June 2012

LIN-53, a nucleosome remodeling protein, is a potential interactor with C. elegans cytoplasmic signaling protein MIG-10

Erin Eileen Maloney
Worcester Polytechnic Institute

LIN-53, a nucleosome remodeling protein, is a potential interactor with *C. elegans* cytoplasmic signaling protein MIG-10

A Major Qualifying Project
submitted to the Faculty of
Worcester Polytechnic Institute
in partial fulfillment of the requirements for the
degree of Bachelor of Science

by
Erin Maloney

Date:
June 1, 2012

Report Submitted to:
Professor Elizabeth Ryder
Worcester Polytechnic Institute

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Abstract

The *C. elegans* protein MIG-10 is a cytoplasmic signaling protein known to facilitate neuronal migrations during development of the worm. LIN-53 is a nucleosome remodeling factor associated with transcriptional repression in *C. elegans*. Biochemical analyses were performed using co-immunoprecipitation and western blotting techniques to assess the interaction of these two proteins. Preliminary results suggest a potential interaction between LIN-53 and MIG-10. Work is in progress to determine whether *lin-53* mutations affect neuronal migrations in *C. elegans*.

Acknowledgements

I would like to thank the Duffy Lab for providing the *Drosophila* cells utilized in this project. In particular, much thanks is given to Harita Menon for her patience and willingness to teach me to maintain the *Drosophila* cells.

I would like to thank the Vidali Lab for the use of their magnetic board for Co-IP experiments.

I would like to thank Karen Tran and Molly McShea, alumni of the Ryder Lab, for their guidance when learning how to do the Co-IP and western blotting experiments and their expertise when troubleshooting.

My lab-mates Meagan Sullender and Andi Reis are owed special thanks for making my MQP experience enjoyable and truly fun. Their constant encouragement and persistence was fundamental to the success of this project.

I cannot thank Professor Liz Ryder enough for all that I have learned both academically and personally during the course of this project. The practical lab and scientific writing skills I have learned under her guidance have laid a solid foundation for the rest of my scientific career to build upon.

Introduction

Neurobiology is the study of the cells of the nervous system, called neurons, and how they are organized into an intricate network responsible for processing information and managing behaviors. The formation of connections between neurons through axon migration is vital for establishing an effective communication framework. Understanding how these connections are formed during development and what happens when things go wrong is essential for understanding nervous system injuries and disorders. Research in this area can provide insight into how our neural architecture is formed; pointing the scientific community towards the development of new treatments and therapies for traumatic brain and spinal cord injuries and neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's.

Neural connections are formed during development through axon outgrowth. Axon outgrowth is led by a structure called the growth cone that is located at the tip of the growing axon (Quinn and Wadsworth, 2008). Extracellular guidance cues are responsible for signaling the growth cone and guiding the axon to its destination (Chang et al. 2006, Quinn and Wadsworth, 2008). There are many different types of guidance cues; each will signal the growth cone to move either towards or way from the source of the guidance cue (Quinn and Wadsworth, 2008). For example, the *C. elegans* guidance cues SLT-1 (Slit) and UNC-6 (netrin) are repulsive and attractive guidance cues respectively (Chang et al. 2006). Rearrangement of the cytoskeleton is necessary for pointing axon outgrowth in the proper direction (Chang et al. 2006). Guidance cues are associated with the

asymmetrical accumulation of F-actin and microtubules that points the growth cone in direction it is migrating (Quinn and Wadsworth, 2008).

In the nematode worm *C. elegans*, extracellular guidance cues are responsible for directing neuronal migration and axon outgrowth during development. These cues can act as repellants or attractants, which signal the worm's neurons to localize to specific locations within the body of the worm (Quinn et. al 2006). The *C. elegans* protein MIG-10, encoded by the gene *mig-10*, is known to facilitate neuronal migrations in the nematode worm. Research has show that MIG-10 is localized in the growth cone and has outgrowth-promoting activity that can be polarized by guidance cues (Quinn and Wadsworth, 2008). In this project the *C. elegans* protein LIN-53 and its interaction with MIG-10 will be studied to determine if it influences axon outgrowth.

Caenorhabditis elegans as a genetic model organism

The nematode worm *Caenorhabditis elegans* is an ideal model organism for genetic exploration of neuronal migration (Manser and Wood, 1990). Presently, all of the developmental processes that occur in a wild-type worm have been documented and are generally constant across a wild-type population allowing wild-type *C. elegans* to provide a sound control for neuronal migration analysis (Manser et al. 1997). Dr. Sydney Brenner began using *C. elegans* as a genetic model organism in the 1960s to study development and neurobiology (*Caenorhabditis Elegans WWW Server*). Since then, *C. elegans* has become a common model

organism for genetic studies due to ease in genetic manipulation allowing for the study of specific mutations (Manser and Wood, 1990).

