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DEPARTMENT OF BIOLOGY

AN INVESTIGATION OF THE EFFECTS OF SELENIUM ON HISTONE ACETYLATION

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## **ABSTRACT**

Histones are an important component of DNA, affecting both transcription and translation within the cell. DNA in our cells is wound around an octomer of four core histone proteins. Post-translational covalent modifications of these histones at specific residues, including acetylation, methylation, and phosphorylation, can lead to the activation or repression of gene transcription. Histone acetylation, performed by histone acetyltransferases (HATs), is of particular interest because it can lead to the activation of inflammatory genes and pathways in the body. Mutated or malfunctioning HATs can also lead to disease, and may be future targets for cancer therapies. Because of these links between inflammation and disease, it is important to understand histone acetylation and how it is regulated.

It has been shown that selenium has anti-inflammatory properties by serving as an antioxidant and by increasing the production of anti-inflammatory prostaglandins in macrophages. Because of the effects of selenium on inflammation, it is believed that selenium may affect HATs and cause a reduction in histone acetylation, decreasing the activation of inflammatory genes. This investigation takes a look at the effects of different concentrations of selenium on histone acetylation on H4K5, H4K8, H4K12, and H4K16 residues in macrophages and hepatocytes. Our results do in fact show that selenium supplementation reduces histone acetylation on H4 residues in both macrophages and hepatocytes.

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**LIST OF ABBREVIATIONS**

|                      |  |
|----------------------|--|
| HAT                  | Histone acetyltransferase                                |
| HDAC                 | Histone deacetylase                                      |
| NF- $\kappa$ B       | Nuclear factor $\kappa$ B                                |
| COX                  | Cyclooxygenase   |
| H-PGDS               | Hematopoietic prostaglandin D synthase                   |
| 15d-PGJ <sub>2</sub> | 15-deoxy- $\Delta^{12,14}$ -prostaglandin J <sub>2</sub> |
| DMEM                 | Dulbecco's Modified Eagle's medium                       |
| FBS                  | Fetal bovine serum                                       |
| LPS                  | Lipopolysaccharide                                       |
| KCl                  | Potassium chloride                                       |
| MgCl <sub>2</sub>    | Magnesium chloride                                       |
| EDTA                 | Ethylenediaminetetraacetic acid                          |
| HCl                  | Hydrochloric acid  |
| TBS                  | Tris Buffered Saline                                     |
| TBST                 | Tris Buffered Saline Tween-20                            |
| DMSO                 | Dimethyl Sulfoxide                                       |

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## **Chapter 1**

### **Introduction**

#### **Histones**

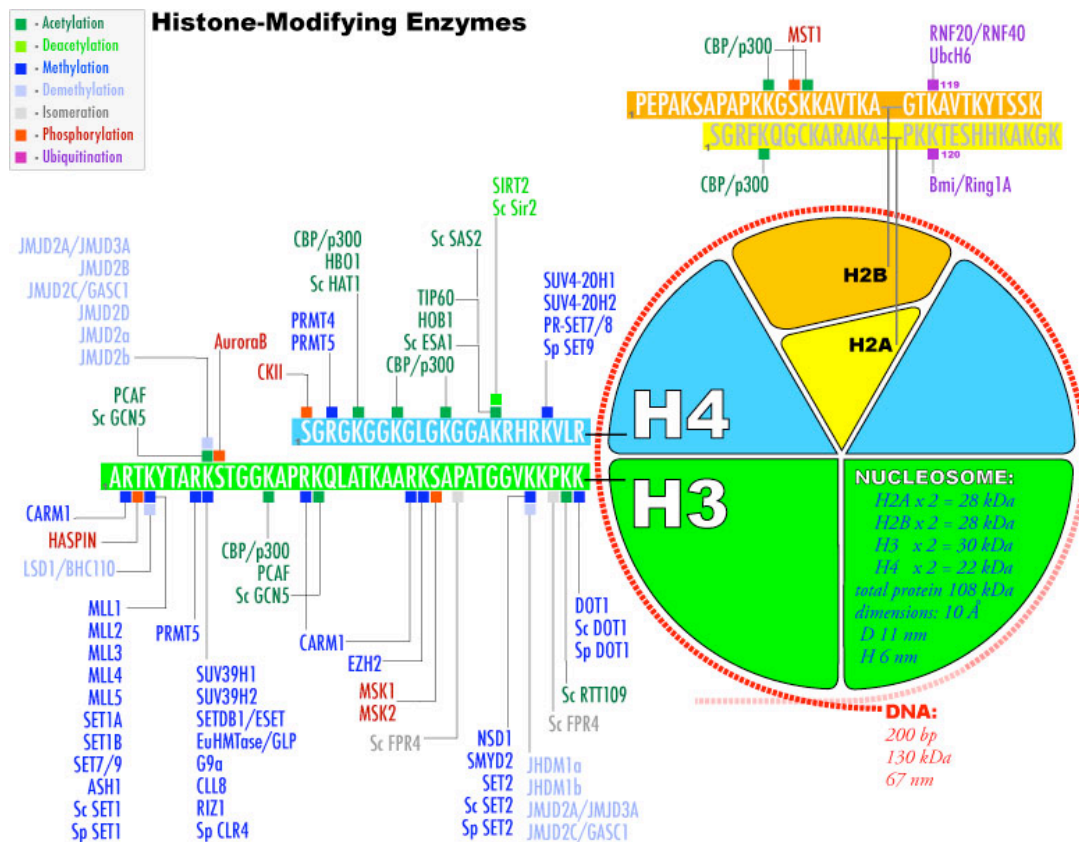
The nucleus of a eukaryotic cell contains a highly organized genome in the form of chromatin. The basic unit of chromatin is the nucleosome, an octamer comprised of two copies each of four core histones: H2A, H2B, H3, and H4 (Selvi et al., 2009). An H1 histone is also present within the chromatin structure, and acts as a spacer between adjacent nucleosomes. Two turns of DNA are wrapped around each nucleosome to form bead-like structures, but the amino-terminal tails of the histones are less structured and project out of the core nucleosome (Zoroduu et al., 2009). These protruding amino-terminal tails can be modified by enzymes through acetylation, deacetylation, methylation, demethylation, phosphorylation, ubiquitination, or isomerization at specific residues along each of the proteins (see Figure 1-1 on page 2). Post-translational modification of the histones at specific sites can then cause conformation changes in the proteins, activating or deactivating transcription and translation within the cell. There is increasing evidence that these histone modifications play an important role in both transcription regulation and normal or abnormal cell function (Dekker et al., 2009).

#### **Histone Acetyltransferases**

Acetylation is a common post-translational modification of histones catalyzed by histone acetyltransferases (HATs). HATs can be classified into two different classes based on their function in the cell: type A HATs and type B HATs. Type A HATs are found in the nucleus while type B HATs are found in the cytoplasm and modify newly translated histones before being incorporated into DNA (Selvi et al., 2009). Type A HATs are the main focus of this study, four families of which have been extensively studied: The GNAT family, the p300/Cbp family,



the MYST family, and the Rtt109 family (Lau et al., 2000). HAT families are characterized by their specific functions and structural folding, and have their own respective acetylation sites on different core histones (see Figure 1-1 below). HATs only acetylate lysine residues on the four core histones by transferring an acetyl group from acetyl coenzyme A onto specific lysine residues along each of the amino-terminal tails of the histones (Zoroddu et al., 2009). Literature has shown that histone acetylation is generally linked to transcriptional activation by depositing a partial negative charge on the histones (Golebiowski et al., 2005). The partial negative charge on the histone repels the negatively charged DNA, displacing the N-terminal of the histone and causing the nucleosome to unfold (Selvi et al., 2009). Because the DNA becomes less compact after acetylation, transcription factors can gain access to the DNA to carry out transcription.



