The functional role of nucleosome remodeling factor (NURF) subunits during metal-induced transcription in Drosophila

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Abstract

The functional role of nucleosome remodeling factor (NURF) subunits during metal-induced transcription in *Drosophila*

A thesis presented to the Graduate Program in Molecular and Cell Biology

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Gene expression is greatly dependent on whether the chromatin is in an open or closed state. ATP-dependent chromatin remodeling complexes, such as the nucleosome remodeling factor (NURF), are important to reorganize chromatin to either activate or repress a gene. To examine the functional role of NURF subunits more closely, dsRNAi experiments in *Drosophila* cells were performed. An analysis was conducted on the effects of a metal-dependent transcriptional gene (MtnA), when major subunits of NURF were knocked down under copper stress. qPCR analysis revealed that in the presence of copper, there was a greater impact when the NURF301 subunit was knocked down compared to the ISWI subunit. This result suggests that NURF301 may have a distinctive role separate from chromatin remodeling under copper stress, unlike ISWI.
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Introduction

ATP-dependent chromatin remodeling complexes are crucial to the regulation of gene expression by repositioning nucleosomes to either activate or repress genes. There are multiple families of these remodeling complexes that all originate from the Snf2 superfamily. Each member is a complex made up of a group of subunits that work together to function. All members of the Snf2 superfamily contain an ATPase domain to supply energy to remodel the chromatin. The varying remaining subunits that make up these complexes are what distinguish each member of the Snf2 superfamily for specific functions. One subfamily of interest from the Snf2 superfamily is the imitation switch (ISWI) family. This work will focus on one member of the ISWI family named the nucleosome remodeling factor (NURF) (Clapier and Cairns, 2009).

The NURF complex along with the ACF and CHRAC complexes make up the three members of the ISWI family of chromatin remodeling complexes. *Drosophila* NURF is composed of four subunits named ISWI, NURF55, NURF38, and NURF301 (Figure 1). The driving force of the complex is the ISWI subunit, which contains the ATPase domain. Each member of the ISWI family contains the ISWI ATPase domain, giving it its family name (Clapier and Cairns, 2009). In mammalian NURF, SNF2L is the ISWI equivalent (Alkhatib and Landry, 2011). The NURF55 subunit is a member of the WD40 repeat proteins involved in histone metabolism. It is a common protein found in other complexes such as the chromatin assembly factor (CAF-1) (Martinez-Balbas et al, 1998). The mammalian ortholog of NURF55 is RbAp46/48 (Alkhatib and Landry, 2011). NURF38 is an inorganic pyrophosphatase (Gdula et al,
Interestingly, in the mammalian NURF complex, there is no NURF38 equivalent (Alkhatib and Landry, 2011). The final and largest component of the NURF complex is NURF301, which is required to structurally bring all the components of the complex together (Xiao et al, 2001). NURF301 is unique in that it distinguishes NURF from ACF and CHRAC, which contain an ACF1 subunit instead. The NURF301 subunit shows similarities to BPTF in the mammalian NURF complex (Clapier and Cairns, 2009).

**Figure 1: Structural breakdown of fly and human NURF.** Fly NURF is made up of ISWI, NURF38, NURF55, and NURF301 subunits. Human NURF is composed of SNF2L, RbAp46/48, and BPTF subunits (Adapted from Alkhatib and Landry, 2011).

As an ATP-dependent chromatin remodeling complex, NURF uses ATP to adjust the spacing of nucleosomes to regulate the accessibility of the DNA to transcription factors (Figure 2). Nucleosome remodeling assays have shown that in the presence of ATP, NURF slides nucleosomes in a bidirectional manner. In the assay, a mononucleosome reconstitution was performed of a fragment of the hsp70 promoter in *Drosophila*. When ATP was added, NURF slid the nucleosomes a short distance to a preferred position upstream of the hsp70 promoter (Hamiche et al, 1999). Further analysis of individual *Drosophila* NURF subunits indicated that both the ISWI and NURF301 subunits are crucial to the nucleosome sliding function. NURF38
and NURF55 are not required for reorganizing the nucleosomes directly. The N-terminal HMGA domain of NURF301, which contains 2 AT hooks, has proven to be important in its nucleosome sliding capability (Xiao et al, 2001). Additionally, experiments mutating different histone tails revealed an importance of the 16-KRHR-19 sequence on histone H4 in nucleosome sliding (Hamiche et al, 2001).