C. elegans has two sexes, males and hermaphrodites. The presence of hermaphroditic worms makes it easy to maintain strains of the worm in a laboratory setting because the hermaphrodites can self fertilize and produce offspring exclusive of males. Males can be used as a tool in genetic crosses to introduce desired alleles of genes into a strain. In appearance, the worms are small, about 1 mm in length, and translucent making them easy to manipulate and view under a microscope (WormClassroom). Furthermore, this allows for uninhibited visualization of the worms' internal structures, such as neurons and excretory canal. *C. elegans* are an inexpensive and low maintenance specimen. They feed on bacteria such as *E. coli*, which can be easily cultured on the Petri dishes the worms inhabit in the laboratory. They have a rapid life cycle that aids in the scientific process by reducing the time a given experiment takes (Manser et al. 1997).

MIG-10

The protein MIG-10, encoded by the gene *mig-10*, is a cytoplasmic adaptor protein known to mediate neuronal migrations in *C. elegans* (Quinn et al. 2006). The protein contains a Ras association domain, a pleckstrin homology (PH) domain, and several FPPPP proline-rich motifs (Manser et al. 1997). MIG-10 is homologous to two vertebrate proteins, lamellipodin (Lpd) and RIAM, that have been indicated to promote and help orient axon outgrowth (Krause et al. 2004, Lafuente et al. 2004). Both lamellipodin and RIAM have a Ras/Rap GTPase association domain, a PH

domain, and many FPPPP proline-rich motifs similar to those of MIG-10 (Chang et al. 2006). MIG-10 has been shown to act in a complex with UNC-34 to guide axons by responding to the repulsive guidance cue SLT-1 (Slit) and the attractive guidance cue UNC-6 (netrin) (Chang et al., 2006).

MIG-10 was first identified in a screen aiming to distinguish mutations that disrupted long-range embryonic migration of canal-associated neurons (CANs) in *C. elegans* (Manser and Wood, 1990). In this screen, Manser and Wood were successful in determining that the *mig-10(ct41)* mutant allele is associated with shortened migration of the CANs, anterior lateral microtubule neuron (ALM), and hermaphrodite-specific neuron (HSN) as well as truncation of the execratory canal, the waste elimination and renal organ in *C. elegans* (Manser and Wood, 1990). There are three known splice forms of *mig-10*, called *mig-10a*, *mig-10b*, and *mig-10c* (Manser et al., 1997). In all three of these transcripts the *mig-10(ct41)* mutation produces an early stop codon (Manser et al., 1997).

The MIG-10 Pathway

Research regarding the MIG-10 pathway has revealed that MIG-10 likely functions downstream of guidance cue SLT-1/Slit and its receptor Robo, as well as guidance cue UNC-6/netrin and its receptor UNC-40/DCC (Quinn et al., 2006; Chang et al., 2006). In *C. elegans*, overexpression of MIG-10 in the absence of these guidance cues results in an animal with misguided processes in the AVM neuron, indicating that MIG-10 possesses outgrowth-promoting activities on its own that require guidance cues in order to be oriented (Quinn and Wadsworth 2008).

Additionally, the presence of SLT-1/Slit and UNC-6 /netrin along with MIG-10 allow the outgrowth-promoting activity of MIG-10 to be oriented by these guidance cues (Quinn and Wadsworth 2008). Further scrutiny of the MIG-10 pathway has led to the conclusion that MIG-10 works in conjunction with UNC-34 to promote axon outgrowth and respond to guidance cues (Chang et al. 2006; Quinn et al., 2006). UNC-34 and MIG-10 are both required in the growth cone for proper reorganization of the cytoskeleton directed by guidance cues to occur. It has been demonstrated that *unc-34* is necessary for the formation of filopodia and that *mig-10* is responsible for increasing the number of filopodia (Chang et al. 2006). This indicates that MIG-10 and UNC-34 have overlapping but distinctive roles in organizing the growth of filopodia and lamellipodia during axon outgrowth (Chang et al. 2006). Studies of mammalian MIG-10 homologue lamellipodin and mammalian UNC-34 homologue Ena/VASP have suggested that lamellipodin and Ena/VASP both have distinct functions, but work together to organize cytoskeletal fibers during axon outgrowth similar to the relationship between MIG-10 and UNC-34 (Chang et al. 2006).

In a study by Chang et al. (2006) it was established that *age-1*, which encodes the lipid kinase PI3K, stimulates MIG-10 activity in developing axons. When studying defects in axon outgrowth for MIG-10 overexpression in *unc-34* null mutants it was found that defects were not suppressed by the *unc-34* null mutation (Chang et al. 2006). This further indicating that *unc-34* does not act upstream of *mig*-10, but rather in conjunction with *mig-10*. However, upon examining *age-1* null mutants with MIG-10 overexpression axon guidance defects were almost entirely

suppressed suggesting that *age-1* acts upstream of *mig-10* in the same pathway (Chang et al. 2006).

Currently enough is known about the function of MIG-10 for a proposed mechanism of its action to be generated. This proposed mechanism is illustrated in Figure 1.

