’’).

Panel (A) was taken with 10X magnification and A’’ had 20X magnification. The retina Outer Nuclear Layer (ONL), Inner Nuclear Layer (INL), Ganglion Cell Layer (GCL), and lens (L) are indicated. Panel (B) shows the section of the retina stained with DAPI (blue) in 10X magnification and panel (B’’) is 20X magnification of retina section stained with DAPI.
Figure 10.

In Situ Localization of Xenopus RdCVF1: Sense Probe
Figure 10 shows *In Situ* hybridization results using a XLRdCVF sense probe (Panels A-A’’). RdCVF1 expression is not detected, and purple staining was not observed (arrow on panel A’’) on panels (A) and (A’’). Panel (A) was taken with 10X magnification and A’’ had 20X magnification. The retina outer nuclear layer (ONL), Inner Nuclear Layer (INL), Ganglion Cell Layer (GCL) and lens (L) are indicated. Panel (B) shows the section of the retina stained with DAPI (blue) in 10X magnification, and panel (B’’) is 20X magnification of retina section stained with DAPI.
XOPNTR-Rod Ablation System

*In situ* hybridization detected RdCVF1 expression in the *Xenopus laevis* retina. This result is consistent with the hypothesis that RdCVF1 is expressed in the *Xenopus* retina in a rod dependent manner. Rene Choi from the Zuber lab generated transgenic *Xenopus laevis* expressing the *Escherichia coli* enzyme nitroreductase (NTR) in retinal outer nuclear layer, under the control of a rod-specific rhodopsin (XOP) promoter (*Figure 11a*). Treating transgenic animals with metronidazole resulted in complete ablation of rod photoreceptors on the 12th day of treatment. I decided to test this rod-ablation system for two purposes: to test whether RdCVF1 expression will be lost in XOPNTR metronidazole treated animals, and also to determine if RdCVF1 is only expressed in rods of the retina outer nuclear layer. I performed PCR to identify transgenic tadpoles using primers specific for the transgene (*Figure 11b for PCR results*).
Figure 11a.

Circular plasmid of XOP-NTR System. This figure shows the *Xenopus laevis* rhodopsin promoter driving the expression of *E. coli* nitroreductase enzyme.

Forward and reverse primers are denoted (red triangles,) and the open reading frame of the DNA is shown (black arrow).
Figure 11b.

**PCR Identification of Transgenic *Xenopus laevis* Tadpoles.**

Lane 1, 10 kb ladder; lane 2, +control (white arrow); lane 3, -control (white arrow); lanes 4-25, DNA samples extracted from tadpoles. Circled bands indicate PCR positive animals.
**Immunohistochemistry**

After identifying transgenic tadpoles, wild type and transgenic tadpoles were treated with metronidazole for 10 days. At the same time, a group of transgenic tadpoles was left untreated (placed in DMSO solution) for ten days. I sectioned the retina of these tadpoles and performed immunohistochemistry using anti-Transducin polyclonal (1:100; product # Sc-389, Santa Cruz Biotechnology, Santa Cruz, CA). All the sections were also stained with DAPI to label the nuclei. Wild type, metronidazole treated animals had their retina outer segments intact after 10 days of treatment (Figure 12). Transgenic animals that did not receive treatment also didn’t exhibit any loss of retina outer segments (Figure 13). On the hand, transgenic animals that were treated with metronidazole showed outer retina outer segment degeneration (Figure 14). These results confirmed that the lab’s XOPNTR- rod ablation system is working. In future experiments, we will examine the effect of rod ablation on *Xenopus laevis* RdCVF1 expression and also determine if RdCVF1 is only expressed by rods in the retinal outer nuclear layer.
Figure 12. Transducin Staining of Wild Type Xenopus laevis Treated with Metronidazole.
Panel (A) is a 10X magnification of retina section of treated wild type animals. The lens is labeled (L). Panel (B) is 20X magnification of the same retina section and the arrow point to the retina outer segment. Panels (B) and (B”) are the same retina sections stained with transducin and panels (C) and (C”) are the same retina sections stained with DAPI.