Figure 2: Schematic of nucleosome sliding. In the presence of ATP, NURF slides nucleosomes in a bidirectional manner (Adapted from Hamiche et al, 1999).

In addition to nucleosome sliding, NURF301 has been shown to be involved in transcription. NURF301 binds to the GAGA transcription factor as well as the HSF and VP16 transcriptional activators (Xiao et al, 2001). NURF has also been shown to be involved in signaling pathways. In the presence of ecdysone, NURF binds to the ecdysone nuclear receptor, which is important to promote *Drosophila* metamorphosis from larval to pupal stages (Badenhorst et al, 2005). Additionally, NURF may play a role in the immune system through the JAK/STAT signaling pathway. A gain-of-function mutation in HOP (*Drosophila* ortholog of JAK) showed increased levels when NURF levels were decreased through mutation. This model of constitutively active HOP results in tumor formation, indicating a possible negative regulatory role of NURF during HOP signaling (Badenhorst et al, 2002).

An important aspect of NURF function relies on the structure of its specific subunits. The ISWI subunit is unique compared to helicase domains from other ATP-dependent chromatin remodeling Snf2 superfamily members. ISWI contains a C-terminal domain named the HAND-
SANT-SLIDE (HSS) domain. Crystal structures of the HSS domain revealed a protein composed of 12 α-helices and a spacer section. Specifically, ISWI has been shown to interact with both histone tails and linker DNA through its SANT and SLIDE domains, respectively. The HSS domain of ISWI is crucial for NURF’s remodeling ability (Grune et al, 2003). One specific model suggests that the SLIDE domain must work together with the ATPase domain (ISWI) to remodel the nucleosomes (Bartholomew, 2014). Further, the NURF301 domain structure is what separates the functions of NURF from its fellow ISWI members, ACF and CHRAC. NURF301 shares many common domains with ACF1, but also has additional features. As seen in Figure 3, these shared domains include DDT, WAC, WAKZ, PHD and bromodomain. NURF301 has an additional N-terminal HMGA domain and a glutamine-rich region (Xiao et al, 2001).

Figure 3: Domain structure of NURF301. NURF301 contains HMGA, DDT, PHD, WAC, WAKZ, glutamine-rich, and bromo domains (Xiao et al, 2001).

The goal of my project is to examine the function or significance of each of the Drosophila NURF subunits in metal-dependent transcription. When excess copper is present in Drosophila cells, the metal-regulatory transcription factor 1 (MTF-1) gene is activated. MTF-1 functions by activating metallothionein genes, which then sequester the excess copper. This helps combat the heavy metal stress to assist the cells back to normal conditions (Southon et al, 2004). There are four metallothionein genes referred to as MtnA, MtnB, MtnC, and MtnD. MtnA has increased sensitivity to sensing copper, while MtnB is more efficient at sensing cadmium. MtnC and MtnD appear to be less essential, and have no specific metal sensing preference (Egli et al, 2006). The transition between the two copper states is very important in reactions involving
Cu/Zn superoxide dismutase and cytochrome oxidase. Therefore, it is important that MTF-1 and the metallothionein genes function properly because excess copper can lead to toxicity through reactive oxygen species. For this project, I performed dsRNAi on the NURF subunits, and conducted qPCR to analyze the expression levels of MtnA when exposed to copper.

Of specific interest to this project was the NURF301 subunit. A previous study using an RNAi screen showed that NURF301 is essential for metal-induced transcription. This data suggests that NURF301 may have a specific non-chromatin remodeling role. My work aims to further examine whether this was a true hit, and confirm whether NURF301 has a similar chromatin remodeling role as ISWI or whether NURF301 has a distinctive role outside of chromatin remodeling.

Significantly, experiments mutating the Drosophila NURF complex have shown to result in cancer symptoms. It is important to better understand NURF in order to potentially work towards treatments surrounding these types of cancers. The similarities between Drosophila and mammalian NURF could be helpful toward human cancer research. For example, mutations of either NURF301 or ISWI subunits resulted in the formation of melanotic tumors, disrupting the differentiation of hemocytes in larval blood. The tumors therefore affect proper development of the hemocytes (Badenhorst et al, 2002). On the contrary, research on SNF2L, the mammalian equivalent of Drosophila ISWI, may be of specific interest in treating cancer. Experiments to knock down SNF2L displayed reduced cell growth, increased apoptosis, and increased DNA damage in a malignant cancer cell line, but not in a normal control line. These results show a potential target area to investigate for cancer therapy (Ye et al, 2009). This project aims to examine the function of NURF more closely to help continue disease research.
Materials and Methods