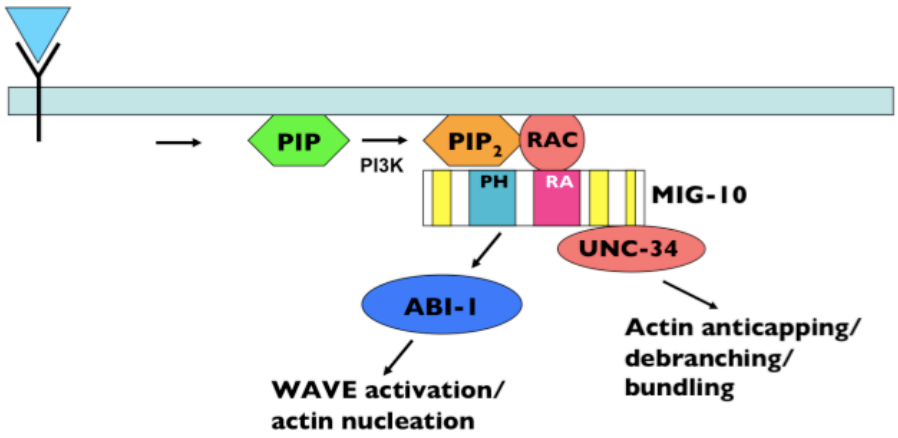

Figure 1: Speculative Model for MIG-10 Function (Ficociello and Ryder, 2007)
When stimulated by an extracellular guidance cue CED-10/Rac GTPase and PI3 kinase are activated, causing the localization of MIG-10 and other proteins related to actin polymerization to this region of the cell's membrane. (Zhang 2009)

In the proposed mechanism an extracellular guidance cue, such as SLT-1 or UNC-6, stimulates the UNC-40/DCC receptor, which then triggers a PI3K and the CED-10/Rac GTPase. *age-1* most likely codes for this PI3K, because *age-1* was shown to suppress uncontrolled outgrowth seen in worms with overexpression of MIG-10 (Chang et al. 2006). The activation of PI3K and CED-10/Rac GTPase prompts the localization of MIG-10 to the membrane, through association with the activated Ras-related protein and PI(3,4)P2 phospholipids. UNC-34 is also localized via binding to MIG-10 and results in actin polymerization (Quinn et al. 2006).

LIN-53: A candidate for the MIG-10 pathway

LIN-53 was identified as a possible interactor in the MIG-10 pathway by a previous MQP through a yeast 2 hybrid screen (Gossellin and O'Toole, 2008). LIN-53 is a known nucleosome remodeling factor associated with chromatin remodeling in *C. elegans*. Encoded by *lin-53*, the protein is similar to the Rb binding protein RbAp48 in mammals (Xiaowei and Horvitz, 1998). Previously, RbAp48 had been identified as a negative regulator of the Ras signaling pathway (Xiaowei and Horvitz, 1998). However, recent research suggests that RbAp48 acts to up-regulate K-Ras and down-regulate H-Ras and N-Ras (Scuto et al. 2007). RbAp48, also known as RbBP4 and CAF-1, has been identified as part the nucleosome-remodelling and histone deacetylase (NURD) complexes (Ho and Crabtree, 2010).

If LIN-53 were found to be an interactor in the MIG-10 pathway, it is likely that LIN-53 would act downstream of MIG-10, since LIN-53 is involved in nucleosome remodeling, an activity that occurs in the nucleus. If MIG-10 were to interact with LIN-53, it could suggest that MIG-10 has a potential influence on transcription. This would be a surprising finding because MIG-10 has only been known to regulate actin in the cytoplasm. The LIN-53 homolog RbAp48 has been shown to regulate cytoskeletal organization in mammals through the Ras signaling pathway (Scuto et al., 2007). Although it is possible that this regulation could occur in the cytoplasm of the cell, there is currently no evidence that RbAp48 is located in the cytoplasm, and the mechanism is thought to be through transcriptional

regulation (Scuto et al., 2007). Thus, confirmation of an interaction between LIN-53 and MIG-10 would indicate the existence of a novel and interesting pathway.

Project Goals

The overall goal of this project is to consider the *C. elegans* protein LIN-53 as a possible interactor in the MIG-10 pathway and investigate its potential role in the pathway. This goal will be approached through both biochemical and genetic analyses. Biochemically, the interaction of LIN-53 and MIG-10 will be investigated in a *Drosophila* cell expression system through the use of Co-Immunoprecipitation (Co-IP) and western blotting experiments. To assess the presence of a mutant phenotype, *lin-53* mutant *C. elegans* strains will be developed for comparison to a wild-type strain in order to establish whether or not LIN-53 is associated with normal axon migration and complete migration of the excretory canal.

Methods

Growing Up Colonies and Miniprep

Cells from frozen down glycerol stocks were streaked onto LB-agar plates containing 50 μg/ml of ampicillin and incubated overnight at at 37°C. Individual colonies were picked and cultured in 5 mL of LB-broth supplemented with 50 μg/ml of ampicillin and were grown overnight at 37°C. Plasmid DNA was isolated from the liquid cultures using the QIAprep Spin Miniprep kit (Qiagen).

Restriction Digests

Digestion of 3 μl plasmid DNA isolated by using the QIAprep Spin Miniprep kit was performed using 1 μl of the restriction enzyme HindIII (New England Biolabs), along with 2 μl 10X Buffer 2 (New England Biolabs) and 14 μl dH_2O. The plasmid DNA was digested for approximately 2 hours by incubating at 37°C. Digested DNA was electrophoresed according to the gel electrophoresis protocol to confirm correct fragment lengths.