Figure 13.

Transducin Staining of Untreated XOPNTR *Xenopus laevis*
Panel (A) is a 10X magnification of retina section of treated wild type animals. The lens is labeled (L). Panel (B) is 20X magnification of the same retina section and the arrow point to the retina outer segment. Panels (B) and (B") are the same retina sections stained with transducin and panels (C) and (C") are the same retina sections stained with DAPI.
Figure 14.

Transducin Staining of XOPNTR *Xenopus laevis* Treated with Metronidazole.
Panel (A) is a 10X magnification of retina section of treated wild type animals. The lens is labeled (L). Panel (B) is 20X magnification of a different retina section and the arrow point to the retina outer segment. Panels (B) and (B”) are the different retina sections stained with transducin and panels (C) and (C”) are the different retina sections stained with DAPI.
As in the mammalian retina, cone degeneration and eventual cell death follows rod ablation in *Xenopus laevis*. I hypothesized that the protein RdCVF1 is expressed in the rods of *Xenopus* retina and cone degeneration and cell death result from the loss of RdCVF1 expression. I report that RdCVF1 ortholog exist in the *Xenopus* genome, and most of RdCVF1 functional domains are conserved in *Xenopus* and other species. Also, *RdCVF1* is expressed in the outer nuclear layer of the *Xenopus* retina and potentially in the inner nuclear layer.

**Conservation of *Xenopus laevis* RdCVF1**

*Xenopus laevis* RdCVF1 amino acid sequence, just like the mouse RdCVF1 sequence, has a conserved “cap region” and hydrophobic residues that are more prevalent in regions before the “cap.” However, unlike the mouse sequence, *Xenopus* RdCVF1 doesn’t have the catalytic (CXXC) region with two distinct residues. Instead *Xenopus* RdCVF1 has (SXX), a cysteine to serine substitution. Although it is proposed that this sequence region confers thiol-reductase activity on RdCVF1 (14), it is unclear if the CXXC region is required for RdCVF1 protein function. In order to determine the importance of this catalytic site, we will need to design an experiment to rescue cone degeneration after rod ablation by expressing *RdCVF1* in a rod-less *Xenopus* retina. If we are not able to stop cone death while expressing *RdCVF1* in the absence of rods, then it may be possible that the cysteine to serine base substitution might be critical to the protein function. Results from the experiment will determine if CXXC region is vital for RdCVF1 protein function. If it is this, we can change the serine to a cysteine at the catalytic site.
Chalmel and colleagues (14) showed that in mouse cone viability is dependent on the production of the RdCVF1-S, and this isoform is generated by the presence of a stop codon at the end of the first exon of RdCVF1. In *Xenopus* RdCVF1 genomic DNA, there is also a stop codon at the end of the first exon. However, this does not prove that RdCVF1-S exists in *Xenopus*. To confirm that RdCVF-S is present in the *Xenopus* retina, we can design an experiment to test specifically for this isoform. We can generate probes that only detect RdCVF1 and perform *in situ* experiments to determine whether there is an RdCVF1 expression. We can generate this probe by isolating RNA and performing PCR to amplify the 3’UTR region of RdCVF1. We can also make primers that will only amplify the region of RdCVF1 gene known to be present in the short isoform only. It is important to determine if RdCVF1-S is present in *Xenopus* retina because this isoform is the cone trophic factor.