Preparation of dsRNA

Five 100µl PCR reactions were performed for each DNA template of interest. The final composition of each 100µl PCR reaction was 1X Thermopol Buffer (20mM Tris-HCl pH8.8, 10mM (NH₄)₂SO₄, 10mM KCl, 2mM MgSO₄, 0.1% Triton®X-100), 800µM dNTPs, 1-3µg DNA, 0.005µM DNA primer, 0.05U Taq. A 1% agarose gel was run to confirm accuracy. The five reactions were then pooled and purified following Qiagen protocol. Samples were eluted with 40µl of TE buffer (10mM Tris pH8.5, 0.1mM EDTA). Two transcription reactions were then set up for each sample (T3 and T7 RNA polymerase strands). The final composition of each 100µl transcription reaction was 1X Transcription Buffer (40mM Tris-HCl pH7.5, 10mM DTT, 2mM spermidine-HCl, 20mM MgCl₂), 30mM rNTPs, 1-3µg RNA, superasin, inorganic pyrophosphatase, T3/T7 RNA polymerase. Transcription reactions were then left to incubate at 37⁰C overnight. After incubation, 100µl of 5M LiCl 33mM EDTA pH8.0 was added and put at -20⁰C for 1 hour to stop the transcription reaction. Samples were then pelleted at 15,000 rpm for 30 minutes at 4⁰C. Pellets were then washed two times with 1mL of 80% EtOH and re-suspended in 100µl of TE buffer (10mM Tris pH7.5, 0.1mM EDTA). In order for complete resuspension, samples incubated at room temperature for 30 minutes before being quantified. T3 and T7 tubes were then diluted accordingly to have the same concentration. Equal volumes of T3 and T7 strands were combined for annealing (NURF301 used two T7 strands). Tubes were
heated for 2 minutes at 95°C, transitioned to 65°C for 30 minutes, and gradually cooled to room temperature. dsRNA samples were then diluted to 1mg/mL to be used in RNAi experiments.

Cell Culture

A line of S2 Drosophila cells was maintained for the course of this project. The cells were incubated at 25°C in Schneider’s media with 10% FBS and 1% pen/strep. Every 3-4 days cells were split using either a 1:5 or 1:3 dilution depending on confluency.

RNAi

Cells were counted and diluted in complete media (containing FBS) to a final concentration of .75 X 10⁶/mL. Each well of a six-well plate was plated with 2mL of the diluted cell media (two wells for each dsRNA sample tested). The plates were then incubated at 25°C for 1 hour allowing the cells to adhere. After incubation, the media was removed and washed twice with 1mL of serum free media. Following the washes, a final 1mL of serum free media was added along with 40µg/mL of dsRNA to each well. The plates were then incubated for another hour at 25°C. A final 2mL of complete media was added to each well after incubation. Cells were then maintained at 25°C for 3 days, and then 0.5mM CuSO₄ was added to one well of each dsRNA sample pair. The plates were then left to incubate at 25°C overnight.

RNA Extraction

After 16-18 hours, the cells were harvested for RNA. Each well of the six-well plate was pipetted repeatedly to re-suspend the cells, and then transferred to a 15mL tube on ice. The wells were then washed with 1mL of 1X PBS, which was then added to the 15mL tube. Then, the
tubes were pelleted at 1000Xg for 10 minutes at 4°C. Pellets were then re-suspended in 1mL of 1X PBS, and transferred to an eppendorf tube. The tubes were then pelleted at 4°C for 30 seconds at 15,000 rpm. Each pelleted tube then was re-suspended in 500µl of Tri Reagent (MRC), followed by the addition of 100µl of chloroform. Samples were then spun at 15,000 rpm for 15 minutes at 4°C to separate the components into three layers. The top clear layer (RNA) was separated from the genomic DNA and protein layers by removing 200µl of the clear phase into a new tube. Then to each tube, 200µl of isopropanol was added, and spun at 4°C for 30 minutes at 15,000 rpm. Pellets were then washed two times with 850µl of 80% EtOH and re-suspended in 40µl TE buffer (10mM Tris pH7.5, 0.1mM EDTA). In order for complete resuspension, samples incubated at room temperature for 30 minutes before being quantified.