Gel Electrophoresis

DNA samples or 10 Worm PCR products were electrophoresed in a 0.8% Agarose gel in 1X TAE (Tris-acetate-EDTA) buffer. The New England BioLabs 1kb or 100bp DNA Ladder was used as a marker. The gels were run at 120 Volts for approximately 1 hour.

Cell Culture and Transfection

Cells from the S3 line (provided by J. Duffy, Worcester Polytechnic Institute) were maintained at 25°C in Schneider's medium (Gibco) + 12.5% FBS (Valley Biomedical). When cells reached confluence they were diluted 1:10 in fresh Schneider's + 12.5% FBS. In preparation for transfection, 2 mL of a 1:10 dilution of confluent cells was added to each well of a 6-well plate and incubated at 25°C for 2 to 3 days. Once cells reached approximately 90% confluence co-transfections were performed using Effectene reagent (Qiagen). Effectene was added to tubes containing 133 ng Arm-

GAL4 (provided by J. Duffy, Worcester Polytechnic Institute) and 133 ng of each construct(s) drop wise and stirred gently with a pipet tip. The constructs were allowed to incubate in the Effectene reagent for 8 to 10 minutes before transfection into the cells. Transfected cells were harvested 4 to 5 days after transfection for co-immunoprecipitation.

Co-Immunoprecipitation

Each well (of a 6-well plate) of transfected S3 cells was resuspended by pipetting up and down. In order to prepare the whole cell lysate (WCL) samples, 200 µl was removed from each well and microcentrifuged at 3,900 g (8000 rpm) for 5 minutes. The supernatant was discarded and the pellet of cells was resuspended with 100 µl of 1X Sample Buffer (20 µl 5X Sample Buffer, 80 µl TBS, 5 µl BME). The remaining cells were removed from the wells, pipetted into 15 ml conical tubes, and centrifuged at 8,960 g (2000 rpm in a tabletop centrifuge) for 2 minutes at 4°C. Cells were resuspended by vortexing in 1mL of Lysis Buffer (160 µl 2X Protease Inhibitor, 100 µl 5mM Phosphatase Inhibitor, 10 µl 1mM Sodium Orthovanadate, 1.730 mL EBC Buffer) on ice for 15 minutes. The lysates were transferred to new tubes and microcentrifuged at 16,464 g (14,000 rpm) for 10 minutes in the cold room (4°C). The supernatant was transferred to new tubes and 2 µl of anti-GFP (Rabbit polyclonal from Clontech) was added and was incubated at 4°C on a nutator for 30 minutes. To each tube, 100 µl of Protein A Magnetic Beads was added and incubated for 30 minutes at 4°C on a nutator. Micro columns (Miltanyi) were assembled on a magnetic board and rinsed with 200 µl of Lysis Buffer. Cell lysates were applied to the columns and allowed to run through. The columns were washed 4 times with 200 µl of Lysis Buffer; each wash was allowed to flow through completely before the addition of the next wash. Each column was rinsed with 100 µl of Buffer X (50 mM Tris pH 8.5, 250 mM NaCl, 2 mM EDTA, 1% NP-40) followed by 100 µl of Final Wash Buffer (50 mM Tris pH 8). Lastly, the lysates were eluted by applying 20 µl of 2X Sample Buffer (30 µl 5X Sample Buffer, 60 µl TBS, 1.5 µl BME) preheated to 95°C to each column and incubating for 5 minutes at room

temperature. An additional 50 µl of 2X Sample Buffer was added and allowed to run though the column. Before loading onto a polyacrylamide gel both WCL and Co-IP samples were boiled for 5 minutes.

Western Blotting

For whole cell lysates and Co-IP products, 6 µl of WCL samples, 12 µl samples of Co-IP samples, and 10 µl of ladder (Novex® Sharp Pre-Stained Protein Standards) were loaded onto a 10% polyacrylamide gel and separated under a constant current of 20 mA for approximately 3 hours. Proteins from the gel were transferred under a constant voltage of 100V for 1 hour to a nitrocellulose/ECL membrane. The membrane was blocked for 1 hour in 5% non-fat dry milk (NFDM) in TBST (TBS + 0.1%Tween) at room temperature. After blocking, the membrane was rinsed briefly with TBST. The membrane was then incubated overnight at 4°C in primary antibody. For anti-V5 antibody (mouse monoclonal, Invitrogen), the antibody was diluted 1:5,000 in 1% NFDM in TBST, and diluted 1:1,000 in 0.5% NFDM in TBST for anti-GFP antibody (mouse monoclonal JL-8, Clontech). The next day, the membrane was washed for 5 minutes in TBST 5 times. Following the washes, a 1:20,000 dilution of secondary antibody (HRP conjugated goat anti-mouse, Jackson Immuno Research) in 5% NFDM was prepared and used to incubate the membrane for 1 hour at room temperature. The membrane was again washed for 5 minutes in TBST 5 times. Lastly the membrane was incubated in a 1:1 peroxide:luminol solution (SuperSignal® West Femto Maximum Sensitivity Substrate, Thermo Scientific) for 5 minutes and exposed to a piece of film and developed.