**Figure 1-1.** Histone Modifying Enzymes and Their Targeted Residues for Modification  
**Source:** Gramatikoff, K. Histone-Modifying Enzymes. *Trends Genet.* 2010

### **Histone Acetyltransferases and Disease.**

Histone acetylation by HATs is an important transcription regulator, but histone deacetylases (HDACs) are also important for reversing the effects of acetylation. Histone deacetylases catalyze the removal of the acetyl group from the lysine residues on histones, returning the chromatin to a more compact state not accessible to transcription. The proper balance of histone acetylation and deacetylation is important to maintain cell homeostasis, and imbalances in this process can lead to abnormal acetylation patterns. Hypoacetylation can occur at residues that should be transcriptionally active, or hyperacetylation can activate genes that should be repressed, which can lead to disease. Mutations in HATs or HDACs, or loss of function of these enzymes can also have implications in disease (Selvi et al., 2009).

Specific HATs, including p300 and the GNAT family enzymes GCN5 and PCAF may also play a role in cancer. Interestingly, it has been reported that inhibiting p300 can suppress tumor growth, and that HAT activity is an important regulator of the G1 to S transition stage of the cell cycle (Iyer, 2007). It has also been noted that GCN5 and PCAF are important in cell cycle checkpoints, and may be targeted for cancer therapies (Dekker et al., 2009).

### **Histone Acetyltransferases and Inflammation**

Histone acetyltransferases not only play a role in cancer and disease, but also are important in regulating inflammatory pathways. It has been shown that histone acetylation by HATs activates inflammatory genes (Bayarsaihan, 2011). HATs are an important cofactor for nuclear factor  $\kappa$ B (NF- $\kappa$ B), a regulator of gene transcription in inflammatory pathways. Increased acetylation of histones at the promoter regions of inflammatory genes activates the NF- $\kappa$ B pathway, leading to an increased production of thromboxane A<sub>2</sub> (an inducer of platelet aggregation and vasoconstriction) and the pro-inflammatory prostaglandin E<sub>2</sub> (See figure 1-2

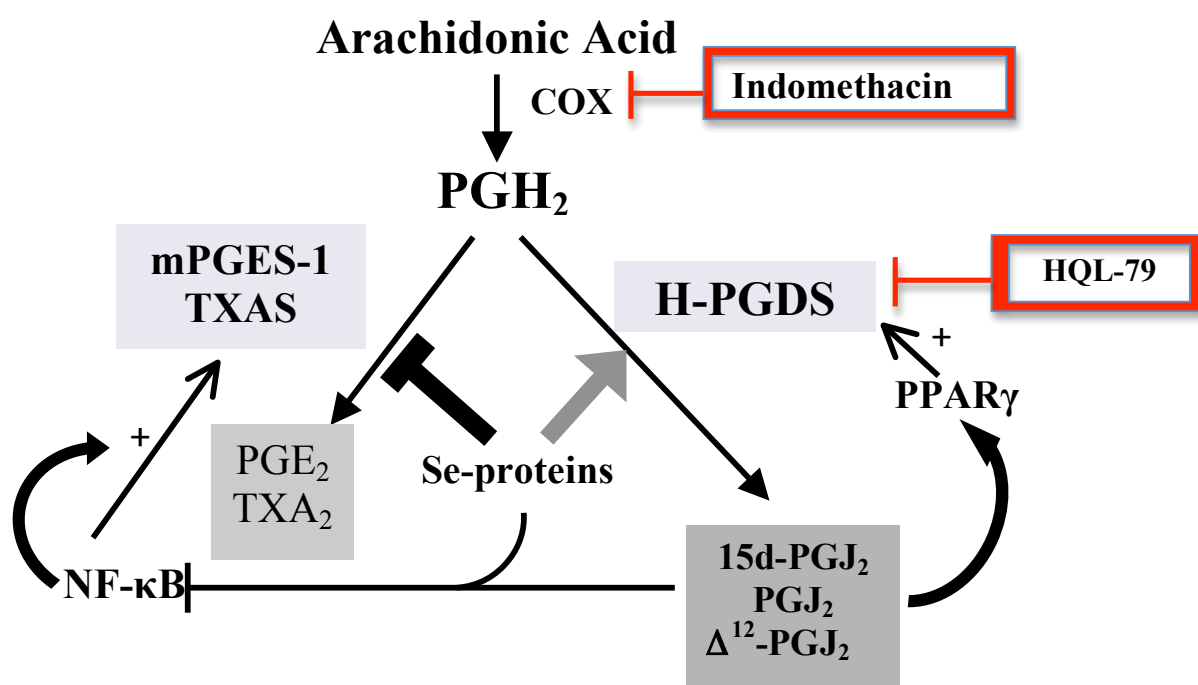
below). An increase in HAT activity has also been associated with asthma and COPD while histone deacetylation has shown to decrease inflammatory responses, further providing evidence that histone acetylation can regulate inflammatory pathways (Dekker et al., 2009).

### **Selenium and Inflammation**

While literature has shown that decreased HAT activity can play a role in reducing inflammation, our lab has also shown that selenium supplementation can activate anti-inflammatory pathways in macrophages. Inflammation in the body can be triggered when arachidonic acid is released by membrane phospholipids through hydrolysis catalyzed by phospholipase A<sub>2</sub>. Through cyclooxygenase (COX) 1 or 2 activities, the arachidonate is converted to prostaglandin (PG) G<sub>2</sub> (via the cyclooxygenase activity of COX enzymes), and then to PGH<sub>2</sub> (via the peroxidase activity of COX enzymes). Without selenium supplementation, PGH<sub>2</sub> is converted to pro-inflammatory prostaglandins and thromboxanes mediated by NF-κB, as mentioned earlier. However, in the presence of selenium, the hematopoietic prostaglandin D synthase enzyme (H-PGDS) catalyzes the production of PGD<sub>2</sub> from PGH<sub>2</sub>, which finally undergoes spontaneous dehydration to form PGJ<sub>2</sub>, Δ<sup>12</sup>-PGJ<sub>2</sub>, and 15-deoxy-Δ<sup>12,14</sup>-prostaglandin J<sub>2</sub> (15d-PGJ<sub>2</sub>). 15d-PGJ<sub>2</sub> acts as an endogenous ligand for the nuclear hormone receptor PPARγ, which can depress the inflammatory responses mediated by the NF-κB pathway (Vunta et al., 2008). Our lab has shown that selenium supplementation increases the production of 15d-PGJ<sub>2</sub> in macrophages as an anti-inflammatory response, reducing pro-inflammatory gene expression and protecting the cells against oxidative stress (Vunta et al., 2007). See figure 1-2 below for an overview of the arachidonic acid pathway.

Interestingly, literature has shown that 15d-PGJ<sub>2</sub> and other H-PGDS metabolites (PGJ<sub>2</sub> and Δ<sup>12</sup> PGJ<sub>2</sub>) are not only potent inhibitors of the NF-κB pathway, but also inhibit the function

of HATs (Hironaka et al., 2009). It has been shown that the soluble forms of the HATs p300, Cbp, and PCAF were reduced and converted to insoluble forms in the presence of 15d-PGJ<sub>2</sub>, reducing H3 and H4 histone acetylation and preventing gene expression (Hironaka et al., 2009). It is suggested that 15d-PGJ<sub>2</sub> mediates the reduction of pro-inflammatory NF-κB- dependent gene expression by both inactivating NF-κB while also decreasing the amount of functional HATs (Hironaka et al., 2009).



**Figure 1-2.** Overview of the Arachidonic Acid Pathway with Selenium Supplementation

### Indomethacin and HQL-79

Indomethacin, a non-steroidal anti-inflammatory drug (NSAID), is commonly used to reduce fever, swelling, and pain. Indomethacin functions by blocking the production of pro-inflammatory prostaglandins, but also anti-inflammatory prostaglandins like 15d-PGJ<sub>2</sub> (see figure 1-2 above). It is an inhibitor of both COX-1 and COX-2 pathways. Our laboratory

specifically has shown that COX-1 is responsible for an increase in production of 15d-PGJ<sub>2</sub> and other metabolites of H-PGDS upon selenium supplementation (Vunta et al., 2007). Therefore, COX-1 inhibition by indomethacin can reduce the production of H-PGDS products.