**Expression Pattern of *Xenopus laevis* RdCVF1**

The *In situ* hybridization experiment localized RdCVF1 expression in the outer nuclear layer of the *Xenopus* retina, where rods and cones are located. I also observed a light expression in the inner nuclear layer. Although I know that RdCVF1 is expressed in the *Xenopus* retina, my result does not indicate the specific retina cell type that expresses the protein. To determine whether rods or other retinal cell types are expressing RdCVF1, we can perform *in situ* hybridization using a XIRdCVF1 RNA probe and use cell specific markers to label rods, cones and bipolar cells. Also, we can test the loss of RdCVF1
expression in the retina of transgenic XOPNTR metronidazole treated animals by performing in situ hybridization on retina sections of untreated XOPNTR and treated XOPNTR animals to detect the levels of RdCVF1 expression. Cones could be labeled with calbindin to detect the loss of cones in the rod-ablated retina. We expect undetected RdCVF1 expression in the outer nuclear layer of XOPNTR treated, since rods are ablated, and also we expect to see fewer cone cells in that retina.

Our hypothesis that RdCVF1 is expressed in the rods and the loss of RdCVF1 in the Xenopus retina leads to cone death could be further tested by knocking down RdCVF1 in the rods of Xenopus retina without ablating rods to determine if cones will degenerate. However, there isn’t a good technique that could be used to turn gene expression on and off in Xenopus laevis later on in development. Therefore, the best way to determine the effect of RdCVF1 expression on cone viability is to develop double transgenic Xenopus animals expressing the E. coli nitroreductase enzyme and RdCVF1. This model will provide an opportunity to treat XOPNTR-RdCVF1 animals with metronidazole to ablate rods and at the same time see if the over-expression of RdCVF1 will delay or even inhibit cone death.

In this thesis, I report that RdCVF1 is present in the Xenopus and the functional domains of the proteins required for its function are conserved. Also, RdCVF1 is expressed in the Xenopus retina and using the Zuber lab XOPNTR model; it could be determined whether RdCVF1 can ultimately save cone death.
Further findings from this project could present a unique therapeutic avenue for patients suffering from Retinitis Pigmentosa.
Works Cited

1. Vision Problems in the U.S. report, developed by the National Eye Institute and Prevent Blindness America, 2002

2. The National Health and Nutrition Examination Survey (NHANES), 2002, reported in JAMA, 2006; 295:2158-2163

3. Ophthalmology Times, 8/1/01, page 38


## Appendix A

### RdCVF1 Amino Acid Sequences

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Capstone Summary

In the United States alone, more than one million people are blind. One of the leading causes of blindness is Retinitis Pigmentosa (RP). RP is a group of inherited disorders that results from the death of photoreceptor cells and leads to the decline of vision. Approximately 100,000 people in the United States and 1.5 people worldwide are affected by Retinitis Pigmentosa. Currently, there is no cure.

The human retina is the light sensitive tissue lining the inner surface of the eye. The retina has many cells including the two types of photoreceptors, rods and cones. Rods are very sensitive to low light conditions, and are responsible for vision under low light conditions and also periphery vision. Rods are concentrated on the outer edges of the retina, and on average there are about 90 million rods in the retina. On the other hand, cones require more light for functioning, and they provide information about color in a very sharp detail. Cone cells are concentrated around the retina’s center. On average there are about 4.5 million cone cells in the retina. Rod cell death results in night-blindness, leaving daylight vision intact.

Much more devastating to patients is the subsequent death of the cone photoreceptors. Although cones represent no more than 5% of all photoreceptors in the human eye, their role in vision is crucial. Cones are required for color vision, and their death leads to daylight vision loss and ultimately complete blindness. Uncoupling the cellular and molecular mechanisms that link rod cell
loss to cone death would allow cones to survive and patients afflicted with Retinitis Pigmentosa to retain functional vision.

The progressive loss of cones following the initial rapid period of rod photoreceptor death raises the possibility of rod-cone interactions playing a vital role in photoreceptor development and survival. Understanding why rod death leads to cone death is crucial. I hypothesized that rods produce some kind of signal essential for maintaining cone viability, and the disappearance of rods deprive cones of this signal and trigger their degeneration. This factor is a protein known as Rod-derived Cone viability factor 1 (RdCVF1), a protein known to maintain the function and viability of cone photoreceptor cells in the retina. 