Ten Worm PCR

At least ten mixed stage worms were picked into the cap of a PCR tube containing 2.5 µl of Lysis Buffer (50 mM KCl, 10mM Tris pH 8.2, 2.5 mM $MgCl_2$, 0.45% NP-40, 0.45% Tween 20, 0.01% gelatin) + Proteinase K (7.5 µl of 10mg/mL Proteinase K per 0.5 mL of lysis buffer). Worms were pelleted in tubes by centrifuging, 50 µL mineral oil was added and tubes were placed at -80°C for at least 30 minutes. Once removed from the -80°C freezer, tubes were placed directly into the thermocycler

and run on the Lysis Program. A master mix containing 16.5 µl dH_20, 2.5 µl 10x Thermopol Buffer (New England Biolabs), 0.5 µl dNTP mix (10 mM in each dNTP), 1.0 µl Lin-53 forward primer, 1.0 µl Lin-53 reverse primer (5 µM stock), and 1.0 µl Vent for each sample was prepared. After the completion of the Lysis Program 22.5 µl of the master mix was added to each tube and the Short PCR Program was run.

Table 1: 10 Worm PCR Programing

Lysis Program	
Temperature (°C)	Time
65	1 hour
95	15 minutes
4	Hold forever
Short Program	
Temperature (°C)	Time
94	10 minutes
Cycle 30x	
94	30 seconds
60	1 minute
72	2 minutes
77	10 minutes
4	Hold forever

Sequencing

Each ten worm PCR product was diluted to a concentration of 2 ng/µl for sequencing in dH_20 in accordance with the sequencing guidelines provided by Genewiz, Inc to make the sequencing template. After preparation of the template, 10 µl were added to an 8 strip tube along with 5 µl of 5 µM sequencing primer, Lin-53F02, and sent to Genewiz, Inc. for sequencing.

Maintaining Worm Strains

C. elegans strains pertinent to this project were maintained on Nematode Growth Medium (NGM) agar plates spotted with *E.coli* OP50 bacteria. Strains were maintained by transferring three L4 hermaphrodite worms to new plates once a week. Plates were stored at 15°C.

Cleaning Worm Strains

A 1mL bleach solution (675 µl M9, 200 µl bleach, 125 µl 5M NaOH) was prepared and a drop was placed on a new NGM agar plate spotted with OP50. About 10 gravid hermaphroditic worms were transferred from a contaminated plate into the bleach spot on the new plate. The plate was checked 1 to 2 days after bleaching and L1 worms, from hatched eggs, were transferred to new plates.

Results

Previously, LIN-53 was identified as a possible interactor with MIG-10 in the yeast two-hybrid system (Gossellin and O'Toole, 2008). Through co-transfection, co-immnuoprecipitation (Co-IP), and western blotting experiments expression of LIN-53 was shown in S3 *Drosophila* cells, in addition to an interaction with MIG-10. If MIG-10 and LIN-53 interact in *C. elegans*, it is expected that the *lin-53* mutant would possess a phenotype resembling that of *mig-10* mutants. Mutant *C. elegans* strains were developed and ten-worm PCR and DNA sequencing were used to determine the DNA sequence of these mutants to confirm that the correct copy of *lin-53* is present in each strain before moving on to phenotypic analysis of the mutants.

LIN-53 is a potential interactor with MIG-10

During work conducted in summer 2011, LIN-53::GFP and MIG-10A::V5 constructs were developed using the Gateway Cloning system (Invitrogen). The hypothesis that LIN-53 interacts with MIG-10A was tested using Co-IP and western blotting techniques. Both LIN-53::GFP and MIG-10A::V5 were co-transfected into S3 *Drosophila* cells. The cells were harvested 4 to 5 days after transfection for use in the Co-IP experiment. Both whole cell lysate (WCL) and Co-IP samples were used in the western blotting experiments to assess the interaction of LIN-53 and MIG-10A.

Western blots were performed for each set of Co-IP and WCL (Figures 1, 2). The WCL samples showed the expression of each protein without immunoprecipitation. The anti-GFP Co-IP samples showed the presence of LIN-53 on the anti-GFP blot, while the anti-V5 blot showed MIG-10 being pulled down by LIN-53, confirming their interaction. ABI-1 wild type and ABI-1 mutant constructs were also co-transfected with MIG-10 to serve as positive and negative controls respectively. The controls should show MIG-10 being pulled down by ABI-1 wild type, but not by ABI-1 mutant.

In Figure 2, lanes 1 through 3 show the WCL samples, which indicated that the proper proteins were present in each lane. Lanes 4 through 8 show the Co-IP samples. In this blot the positive and negative controls were as expected, showing MIG-10 being pulled down by ABI-1 wild type, but not ABI-1 mutant (lanes 4 and 5). Lanes 6, 7, an 8 are experimental lanes. In lane 6 both LIN-53 and MIG-10 were transfected. The presence of MIG-10 is seen on the anti-V5 blot in lane 6 (yellow box/arrow). This result indicated that MIG-10 is interacting with and being pulled down by LIN-53. However, the band is faint, suggesting a weak interaction between the two proteins. The anti-GFP blot in Figure 2 showed that LIN-53 was present in the Co-IP sample indicating that in order to appear on the anti-V5 blot, MIG-10 must be pulled down by LIN-53 (pink arrow). In lanes 7 and 8 the intention was to have samples of LIN-53 and MIG-10 were singly transfected respectively. MIG-10 is not seen on the anti-V5 blot in in lanes 7 which is consistent with the single transfection of LIN-53, but it appears that LIN-53 was present in lane 8 suggesting that MIG-10 was not singly transfected, thus leading to the repetition of this experiment.