**cDNA Synthesis**

RNA samples were then prepared for cDNA synthesis by digesting using DNase I enzyme. Tubes were then incubated at 37°C for 1 hour. After incubation, 5mM EDTA was added to each tube and heated to 65°C for 10 minutes. First strand cDNA synthesis was conducted using a mix of random hexamers and oligo dT primers. The final composition of a 25µl cDNA reaction was 1X RT Buffer (50mM Tris-HCl pH8.3, 50mM KCl, 3mM MgCl2), 10mM DTT, 500ng cDNA primer, 400µM dNTPs, 1-3µg RNA, 200 units MMLV RT. The cDNA was then diluted 1:10 in TE buffer (10mM Tris pH8.0, 0.1mM EDTA).

**qPCR**

Original digested RNA (0.5µg) was diluted in 100µl of TE buffer (10mM Tris pH8.0, 0.1mM EDTA) to be used as a control in qPCR. Triplicates of each cDNA sample were loaded
in a 96 well plate. The final composition of a 20µl qPCR reaction was 1X Thermopol Buffer (20mM Tris-HCl pH8.8, 10mM (NH₄)2SO₄, 10mM KCl, 2mM MgSO₄, 0.1% Triton®X-100), 200µM dNTPs, 0.5µM qPCR primer, 0.5X Syber Green, 1U/20µl reaction of Taq. Primer pairs for RP49 were used as a control. Experimental primer pairs for metallothionein A (MtnA) were investigated. Opticon Monitor 3 was used to analyze curves and C₇ values of the samples.

**Western Blotting**

Concurrently with RNAi experiments, 10% of cells were harvested and used for western blotting. The pelleted cells were re-suspended in 50µl of RIPA buffer (1X PBS, 0.1% SDS, 0.5% deoxycholate, 10% glycerol, 5mM EDTA). Extracts then received 1µl of Benzonase and incubated on ice for 30 minutes. Samples were then frozen at -20°C until needed. Polyacrylamide gels (6%) were prepared using 1mm plates and 10 well combs. After thawing the samples on ice, 15µl of extract was removed and placed in a new tube with 1X load dye and 1µl dH₂O. Samples were boiled at 95°C for 1-5 minutes and spun at 15,000 rpm for 1 minute. After boiling, 20µl of each sample was loaded into each well along with one well of 1-2µl of All Blue Ladder (Bio-Rad). The gel dock apparatus was filled with running buffer (100mL Tris SDS and 900mL dH₂O) and ran at 100 volts for ~1.5 hours. After the gels finished running, a cassette filled with paper and pads was used to surround the gel and nitrocellulose membrane (Life Technologies) for transfer. Transfer buffer (200mL 5X Tris glyceride, 700mL cold dH₂O, 100mL methanol) was used to fill the membrane cassettes, and placed next to an ice pack. The gel transferred for 1 hour at 80 volts at 4°C. Once complete, the membranes were placed in a closed container filled with blocking solution (1X PBS, 1% non-fat dry milk). Membranes were incubated in the blocking solution at 4°C overnight. The following day, membranes soaked in 1°C
antibody for 1 hour. The antibody/wash solution consisted of 1X PBS, 1% non-fat dry milk, and 0.5% Tween 20. Depending on the sample, a rabbit 1⁰ antibody (1:1000) was added to 10mL of antibody/wash solution to cover the membranes. Membranes probing for ISWI used an ISWI 359 1063 antibody. Antibodies probing for MTF-1 were BRD6 and/or BRD7. Membranes with samples for NURF used NURF A and B antibodies. Each aliquot of 1⁰ antibody also received tubulin antibody (1:1000) as a control. After 1 hour of incubation in 1⁰ antibody, the membranes were washed in antibody/wash solution 5 times for 5 minutes each. Following the washes, the membranes were incubated in 2⁰ antibody for 1 hour, covered. The 2⁰ antibody solution consisted of 10mL of antibody/wash solution, fluorescent goat anti-rabbit 800 (1:5000), and fluorescent goat anti-mouse 680 (1:5000). After 1 hour of incubation in 2⁰ antibody, the membranes were washed in antibody/wash solution 5 times for 5 minutes each. Following the washes, the membranes were briefly rinsed in 1X PBS. Lastly, membranes were scanned using the Li-Cor Odyssey Infrared imaging system and analyzed.
Results