While indomethacin inhibits COXs, HQL-79 is a specific inhibitor for H-PGDS. Research has shown that HQL-79 acts as a competitive inhibitor against PGH<sub>2</sub>, inhibiting the production of PGD<sub>2</sub> catalyzed by H-PGDS (Aritake et al., 2006). HQL-79, like indomethacin, also causes a decrease in the synthesis of 15d-PGJ<sub>2</sub> and other H-PGDS metabolites.

### **Research Hypotheses**

Given that previous research has indicated that selenium treatments increase the production of 15d-PGJ<sub>2</sub> and other H-PGDS metabolites, and that these metabolites inhibit the function of HATs, we have examined the effects of selenium on histone (H4) acetylation. It is hypothesized that selenium supplementation will result in a decrease in histone acetylation in macrophages, which play an important role in immune regulation and inflammatory response. Macrophages will first be treated with bacterial endotoxin lipopolysaccharide (LPS), which causes macrophages secrete pro-inflammatory enzymes. However, selenium supplementation should activate the production of anti-inflammatory H-PGDS metabolites, inhibiting the HATs and causing a reduction in histone acetylation. It is also hypothesized that indomethacin and HQL-79 treatments, by inhibiting COX and H-PGDS respectively, will lead to a decrease in HAT inhibition compared to selenium treatments. The effects of selenium on HepG2 cells, a human liver carcinoma cell line, will also be examined to look for decreases in histone acetylation.

## **Chapter 2**

### **Materials and Methods**

#### **RAW 264.7 Macrophage Cell Culture**

RAW 264.7 cells, a mouse macrophage-like cell line purchased from American Type Culture Collection (ATCC, Manassas, VA), were maintained in a 37°C incubator in a humidified atmosphere with 5% carbon dioxide. Cells were cultured using Dulbecco's Modified Eagle's medium (DMEM), supplemented with 5% fetal bovine serum (FBS), a 1% penicillin/streptomycin solution, and a 1% L-glutamine solution. For routine maintenance, cells were grown to a confluence of 80% in T-75 flasks with 10 mL of DMEM, changing the medium every other day. Cells were passaged every two days also using 10 mL of DMEM. For experimentation, cells were seeded into 6-well plates at a concentration of 250,000 cells per well using 2 mL of DMEM per well, and were allowed to adhere to the plates overnight before beginning treatments.

#### **HepG2 Cell Culture**

HepG2 cells, a human liver carcinoma cell line purchased from American Type Culture Collection (ATCC, Manassas, VA), were maintained in the same incubation conditions as RAW 264.7 cells above. Cells were cultured using GIBCO's Minimum Essential Medium (MEM), supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% L-glutamine. For routine maintenance, cells were grown to a confluence of 80% and were passaged twice a week while changing the medium every other day. Trypsin-EDTA (.25%) was used to remove HepG2 cells and passage them to new T-75 flasks. For experimentation, cells were seeded into 6-well plates at a concentration of 1.0E6 cells per well using 2 mL of MEM, and remained in the incubators

overnight before beginning treatments.

### **Treatments for RAW 264.7 Cells**

For selenium treatments, after overnight incubation, the RAW cells were treated with lipopolysaccharide (LPS) at a final concentration 50 ng/mL per well for 30 minutes. After 30 minutes, the medium with LPS was removed, cells were washed twice with complete media, and replaced with 2 mL of fresh DMEM per well, and each well was then supplemented with a sodium selenite solution at a final concentration of either 0 nM (untreated), 100 nM, 250 nM, 500 nM, or 1  $\mu$ M. Cells were treated with the sodium selenite solution for 3 days, replacing the medium every 24 hours with fresh DMEM and sodium selenite. At the end of three days of treatment, each cell well was scraped, and cells were harvested into 1.5 mL Eppendorf tubes and immediately placed on ice in preparation for histone isolation.

For HQL-79 (an inhibitor of H-PGDS) and indomethacin (and inhibitor of COXs) treatments, RAW cells in 6-well plates were again treated with LPS at a final concentration of 50 ng/mL per well for 30 minutes. After 30 minutes and replacing the medium, each cell well was either supplemented with DMSO, 500 nM HQL-79, 500 nM indomethacin, or 500 nM of sodium selenite. The two wells containing either HQL-79 or indomethacin were additionally treated with a sodium selenite solution at a final concentration of 500 nM. Cells were treated with their respective compounds for 3 days, changing the medium each day. At the end of treatment, cells were again scraped and harvested into 1.5 mL Eppendorf tubes, and placed on ice for histone isolation.

### **Treatments for HepG2 Cells**

After seeding into 6-well plates, HepG2 cells were supplemented with a sodium selenite

solution at a final concentration of either 0 nM (untreated), 100 nM, 250 nM, 500 nM, 1  $\mu$ M, or 2  $\mu$ M for three days. The medium was changed every 24 hours and fresh sodium selenite was added. At the end of treatment, cells were scraped and harvested into 1.5mL Eppendorf tubes and placed on ice for histone isolation.

### **Histone Isolation**

Both RAW 264.7 cells and HepG2 cells were isolated for histones using the same protocol from Balasubramanyam et al., 2004. After being harvested into 1.5 mL Eppendorf tubes, cells were centrifuged at 2000 rpm for 10 minutes at 4°C. The supernatant from each tube was removed, and the cell pellets were washed in 700  $\mu$ L of ice-cold buffer A, made of 150 mM KCl, 20 mM HEPES at a pH of 7.9, .1 mM EDTA, and 2.5 mM MgCl<sub>2</sub>. The resuspended cell pellets were centrifuged again at 2000 rpm for 10 minutes at 4°C. The supernatant from each tube was removed, and cell pellets were resuspended in 700  $\mu$ L of ice-cold buffer B, made of 150 mM KCl, 20 mM HEPES at a pH of 7.9, .1 mM EDTA, 2.5 mM MgCl<sub>2</sub>, 250 mM Sucrose, and 1% Triton-X-100. Cell lysis was allowed to proceed for one hour on ice.

After one hour, each solution was centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatants were discarded, and cell pellets were resuspended in 700  $\mu$ L of buffer A as used earlier. HCl was added to each solution for a final concentration of 0.25 M to allow for acid extraction of the histone proteins. Extraction was continued by incubating the samples on ice for one hour followed by mixing of the samples every 15 minutes. The samples were then centrifuged at 12000 rpm for 20 minutes at 4°C, and the supernatants were collected to obtain the acid soluble proteins. 25% (v/v) trichloroacetic acid was added to each supernatant, and the samples were allowed to incubate on ice for 30 minutes.

Following incubation, samples were centrifuged at 12000 rpm for 30 minutes at 4°C,



supernatants were removed, and the remaining cell pellets were washed with 500  $\mu$ L of ice-cold acidified acetone (20  $\mu$ L of 12 N HCl was added to 100 mL of acetone). The samples were again centrifuged at 12000 rpm for 30 minutes at 4°C. The supernatant from each sample was removed one final time, and the pellets were allowed to air-dry for 20 minutes. The pellets were dissolved in 25  $\mu$ L of distilled water, and incubated in a 37°C water bath for 20 minutes. Protein content in the samples was then estimated as described below.

### **Protein Estimation**

A bovine serum albumin set was used to estimate protein content. 3  $\mu$ L of each sample was added to one well of a 96-well plate, and 7  $\mu$ L of Milli-Q water was then added to each sample well. 10  $\mu$ L of each pre-diluted protein assay standard (at 2000, 1500, 1000, 750, 500, 250, 125, and 25  $\mu$ g/mL concentrations) was added to a separate column of the 96-well plate. 150  $\mu$ L of Pierce 600 nm Protein Assay Reagent was added to each well containing either the sample or standard. The 96-well plate was incubated for 5 minutes and then absorbance was recorded at 620 nm using a Spectra Count plate reader (Packard-Perkin Elmer Instruments). The absorbance values were used to calculate the concentration of protein (in  $\mu$ g/ mL) per sample using a standard curve based on the standard absorbance values.