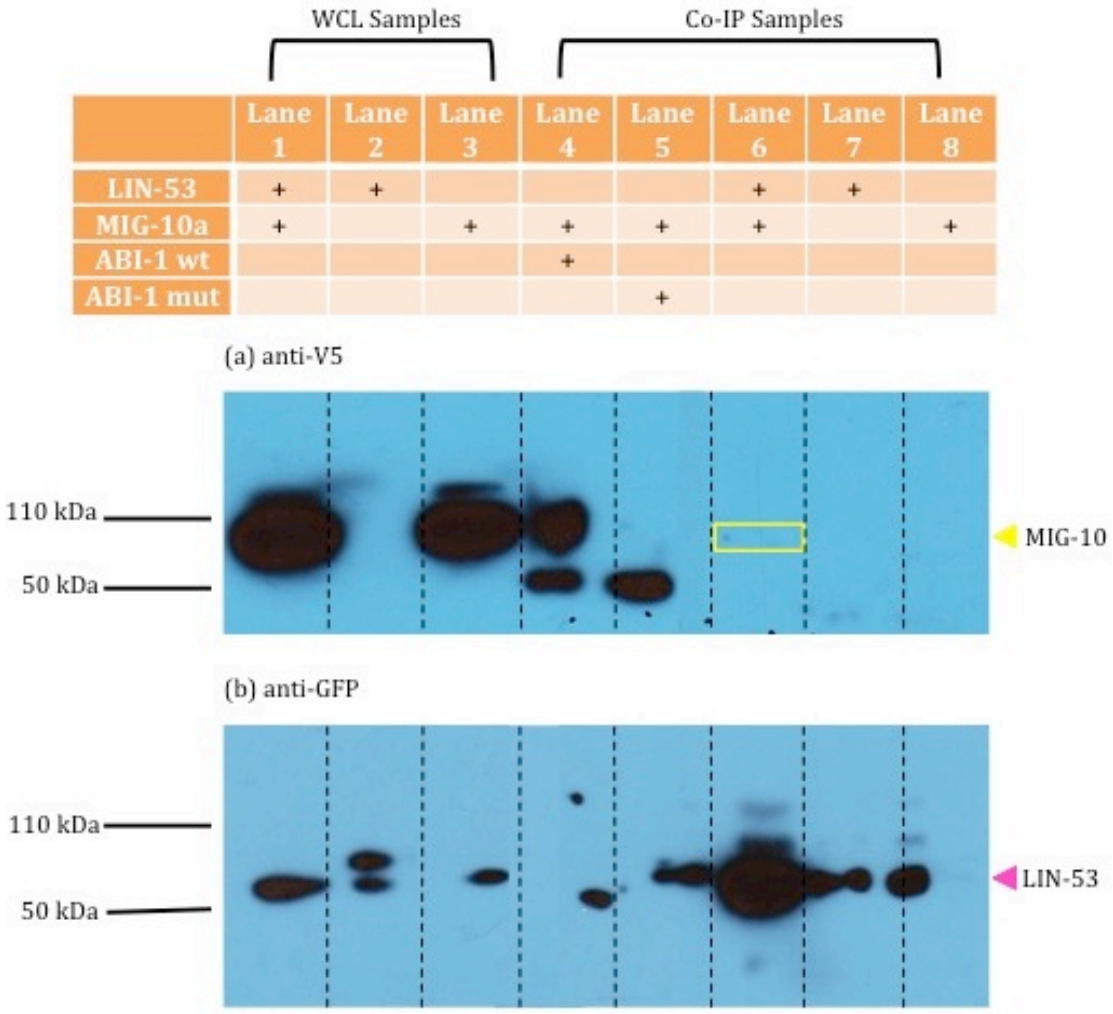

Figure 2: LIN-53::GFP interacts with MIG-10A::V5
S3 cells transfected with various constructs were lysed and immunoprecipitated with an anti-GFP antibody, and then western blotted and probed with either anti-V5 antibody (a) or anti-GFP antibody (b). Transfected constructs are indicated in the table. Lanes 1-3 are WCLs; lanes 4-8 are Co-IPs. (a) anti-V5 probe. Band size expected for MIG-10 is indicated. A faint band was observed in the co-IP where both LIN-53 and MIG-10 were expressed (lane 6, yellow box). (b) anti-GFP probe. Expected band size for LIN-53 is shown.