dsRNAi of NURF subunits

The aim of this project was to examine the individual NURF subunits for functionality during copper-dependent transcription. As the project progressed, the goals were refined to focus mainly on the two most important subunits of NURF, ISWI and NURF301. To achieve these results, dsRNA of NURF subunits were used to knock down these subunits of interest in Drosophila cells. Copper was added after 3 days to analyze expression levels of a specific copper-dependent transcriptional gene, MtnA. qPCR was conducted using primers for MtnA and normalized to RP49. An analysis was performed of MtnA transcript levels in comparison to RP49 levels when either ISWI or NURF301 was knocked down. Figure 4 shows the results of 3 replicate experiments averaged together. In these experiments, lacI and MTF-1 dsRNA samples were used as controls. ISWI and NURF301 dsRNA samples were tested and compared. Transcript levels were analyzed for dsRNA samples with and without copper. The lacI dsRNA control represents normal conditions, which are not affected by copper. MtnA transcript levels of lacI are roughly the same as RP49 transcript levels in the presence of copper. When MTF-1 was knocked down, we see a significant 13 fold decrease in MtnA transcript levels in comparison to RP49 when copper is added. This is the expected result because MTF-1 activates MtnA (Southon et al, 2004). Additionally, when ISWI is knocked down, we see a small 1.4 fold decrease in MtnA transcript levels in comparison to RP49, in the presence of copper. This is expected
because ISWI is the ATPase domain of the NURF complex. Interestingly, when NURF301 is knocked down, we see a larger 2.2 fold decrease in MtnA transcript levels during copper induction. The larger effect in NURF301 compared to ISWI suggests a potential distinctive role of NURF301 outside of chromatin remodeling during metal-dependent transcription. As a control, when no copper is added, we see a much smaller scale of MtnA transcript levels for all dsRNA samples indicating a small level of transcripts under normal conditions.

**Figure 4: qPCR analysis of dsRNAi experiments.** The averages of three replicate experiments are shown. The graphs show MtnA transcript levels as a fraction of RP49 transcript levels for each dsRNA sample tested. Results are separated as with or without the addition of copper. LacI and MTF-1 dsRNA samples served as controls. ISWI and NURF301 dsRNA samples were tested.
Western blotting

To confirm that the dsRNA was functioning properly, western blotting was conducted. Figure 5 shows a blot probing for NURF301. Again, lacI dsRNA samples were used as a control. In lanes where lacI dsRNA samples were loaded, two faint bands can be seen between 250-300 kDa. NURF301 is very sensitive to degradation indicating the bands shown are two slightly degraded NURF301 proteins (Xiao et al, 2001). As shown in the blot, no bands are seen in the lanes where NURF301 dsRNA samples were loaded, confirming that the dsRNA was working.

Primary antibodies probing for NURF301 were made in the Marr lab. A combination of causes including antibodies, NURF301 sensitivity to degradation, and the background on the blot may explain the difficulty in visualizing and interpreting the blot. Unfortunately, issues arose with the tubulin control, but a non-specified band serves a similar purpose as seen in Figure 5. This confirms that protein was loaded into each lane. These results together support the accuracy of the qPCR results through successful knockdown of NURF301 using dsRNA.
Figure 5: Anti-NURF301 western blot. Rabbit anti-NURF301 (1:1000) and goat anti-rabbit 800 (1:5000) primary and secondary antibodies were used. LacI dsRNA samples with and without copper served as a control. NURF301 dsRNA samples with and without copper were tested.

Similar to NURF301, ISWI dsRNA was also tested by western blotting using an anti-ISWI antibody. As seen in Figure 6, it can be confirmed that ISWI was being knocked down. Lanes where lacI dsRNA samples were loaded show distinct bands at ~100 kDa, representing the ISWI protein. This band is not seen in the lanes where ISWI dsRNA samples were loaded. Also, like the NURF301 blot, a non-specified band serves as a control for protein concentration. The ISWI blot therefore also supports the accuracy of the qPCR results through its successful knockdown of ISWI using dsRNA.
Figure 6: Anti-ISWI western blot. Rabbit anti-ISWI (1:1000) and goat anti-rabbit 800 (1:5000) primary and secondary antibodies were used. LacI dsRNA samples with and without copper served as a control. ISWI dsRNA samples with and without copper were tested.