### **SDS- PAGE Sample Preparation**

Given the calculated protein concentration of each sample, further calculations were made to determine load volumes to create a new set of samples for SDS-PAGE, with each new sample containing 10  $\mu$ g of protein. Each new sample set was made with both the calculated load volume for the protein and with Milli-Q water so that the sample contained 15  $\mu$ L of solution. 3  $\mu$ L of 3X SDS-PAGE loading dye was added to each sample for a total of 18  $\mu$ L, and

all samples were heated on a VWR heat block for 5 minutes. Duplicate sets of samples were made and frozen at -80°C for future use.

### **Electrophoresis and Immunoblotting**

Using the prepared sample sets, samples were loaded onto a 12% SDS-PAGE gel. SDS-PAGE was carried out for 60 minutes using 500 mL of a 5% SDS-PAGE running buffer, made of 15.1 Tris base, 72 g glycine, and 5 g SDS per 1000 mL of Milli-Q water. The proteins were then transferred to a nitrocellulose membrane for 90 minutes using a 1X transfer buffer, made of 12.1 Tris base, 57 g glycine, and 800 mL of methanol in 4 L of water.

A 5X Tris-Buffered Saline (TBS) solution was made for blocking the membranes by adding 12.1 g Tris base to 8.7 g NaCl and 1 liter of water, and adjusting the pH to 7.5. 1 mL of Tween-20 was added to every 1 L of diluted (1X) TBS to make a 0.1% Tris-Buffered Saline Tween-20 (TBS-T) solution. After the transfer was complete, membranes were blocked in a 5% milk solution made of 0.1% TBST and powdered milk for one hour. The membranes were then treated with a primary polyclonal rabbit antibody specific for H4K5, H4K8, H4K12, H4K16, or Total H3 overnight at 10°C.

After adding the primary antibody, membranes were washed using 10 mL of 0.1% TBS-T buffer for 10 minutes. Three washes were performed for each membrane for a total of 30 minutes of washing time, and were then probed with a secondary anti-rabbit antibody. The membranes were left on a rocker for one hour, and 3 more 10-minute washes were then given.

### **Autoradiography and Densitometric Analysis**

In order to visualize the bands of proteins on each membrane, a chemiluminescence substrate assay kit was used. 3 mL total substrate was added to each membrane and placed on a

rocker for 10 minutes. The bands were then visualized with HyBlot CL autoradiography film using a medical film processor. After obtaining results, the membranes were stripped using 10 mL of Restore Membrane Stripping Buffer and incubated at 37°C for 45 minutes while shaken at 90 rpm. Three more 10-minute washes with 0.1% TBS-T buffer were given, and then the membrane was treated with a different primary antibody. Membranes were also stored in a 10% (v/v) ethanol solution at 4°C for future use.

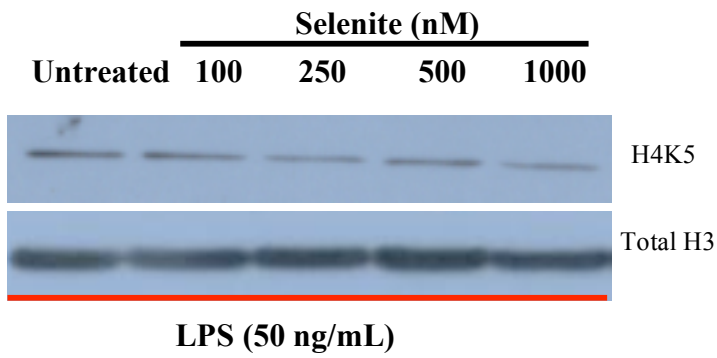
For further analysis, films were scanned and the bands were analyzed using an Image J densitometric analysis program (National Institutes of Health, MD). Each set of protein values were compared to total H3 protein levels to obtain a normalized set of values, which can be used to show trends in histone acetylation. For RAW 264.7 macrophage cell samples, three repetitions were performed. The densitometry results for each sample set were then averaged, and t-tests were performed to show statistically significant differences in treated samples compared to the controls ( $p < .05$ ).

## Chapter 3

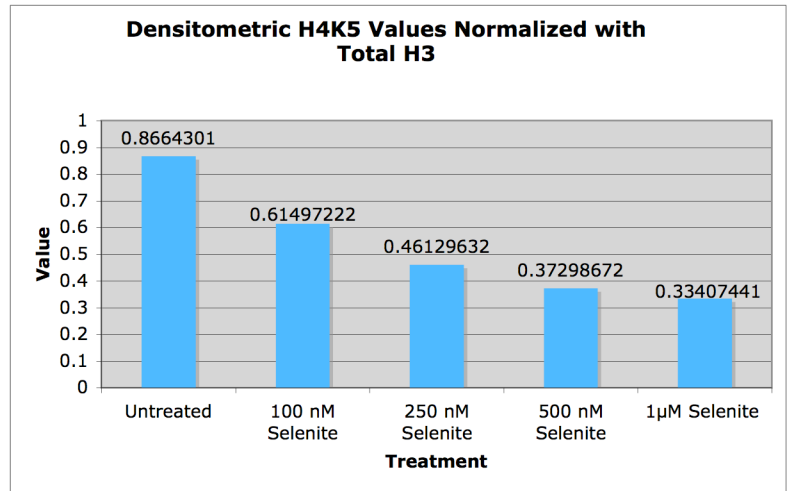
### Results

#### Results for RAW 264.7 Macrophages: Selenium and LPS Stimulation

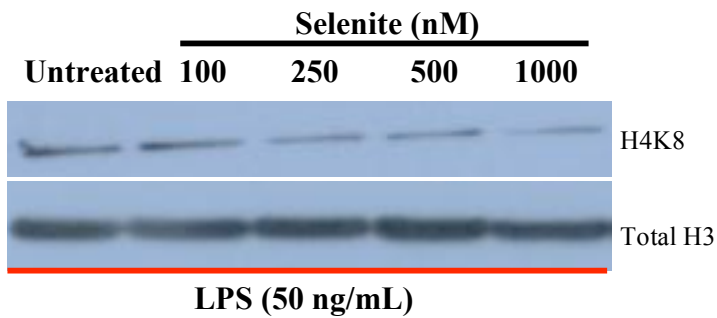
Given that previous studies in the laboratory have shown that selenium treatment in macrophages leads to an increased production of H-PGDS metabolites (PGJ<sub>2</sub>,  $\Delta^{12}$ -PGJ<sub>2</sub>, and 15d-PGJ<sub>2</sub>), and that recent literature has shown that these metabolites serve as endogenous inhibitors of HATs, we have examined HAT activity in selenium supplemented cells. Following selenium and LPS treatments, averaged densitometry results from three westerns showed an overall decline of 57.4% in H4K5 acetylation with increasing sodium selenite concentrations (see Figure 3-9 below). A decrease in histone acetylation at H4K8, H4K12, and H4K16 by 50.1%, 85.9%, and 58.1%, respectively was also seen (see Figure 3-9) when comparing the percent acetylation of untreated samples to the samples treated with the highest levels of sodium selenite (1  $\mu$ M). The individual densitometry results in Figures 3-2, 3-4, 3-6, and 3-8 additionally show a clear decreasing acetylation trend at all four lysine residues with increasing sodium selenite concentrations. It is important to note that there is a statistically significant decrease in histone acetylation levels for all samples treated with either 100 nM, 250 nM, 500 nM, or 1  $\mu$ M of selenite compared to the control (untreated samples). See the following page for Western blot and densitometry results.