The WCL samples in Figure 3 indicated that the proper proteins were present in each lane on the anti-V5 blot, but were not observed on the anti-GFP blot (lanes 1, 2 and 3). Lanes 4 through 8 show the Co-IP samples. In this set of blots the positive control in which MIG-10 was pulled down by ABI-1 wild type was successful. However, the negative control failed because the blot shows that the ABI-1 mutant pulled down MIG-10, which is inconsistent with what is expected from the negative control. In the experimental lane 6 where LIN-53 and MIG-10 were co-transfected, a

strong presence of MIG-10 was seen on the anti-V5 blot, indicating that MIG-10 was interacting strongly with and was being pulled down by LIN-53 (yellow arrow). The anti-GFP blot in Figure 3 shows that LIN-53 was present in the Co-IP sample (lane 6, pink arrow). This result suggests that although there was some problem with the WCL samples for LIN-53, the protein was indeed expressed as expected. The presence of LIN-53 in the same sample signifies that in order to appear on the anti-V5 blot, MIG-10 must be pulled down by LIN-53. In lane 7 and 8 LIN-53 and MIG-10 were singly transfected respectively. MIG-10 was not seen on the anti-V5 blot in in lanes 7 and 8, thus confirming that in order to appear on the blot in lane 6 MIG-10 must have interacted with LIN-53.

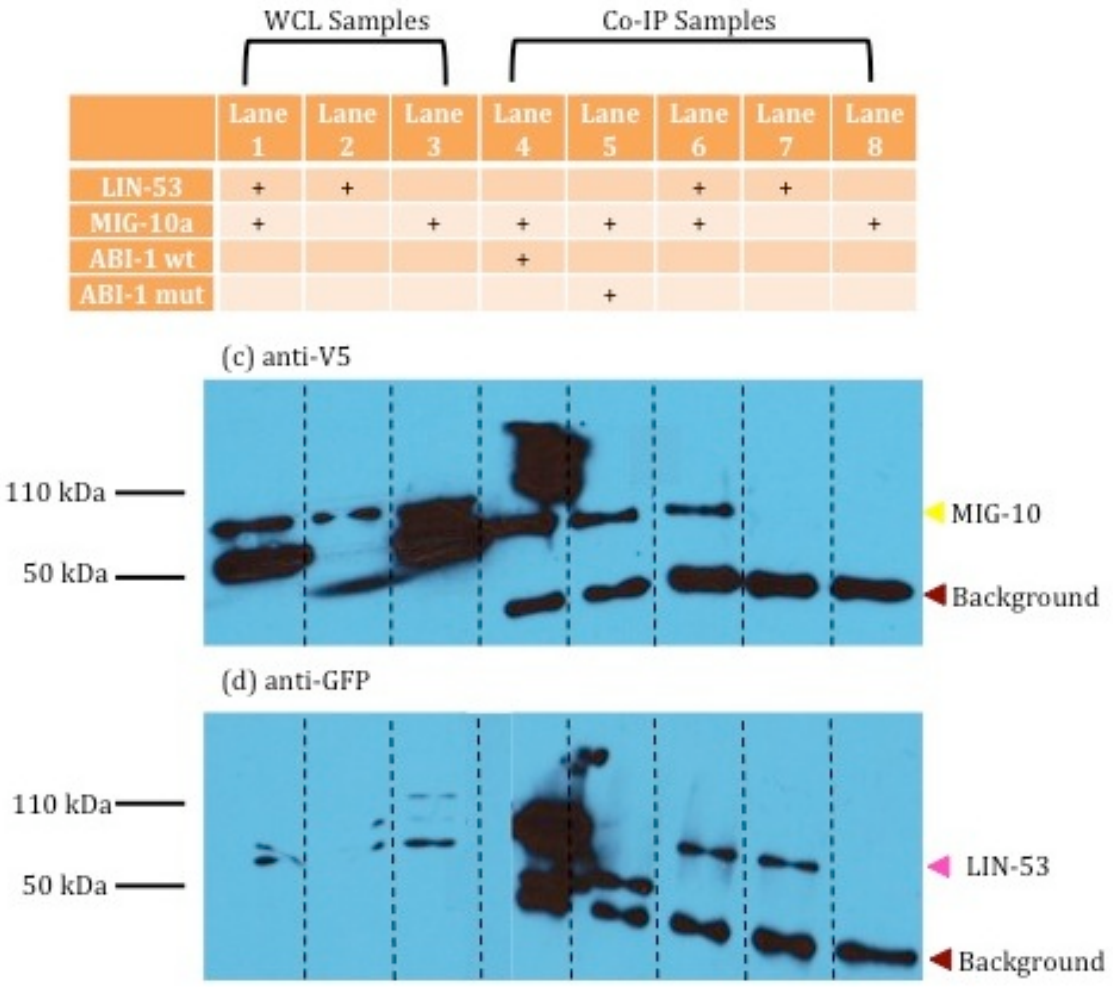

Figure 3: LIN-53::GFP interacts with MIG-10A::V5
S3 cells transfected with various constructs were lysed and immunoprecipitated with an anti-GFP antibody, and then western blotted and probed with either anti-V5 antibody (a) or anti-GFP antibody (b). Transfected constructs are indicated in the table. Lanes 1-3 are WCLs; lanes 4-8 are Co-IPs. (a) anti-V5 probe. Band size expected for MIG-10 is indicated. A band was observed in the Co-IP where both LIN-53 and MIG-10 were expressed (lane 6, yellow arrow). (b) anti-GFP probe. Expected band size for LIN-53 is shown (lane 6, pink arrow). Background bands due to interaction of the antibodies with Protein A are seen in all Co-IP samples in both blots (brown arrows).