Troubleshooting

Throughout the project, slight changes were made to the protocol to troubleshoot. The experiments have multiple parts, and each section needed to be addressed. Early on in the project when harvesting RNA, an agarose gel revealed that the RNA integrity was not ideal. Minor adjustments when harvesting such as keeping samples on ice and working quickly helped improve the RNA integrity. When experiments weren’t working, the accuracy of the dsRNA was tested by performing western blotting. A few modifications to the western protocol were conducted to help get a clearer blot. Benzonase was added to lysates before running on the gel to help get rid of any DNA or RNA present. Originally, a 10% polyacrylamide gel was used to run the samples to be transferred for blotting, but because of the large size of NURF301 the gel was switched to 6%. Also, to try to remove background on the blots the wavelengths of the secondary antibodies were switched. Lastly, alterations were made to improve cDNA synthesis. The amount of MMLV reverse transcriptase was doubled from the original protocol. Additionally, a combination of random hexamers and oligo dT primers was used during synthesis instead of only
using random hexamers. The final alteration from the original protocol was to make the cDNA for qPCR more concentrated by changing the dilution in TE buffer from 1:20 to 1:10.
Discussion

Through this project, the nucleosome remodeling factor complex (NURF) was examined during metal-dependent transcription. A previous RNAi screen selected NURF301 as playing an essential role during metal-induced transcription. This work aimed to verify if this was a true hit. After completing three repetitions of the dsRNAi experiments, we can confirm that NURF301 does have a distinctive role during metal-dependent transcription separate from the chromatin remodeling role typically seen in NURF. The main component functioning in chromatin remodeling has previously been shown to be the ATPase subunit, ISWI (Clapier and Cairns, 2009).

Three individual repetitions of qPCR results from the RNAi experiments revealed that when either ISWI or NURF301 is knocked down, we see a stronger effect when NURF301 is not present. Due to the <2 fold decrease in MtnA transcript levels when ISWI is knocked down, it can be concluded that ISWI does not play an important role during metal-dependent transcription. When the three experiments were averaged, we saw a 2.2 fold decrease in MtnA transcript levels when NURF301 was knocked down. Unlike ISWI, NURF301 appears to play a similar role to MTF-1 which is greatly involved in the metal-dependent transcription pathway. MtnA transcript levels were significantly decreased when MTF-1 was knocked down. This is not surprising since, MTF-1 activates MtnA when copper is present (Southon et al, 2004). The role of NURF301 is not as significant as MTF-1, but does play a role in the metal-dependent transcription pathway.
Individual dsRNAi experiments all revealed the same trend in MtnA transcript levels, but showed slightly different values. The strongest effect seen when NURF301 was knocked down was a 3.8 fold decrease in transcript levels. In this same experiment, ISWI showed a 1.5 fold decrease in transcript levels when it was knocked down. The remaining two experiments showed ~2 fold decrease in transcript levels when NURF301 was knocked down. Similar to the previous experiment described, transcript levels decreased by ~1.3 fold when ISWI was knocked down. When averaging the three repetitions, we can conclude similar results as the previous high-throughput screen indicating a true hit. Through this project a new distinctive role of NURF301 in metal-induced transcription has been confirmed.

In order to progress this project, the focus was centered on NURF301. It would be interesting for future research to examine NURF38 and NURF55 as well. Since these two subunits are minor, it is expected that they do not have a distinctive role in metal-dependent transcription. Further dsRNAi experiments with NURF38 and NURF55 could verify this prediction. Additionally, this project centered on the use of copper and examining its effects on MtnA transcript levels. An analysis of dsRNAi experiments in the presence of cadmium and its effects on MtnB transcript levels is also of interest. A comparison of the two different metal-dependent transcription pathways could give further insight into the role of NURF301.

Future work examining the different domains of NURF301 could help specify which domains are important to metal-dependent transcription. Specifically, experiments that mutate the N-terminal HMGA domain of NURF301 could be interesting. If the N-terminal domain is crucial to metal-dependent transcription, then this result would provide further evidence that distinguishes NURF from the other two ISWI complex family members in terms of functionality (Xiao et al, 2001). On the other hand, if domains in NURF301 that are shared with its family
members are important to metal-dependent transcription, then these results could help link further similarities between family members. Experiments using mutated NURF301 dsRNA affecting varying domains would help determine which hypothesis is correct.

The work conducted through this project using *Drosophila* NURF serves as a model for mammalian NURF. Strong homologies between NURF301 and BPTF suggest potential similarities in their functions during metal-dependent transcription. A better understanding of NURF’s functions will help in research working towards treatment of diseases involving NURF.
References