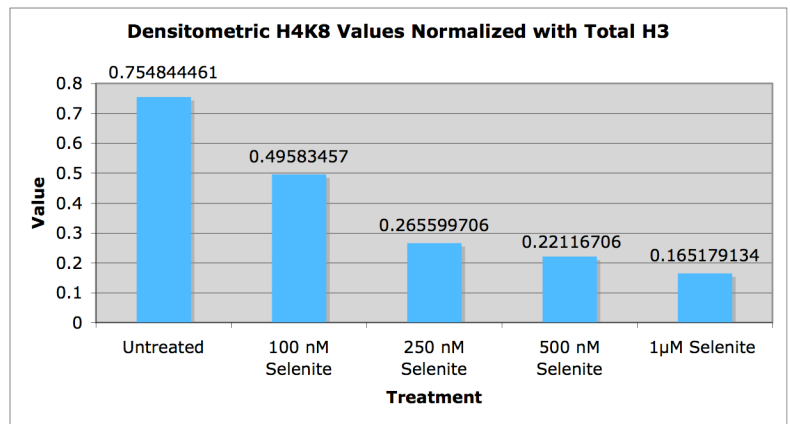
**Figure 3-1.** RAW 264.7 Cells: Western blot for H4K5 after Selenium and LPS Treatments



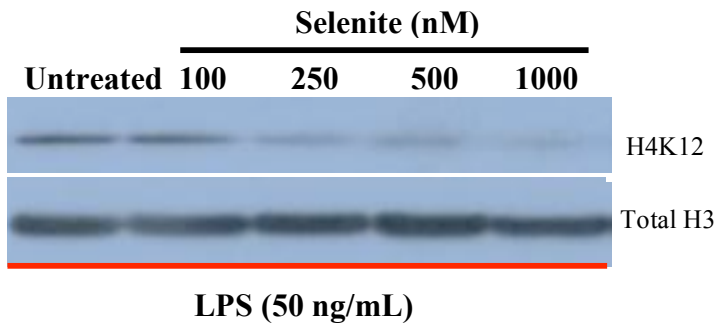
**Figure 3-2.** Densitometry Results for one H4K5 Western blot after Selenium and LPS Treatments



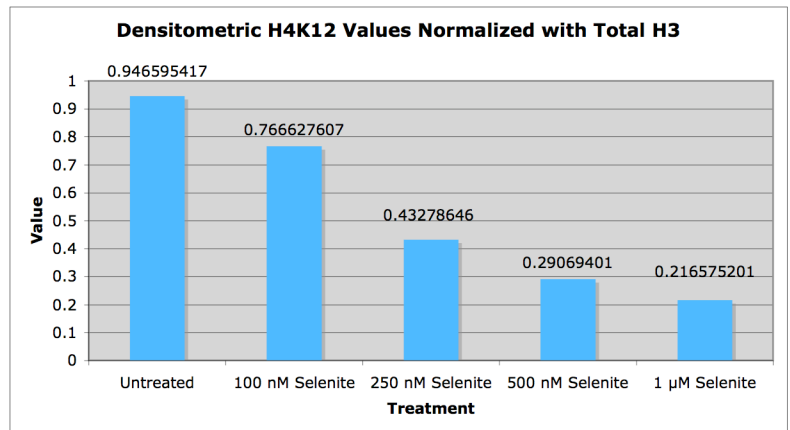
**Figure 3-3.** RAW 264.7 Cells: Western blot for H4K8 after Selenium and LPS Treatments



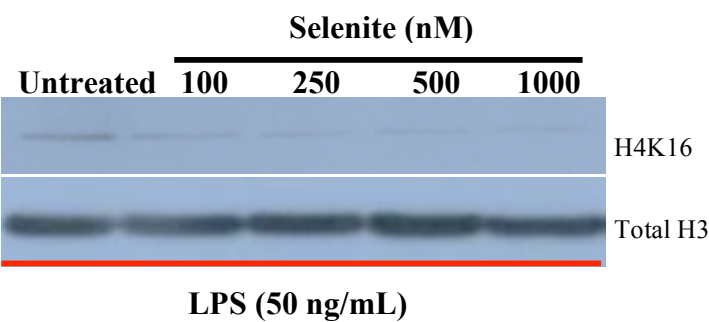
**Figure 3-4.** Densitometry Results for one H4K8 Western blot after Selenium and LPS treatments



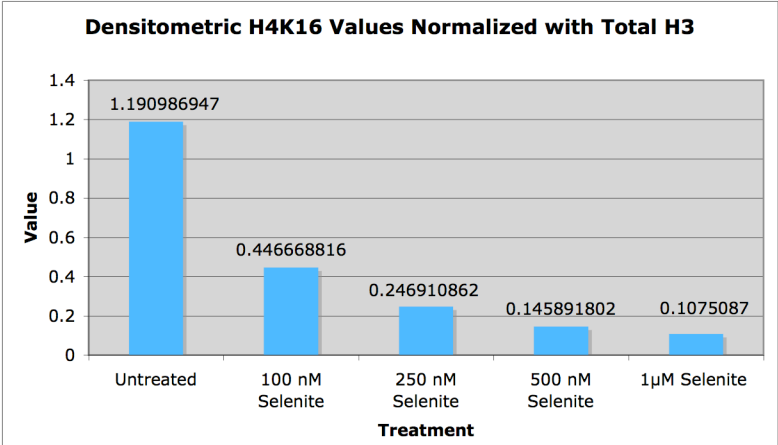
**Figure 3-5.** RAW 264.7 Cells: Western blot for H4K12 after Selenium and LPS Treatments



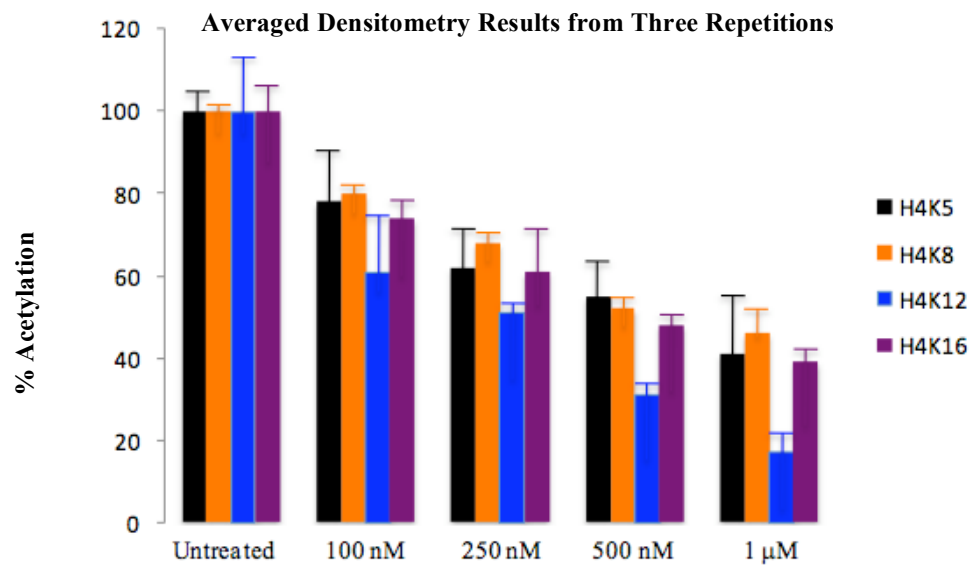
**Figure 3-6.** Densitometry Results for one H4K12 Western blot after Selenium and LPS Treatments