*Nucleoredoxin like1 (Nxnl1)* gene, a protein involved in defense mechanism against oxidative stress, encodes RdCVF1. In mouse, there is a long form (RdCVF1-L) and a short form (RdCVF1-S). There is also a similar protein, RdCVF2, that also has both long and short forms. RdCVF2 expression is not restricted to the retina but also in the brain, testis, placentas and other tissue types. However, RdCVF1 is only expressed in the retina and it is the protein I explore in this project.

In order to explore the importance of RdCVF1 to cone survival in our lab, we needed a model organism. Our lab chose the tadpoles of the African Clawed frog, *Xenopus laevis*, because *Xenopus* has been used in the field of developmental biology for long time and wide variety of molecular biology techniques have been developed for this model organism. Also, many of the genes have been identified. And it is easy to obtain and maintain large numbers of
Xenopus tadpoles in the laboratory. Our lab recently developed a model of Retinitis Pigmentosa in the African Clawed Frog, *Xenopus laevis*, to examine whether *Xenopus laevis* will exhibit the same secondary cellular changes after rod ablation. To answer this question, Rene Choi generated a transgenic line of *Xenopus laevis*, driving the expression of *E. coli* nitroreductase (NTR) under the control of the rod-specific rhodopsin promoter (XOP). Nitroreductase converts nitromidazole prodrugs, such as metronidazole (Mtz), into a cytotoxic DNA cross linker. Using this drug, we demonstrated that rods were dying during metronidazole treatment.

Based on the aforementioned observation, I hypothesized that that RdCVF1 is expressed in the rods of the *Xenopus laevis* retina. Rod ablation using our model system results in the loss of RdCVF1, and, as a result, cone photoreceptors subsequently degenerate and die. If this hypothesis is true, a *Xenopus laevis* RdCVF1 ortholog must be present in the *Xenopus laevis* genome, and the protein’s functional domains must be conserved in *Xenopus* and other species. Also RdCVF1 must be expressed in the rod photoreceptors of the *Xenopus* retina.

To determine if a *Xenopus laevis* RdCVF1 and RdCVF2 ortholog exists, I used the mouse RdCVF1 and RdCVF2 as a probe, to identify RdCVF1 and RdCVF2 DNA and protein sequences for homologs in *Xenopus* and other species. I found that RdCVF1 and RdCVF2 protein sequences are present in the *Xenopus* genome, *Xenopus* RdCVF1 is 60.6% identical to mouse RdCVF1 and *Xenopus* RdCVF2 is 65.2% identical to mouse RdCVF2. The proteins’ functional domains
such as the “cap” regions and hydrophobic residues are conserved in *Xenopus* and other species. The “cap” is a region that is present in the long forms of the proteins but absent in the short forms. Since there are both RdCVF1 and RdCVF2, I needed to confirm that the protein I used in the lab was indeed RdCVF. To do that, I used computer software to generate a phylogenetic tree of RdCVF1 and RdCVF2 using mRNA sequences. I found that RdCVF1 and RdCVF2 grouped separately, and the *Xenopus laevis* RdCVF1 in our lab grouped with other RdCVF1 sequences, indicating that the protein I used in the lab is most likely RdCVF1.

After determining that RdCVF1 exists in our model organism, I performed *in situ* hybridization to detect the protein expression. *In situ* hybridization uses a labeled complementary DNA or RNA strand as a probe to localize a specific DNA or RNA sequence in a tissue. I used the *Xenopus* RNA *RdCVF1* probe to find where the protein is expressed the retina. Expression was detected in the outer nuclear layer of the retina, where rods and cones are located, supporting the postulation that RdCVF1 is secreted by rods.

In this thesis, I report that RdCVF1 is present in the *Xenopus* and the functional domains of the proteins required for its function are conserved. Also, RdCVF1 is expressed in the *Xenopus* retina and using the Zuber lab XOPNTR model; it could be determined whether RdCVF1 can ultimately save cone death. Further findings from this project could present a unique therapeutic avenue for patients suffering from Retinitis Pigmentosa.