Thus, preliminary results from Co-IP and western blotting experiments suggest a potential interaction between LIN-53 and MIG-10.

DNA Sequencing confirmed that transgenic *C. elegans* strains contained appropriate *lin-53* alleles

To examine the *lin-53* mutant phenotype, transgenic *C. elegans* strains containing the *lin-53* mutation were developed during summer 2011. These strains were created by crossing a *lin-53; dpy-5* double mutant with either *pgp-12::*GFP or *flp-*20::GFP strains. The *dpy-5* mutation is tightly linked to *lin-53* and provides a visible marker for worms containing the *lin-53* mutation. During the course of this project transgenic and control strains were prepared for DNA sequencing using the ten-worm PCR procedure. Once prepared, the PCR products were sent for sequencing to confirm the presence of the *lin-53* mutation in experimental strains and wild-type *lin-53* in control strains. The results of the DNA sequencing of each transgenic strain pertinent to this project is summarized in Table 1. Confirmation that the correct copy of *lin-53* is present in each strain was necessary before moving on to phenotypic analysis of mutant *lin-53 in vivo*.

Table 2: Summary of transgenic *C. elegans* strains and their genetic makeup

Strain Name	Genetic Makeup	Proper Sequence
RY 1142	*dpy-(e61) lin-53(n833); pgp-12*::GFP	Yes
RY 1143	*dpy-(e61) lin-53(n833); flp-20*::GFP	In Progress
N2 (control strain)	Wild-type	Yes
MT8840 (control strain)	*dpy-(e61) lin-53(n833)*	Yes

The sequencing results summarized in the above table indicates that the *lin-53* mutant is present in strains RY 1142, and that the control strains both contain wild type *lin-53*. Work to determine the sequence of RY 1143 is in progress.

The results of DNA sequencing analysis show that the proper copy of *lin-53* is present in RY 1142 and the control strains, and that confirmation of the sequence of RY 1143 is in progress.

Discussion

Preliminary results from Co-IP and western blotting experiments suggest a potential interaction between LIN-53 and MIG-10. Based on the results gained during the course of this project Co-IP and western blotting experiments should be repeated with LIN-53 and MIG-10 to gain better quality and more conclusive results. This will allow for a more definitive evaluation of the interaction of between LIN-53 and MIG-10. The results indicate that MIG-10 may interact with LIN-53, suggesting MIG-10 has a potential influence on transcription. This is surprising because MIG-10 has only been known to regulate actin in the cytoplasm and LIN-53 is a known nucleosome remodeling factor. LIN-53 homologs have been shown to regulate cytoskeletal organization in mammals, but the regulation is though to be at the transcriptional level (Scuto et al., 2007). The possibility that LIN-53 could interact with MIG-10 in the cytoplasm of the cell should also be considered in future research.

When working on the biochemistry experiments used in this project it is recommended that future students are consistent and meticulous in their methodology in order to obtain the best possible results. Special care should be taken when maintaining cell lines. The best results were seen in this project when a specific schedule was put in place and followed closely. In the western blotting experiments varying the exposure time when developing films allowed for a better blot to be developed. The exposure time on anti-GFP probed blots were significantly less than those for blots probed with anti-V5 when the clearest blots were obtained.

Some challenges were encountered with the ten worm PCR experiment during this project. For future students using the ten worm PCR protocol, sequencing results seems to be of higher quality when a large quantity of worms was used as opposed to just ten. Additionally, changing the annealing temperature in the short PCR cycle to fit the specific gene that is being investigated is suggested. During this project the annealing temperature was changed to 60°C and more successful PCR and sequencing results were seen after this change.

The phenotype produced by mutations in *lin-53* should be assessed *in vivo* by examining transgenic *C. elegans* strains confirmed by this project. The sequence of the remaining experimental strain, RY 1143, should be confirmed by ten worm PCR and DNA sequencing before proceeding to phenotypic analysis of *lin-53* mutant animals *in vivo*. Determining the phenotype of *lin-53* mutants will allow a comparison to be made to the phenotype of *mig-10(ct41)* mutants. If the *lin-53* mutant phenotype resembles that of *mig-10* mutants, then it is likely that LIN-53 and MIG-10 are interacting as part of the same genetic pathway. It would be a novel discovery if LIN-53 were confirmed as an interactor in the MIG-10 pathway by future research. MIG-10 has only been known to act in the cytoplasm and the results of this project indicate that MIG-10 may influence transcription occurring in the nucleus.

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Appendix I – Solutions

5X Sample Buffer
60mM Tris-HCL, pH 6.8
25% glycerol
2% SDS
14.4 mM Beta-mercaptoethanol
0.1% bromophenol blue
dH_20

Protease Inhibitor Cocktail
Dissolve 1 Roche Complete EDTA-free protease inhibitor cocktail tablet in 2mL dH_20 to produce a 25X stock. Aliquot and store at -20°C.

Phosphatase Inhibitor Cocktail	Stock [C]	Solvent
1 mM Sodium orthovandate	200 mM	dH_20
5 mM Sodium pyrophosphate	100 mM	dH_20
5 mM Sodium fluoride	100 mM	dH_20

EBC Buffer
50 mM Tris, pH 8
150 mM NaCl
2 mM EDTA
0.5% NP-40