**Figure 3-7.** RAW 264.7 Cells: Western blot for H4K16 after Selenium and LPS Treatments



**Figure 3-8.** Densitometry Results for one H4K16 Western blot after Selenium and LPS Treatments

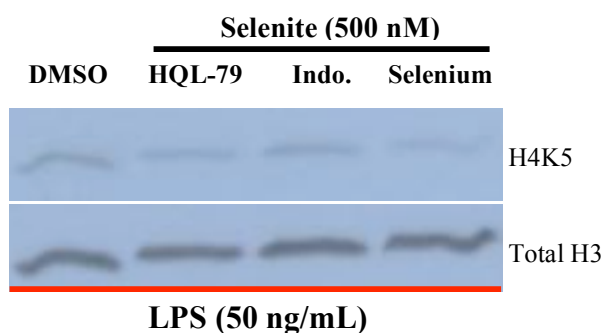


**Figure 3-9.** Densitometric analysis of total H3 versus acetylated H4. Mean  $\pm$  s.e.m. of three independent assays shown.  
**Note:** There are statistically significant differences for all selenium- supplemented samples when compared to untreated samples (statistical significance if  $p < 0.05$ )

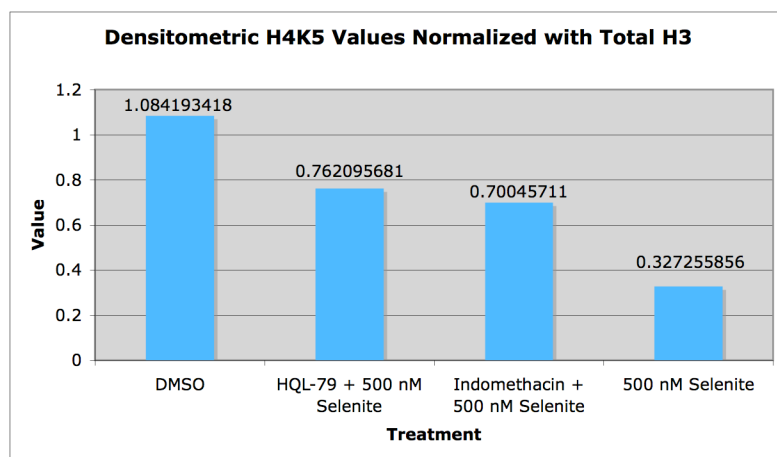
**Results for RAW 264.7 Cells: DMSO, HQL-79, Indomethacin, and Selenium Treatments with LPS Stimulation**

In addition to selenium supplementation, we have also examined the role of COX and H-PGDS inhibitors (indomethacin and HQL-79) on HAT activity. After treatments and averaging

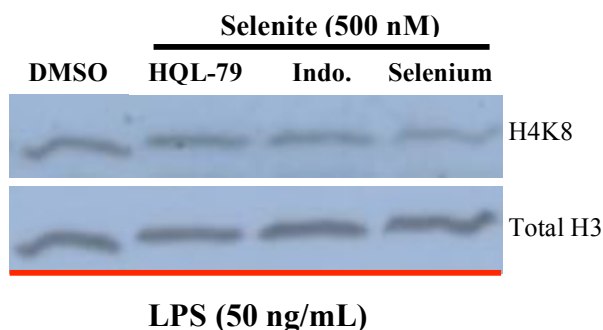
densitometric data for three repetitions, a statistically significant decline of 48.8% in H4K5 acetylation was seen in the 500 nM sodium selenite treated cells compared to the DMSO control (see Figure 3-18 below). H4K8, H4K12, and H4K16 acetylation also significantly decreased by 38.6%, 72.9%, and 52.2% respectively with just selenium supplementation (see Figure 3-18). Interestingly, densitometry results showed a smaller decrease in histone acetylation with indomethacin treatments compared to only selenium treatments. H4K5, H4K8, H4K12, and H4K16 acetylation decreased by only 10.4%, 11.1%, 15.1%, and 6.50% respectively with indomethacin and selenium treatments compared to the DMSO control. Similar results to indomethacin were seen with HQL-79 treatments, with H4K5, H4K8, H4K12, and H4K16 acetylation only decreasing by 13.7%, 9.37%, 13.1%, and 10.4% respectively compared to the DMSO control.



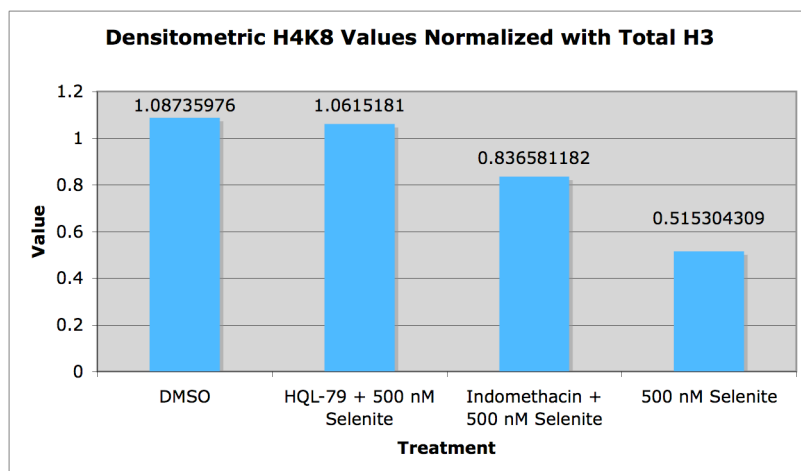
**Figure 3-10.** RAW 264.7 Cells: Western blot for H4K5 after LPS, Selenium, DMSO, HQL-79, and Indomethacin Treatments



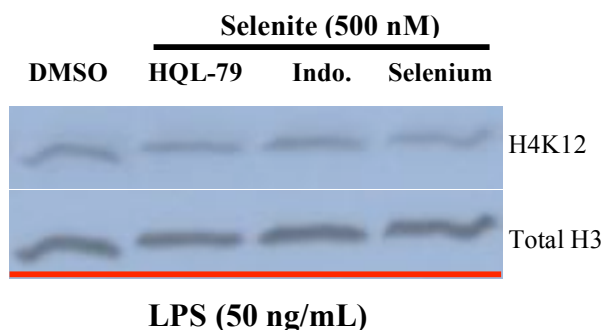
**Figure 3-11.** Densitometry Results for one H4K5 Western blot after LPS, Selenium, DMSO, HQL-79, and Indomethacin Treatments



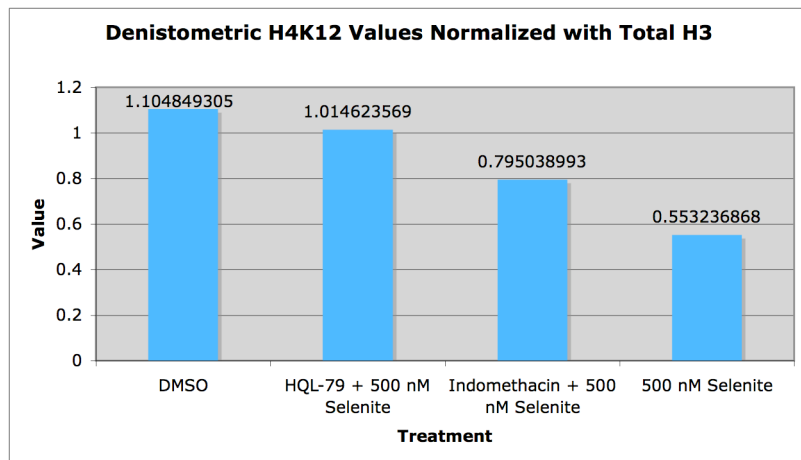
**Figure 3-12.** RAW 264.7 Cells: Western blot for H4K8 after LPS, Selenium, DMSO, HQL-79, and Indomethacin Treatments



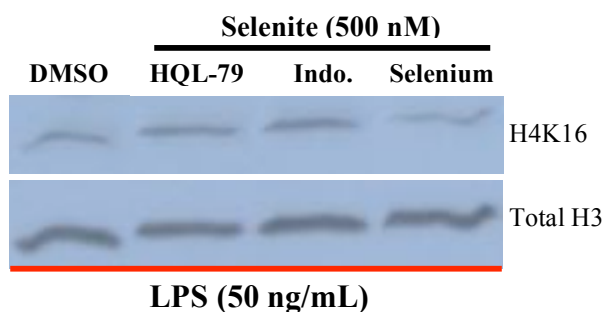
**Figure 3-13.** Densitometry Results for one H4K8 Western blot after LPS, Selenium, DMSO, HQL-79, and Indomethacin Treatments



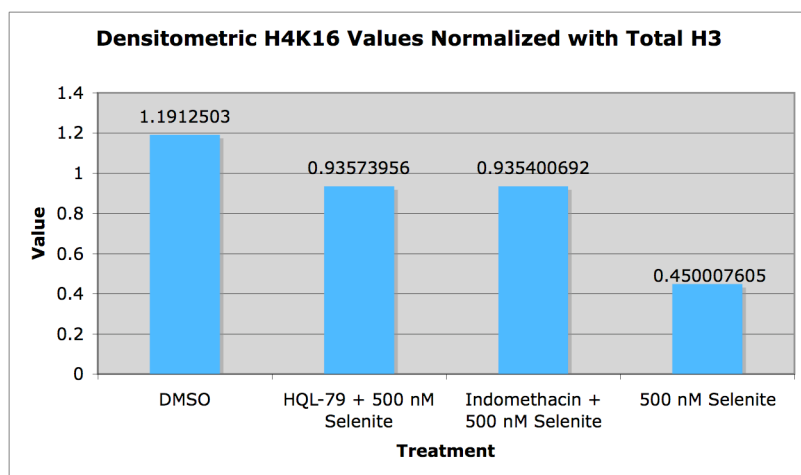
**Figure 3-14.** RAW 264.7 Cells: Western blot for H4K12 after LPS, Selenium, DMSO, HQL-79, and Indomethacin Treatments



**Figure 3-15.** Densitometry Results for one H4K12 Western blot after LPS, Selenium, DMSO, HQL-79, and Indomethacin Treatments

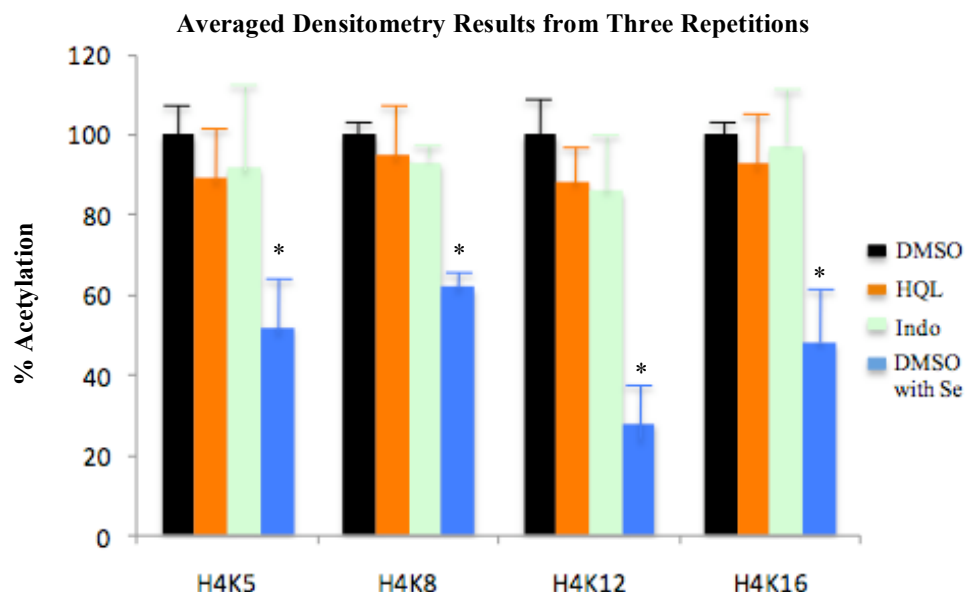


**Figure 3-16.** RAW 264.7 Cells: Western blot for H4K16 after LPS, Selenium, DMSO, HQL-79, and Indomethacin Treatments



**Figure 3-17.** Densitometry Results for one H4K16 Western blot after LPS, Selenium, DMSO, HQL-79, and Indomethacin Treatments

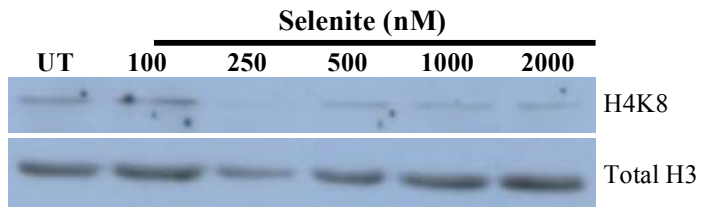




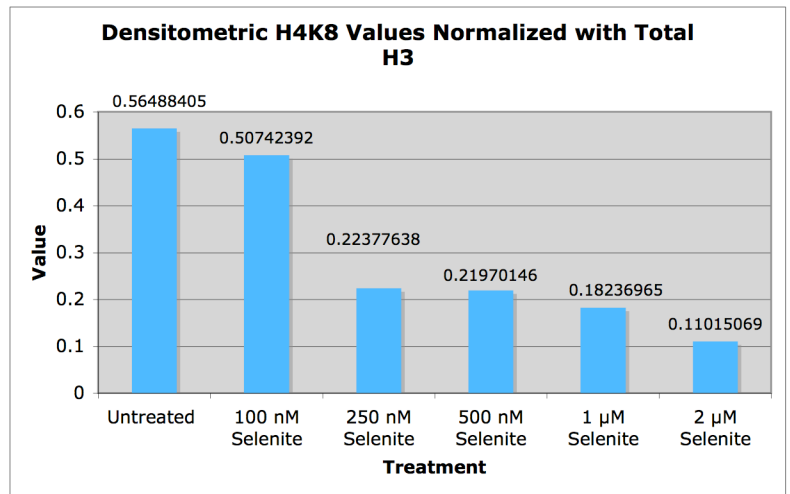
**Figure 3-18.** Densitometric analysis of total H3 versus acetylated H4. Mean  $\pm$  s.e.m. of three independent assays shown. \* Statistical significance compared to the DMSO control if  $p < 0.05$

### Results for HepG2 Cells: Selenium Treatments

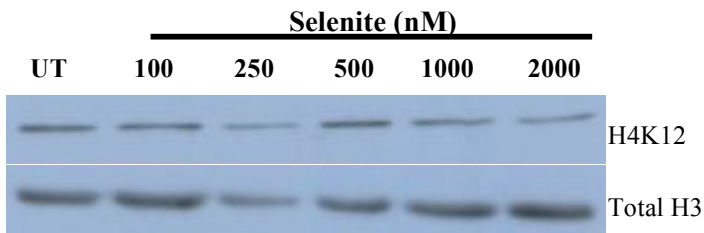
Similar histone acetylation results were expected for HepG2 cells compared to RAW 264.7 macrophages after selenium supplementation. Following selenium treatments, a decrease in histone acetylation at H4K8 and H4K12 was seen with increasing sodium selenite concentrations, like that in RAW 264.7 macrophages. Based on the densitometric evaluation of the Western blots (Figures 3-20 and 3-22), there was a decreasing histone acetylation trend seen with selenium supplementation. The untreated samples showed the highest histone acetylation levels while the samples treated with the highest selenium concentrations (2  $\mu$ M) showed the lowest levels. Comparing the untreated samples and those treated with 2  $\mu$ M of selenite, there was overall an 80.5% decrease in histone acetylation at H4K8, and a 55.4% decrease at H4K12, confirming the ability of selenium to modulate histone acetylation.



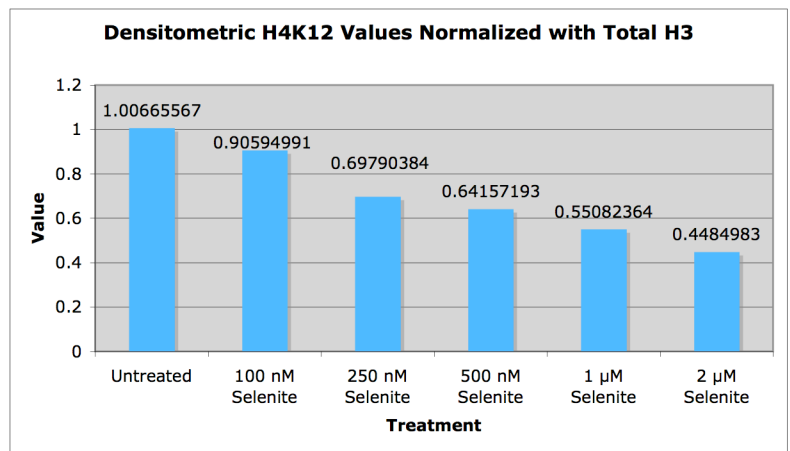
**Figure 3-19.** HepG2 Cells: Western blot for H4K8 after Selenium Treatments



**Figure 3-20.** Densitometry Results for H4K8 Western blot after Selenium Treatments



**Figure 3-21.** HepG2 Cells: Western blot for H4K12 after Selenium Treatments



**Figure 3-22.** Densitometry Results for H4K12 Western blot after Selenium Treatments

## Chapter 4

### Discussion and Future Applications

Recent studies have shown that selenium supplemented macrophages display an increased production of H-PGDS metabolites, which are potent inhibitors of the NF- $\kappa$ B pathway. Published literature has also shown that these metabolites, such as 15d-PGJ<sub>2</sub>, can inhibit HATs and result in a decrease in histone acetylation on lysine residues without selenium supplementation. Further literature has shown that HATs are also important for regulating NF- $\kappa$ B and transcription of inflammatory genes. Given these current studies, this investigation has provided a link between selenium and HAT activity that has not yet been extensively examined.

The findings from this study show that selenium supplementation in macrophages does in fact result in a statistically significant decrease in histone acetylation at H4K5, H4K8, H4K12, and H4K16 residues. Selenium treatments have lead to an increased production of 15d-PGJ<sub>2</sub>,  $\Delta^{12}$ -PGJ<sub>2</sub>, and PGJ<sub>2</sub>; the synthesis of these metabolites has consequently inhibited HAT activity, causing the decrease in acetylation levels seen in Figures 3-2, 3-4, 3-6, 3-8, and 3-9 above. There is an inverse relationship between the concentration of selenite treatment and the level of histone acetylation seen at each residue, showing that the higher the level of selenium supplementation, the further the HAT activity is reduced. Is it assumed that the HATs Cbp/p300, HBO1, Sc HAT1, Sc SAS2, Sc ESA1, and TIP60 are inhibited through increased selenium supplementation because they are responsible for histone acetylation at the K5, K8, K12, and K16 sites (see Figure 1-1 in the “introduction”), but it is possible that other HATs acetylating additional residues within the nucleosome are also affected by selenium treatments. Further analysis should be done to examine if additional HATs are inhibited through selenium supplementation at H3, H2B, and H2A.

HAT inhibition and a reduction in histone acetylation with increasing selenite concentration treatments was not only seen in macrophages, but also in HepG2 cells at H4K8 and H4K12 sites as seen in Figures 3-20 and 3-22 above. A similar decline in histone acetylation can be seen between the two cells lines with selenium supplementation, indicating that selenium does not solely affect cells playing roles in immune regulation and inflammation. Because HepG2 cells are a liver carcinoma cell line, selenium and HAT inhibition in these cells may be relevant for potential cancer therapies by reducing histone acetylation and downregulating oncogenic signaling. Current literature has shown that the expression of the CYP2E1 gene that induces cancer cell apoptosis in hepatocellular carcinoma cell lines is associated with the regulation of histone acetylation (Yang et al., 2009). Given our preliminary data, experiments could be performed to see the role of selenium on histone acetylation and the expression of this gene. Overall, the findings from both selenium supplemented macrophages and HepG2 cells show the importance of selenium in regulating HATs and H4 acetylation.

In addition to selenium treatments, our findings also show that indomethacin supplementation, a COX inhibitor, in addition to selenium supplementation does result in a decrease in the inhibition of HATs as expected. Histone acetylation levels at H4K5, H4K8, H4K12, and H4K16 in macrophages are higher with indomethacin-treated cells than solely selenium supplemented cells. Because indomethacin inhibits COX pathways, the production of the anti-inflammatory H-PGDS products is reduced, leading to a decrease in HAT inhibition and an increase in histone acetylation compared to macrophages treated with just selenium. Similar results were also found when macrophages were treated with HQL-79 and selenium. Because HQL-79 is an inhibitor of H-PGDS, the synthesis of the prostaglandins  $\text{PGJ}_2$ ,  $\Delta^{12}\text{-PGJ}_2$ , and  $15\text{-d-PGJ}_2$  is again reduced, leading to a decrease in the inhibition of HATs and increased levels of

histone acetylation compared to selenium-treated cells. These results add support that selenium and the increased production of H-PGDS products is important in regulating HATs and H4 acetylation in macrophages.

The results from these experiments may have several important implications in inflammation and disease. On a broader, more general scale, histone acetylation is important for gene activation and transcription. Selenium, by reducing histone acetylation, may be able to downregulate transcription by preventing DNA from entering a more open conformation state accessible to transcription factors. Specifically, selenium is important because it inhibits the activity of HATs, which are important cofactors for NF- $\kappa$ B. Decreasing histone acetylation at sites on genes involved in pro-inflammatory pathways can also prevent the activation of inflammation pathways in the body, further promoting the anti-inflammatory effects of selenium. Selenium may also play an important role in anti-cancer therapies. By inhibiting HATs like p300, selenium may be important in downregulating the growth of tumor cells by regulating transition states within the cell cycle. (Dekker et al., 2009). It is also interesting to note that while literature has shown that H-PGDS metabolites can lead to decreased levels of histone acetylation, we currently do not know exactly how these compounds inhibit HATs. Future studies should also be done to determine the mechanism for inhibition to see how these prostaglandins reduce histone acetylation. Selenium, HAT inhibition, and histone acetylation regulation therefore have many important applications that can be further explored in future experiments.

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## Academic Vita

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### Education:

The Pennsylvania State University, State College, PA

Bachelor of Science Degree in Biology, May 2011

Minor in Spanish

Honors in Biology

### Employment and Volunteer Experience:

*Undergraduate Research with Dr. K. Sandeep Prabhu*

August 2009- Present

Thesis Title: An Investigation of the Effects of Selenium on Histone Acetylation

*Mount Nittany Medical Center*

March 2008- Present

Emergency Department and Patient Floors Volunteer

- Assist doctors and nurses with patient care and equipment transport
- Transport patients for discharge and medical testing
- Provide a more comfortable setting for family members of patients
- Train new volunteers

*Biology Tutoring at Welsh Valley Middle School (Penn Valley, PA)*

May 2008-August 2008

- Work with students to recognize strengths and weaknesses
- Help middle school students struggling with biology improve their critical thinking skills and grades
- Help recent middle school graduates prepare for high school biology

*Thomas Jefferson University Hospital*

June 2007- July 2007

Extern

- Performed patient rounds and attended office hours with Dr. Michael Sperling, Director of the Jefferson Comprehensive Epilepsy Center
- Observed a temporal lobectomy

### Activities:

Alpha Epsilon Delta- Inducted Member, Honors Member

April 2009- Present

Schreyer Honors College Student Council- Tour Guide

November 2007- Present

THON Rules and Regulations Committee Member

October 2007- Present

- Family Pass Leader

### Honors:

Phi Beta Kappa- Inducted Member

February 2011- Present

Phi Kappa Phi- Inducted Member

January 2010- Present

President's Freshman Award for Academic Excellence

April 2008

Dean's List All Semesters

December 2007- Present

Schreyer Honors College Academic Excellence Scholarship

August 2007- Present