THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

REGULATION OF TRANSCRIPTION ELONGATION BY THE SUPER ELONGATION COMPLEX

KATIE CAMPBELL
SPRING 2014

A thesis submitted in partial fulfillment
of the requirements
for a baccalaureate degree
in Biochemistry
with honors in Biochemistry.

Reviewed and approved* by the following:

David S. Gilmour
Professor of Molecular Biology
Honors Adviser and Thesis Supervisor

B. Franklin Pugh
Willaman Chair of Molecular Biology
Second Reader

Richard Frisque
Interim Department Head for Biochemistry and Molecular Biology

*Signatures are on file in the Schreyer Honors College.

Abstract

Understanding the regulation of gene transcription allows us to better analyze gene activity and function. Using Drosophila as a model system, I am studying the function of specific proteins involved in the transcriptional elongation control checkpoint. Normally, RNA polymerase II is paused at the promoters of hsp70 genes, repressed by negative elongation factors. Positive transcription elongation factor, P-TEFb, is thought to reactivate RNA polymerase II from its paused state in order to allow transcription to occur. P-TEFb associates with ELL and Lilli in the Super Elongation Complex. To investigate the role of these proteins in transcription of hsp70, I used RNA interference to deplete P-TEFb, ELL, and Lilli to monitor the effects on the expression of an hsp70 reporter gene. Depletion of these proteins resulted in marked inhibition of the hsp70 reporter gene. To assess if this inhibition was due to defects in Pol II behavior, I analyzed the association of Pol II with hsp70 in polytene chromosomes derived from the salivary glands of heat shocked larvae. P-TEFb-depleted glands were not amenable to this immunofluorescence analysis because they were too small. ELL- and Lilli- depleted glands did not exhibit any obvious changes in Pol II levels at hsp70, but exhibited a striking loss of phosphorylation of serine 2 in the carboxyl terminal domain of the largest subunit of Pol II. Phosphorylation of serine 2 in the CTD of Pol II has been correlated with the activation of the paused Pol II, but my results suggest that it is not essential for activation of the paused Pol II at the hsp70 gene.

Contents

List of Figures

Chapter 1

Introduction

1.1 RNA polymerase II

An understanding of transcriptional control by RNA polymerase II (Pol II) is essential to understanding gene expression. While it was originally thought that Pol II initiation was the key step to transcriptional regulation, regulation of transcriptional elongation is now recognized as a major point of regulation in higher eukaryotes.[1–3] Transcription is initiated by the recruitment of Pol II to a gene's promoter, but it has been found that Pol II pauses at the 5' end of most protein-encoding genes 30-40 nucleotides downstream of the transcription start site in mammals and *Drosophila*.[4–7] Many of these genes are developmentally regulated, suggesting that the regulation of paused Pol II plays a role in expression and development.[5,8,9] DRB sensitivity-inducing factor (DSIF) and negative transcription elongation factor (NELF) coordinate the pausing of Pol II in the promoter-proximal region.[10,11] The Spt5 subunit of DSIF and the E subunit of NELF are phosphorylated.[12–15] Phosphorylated NELF dissociates from Pol II, while phosphorylated Spt5 remains associated with the elongation complex.[16] This modification to Spt5 converts DSIF into a positive elongation factor, allowing Pol II to proceed past the transcriptional elongation control checkpoint.[17] Additional phosphorylations to Pol II are also thought to contribute to its release from the promoter-proximal region. In particular, phosphorylation of serine 2 in the carboxyl ter-

minal domain of the largest subunit of Pol II by P-TEFb has been found to correlate with activation of the paused Pol II. However, direct evidence showing that the failure to phosphorylate serine 2 of the CTD blocks elongation is lacking, since the phosphorylation has never been uncoupled from the phosphorylation of DSIF or NELF.

The *hsp70* promoter of *Drosophila melanogaster* is one of the best studied examples of promoter-proximal pausing.[18] Heat shock causes transcriptional activation by the heat shock factor (HSF) that induces the release of the paused polymerase from the heat shock promoter.[18] In *Drosophila*, heat shock causes rapid recruitment of HSF and other proteins involved with phosphorylating Pol II, including P-TEFb.[14,19,20] In addition, there is a correlation between polymerase at *hsp70* promoters and phosphorylation patterns on the carboxy-terminal domain (CTD) of the largest subunit in Pol II before and after heat shock. In *D. melanogaster*, the CTD contains the amino acid sequence, Tyr-Ser-Pro-Thr-Ser-Pro-Ser, 42 times in a tandem array.[21] Serine 5 in the sequence is phosphorylated by TFIIH during the initiation step that must precede pausing in nonheat shocked cells.[22–25] Phosphorylation of serine 2 by P-TEFb correlates with the release of the paused Pol II for elongation

1.2 The Super Elongation Complex

The Super Elongation Complex (SEC) is thought to associate with paused Pol II to promote transcriptional elongation. The SEC consists of elongation factors ELL1 to ELL3, kinase P-TEFb, AFF1, AFF4, ENL, and AF9.[26] The SEC is necessary for proper induction of human *hsp70* promoter genes upon heatshock, as well as *Hox* genes during early developmental stages.[5,26–28] In various cancers, components of the SEC are known as translocation partners of the Mixed Lineage Leukemia (MLL) gene, resulting in fusion proteins that misregulate transcription. The human MLL gene, found on chromosome 11q23, participates in chromosomal rearrangements that result in many myeloid and lymphoblastic leukemias.[29,30] The SEC was discovered in part by characterizing MLL chimeric complexes.[26] The ELL1 gene, found on chromosome 19p13.1, participates in

a translocation that has been identified in hematological malignancies.[31] Some of the other partners include AFF1, AF9, ENL, and AF10.[29,30] The fusion partners, once associated with the rest of the SEC, can misactivate *Hox* genes and other MLL target developmental genes.[7,26,32,33] Based upon the properties of translocation partners of MLL, it is suggested that misregulation of elongation and H3K79 methylation could contribute to leukemogenesis in patients with these chromosomal rearrangements.[34–39]

1.2.1 P-TEFb

Positive transcription elongation factor b (P-TEFb) associates with the *hsp70* gene during heat shock and appears to activate the paused Pol II. P-TEFb is generally inactivated within a complex with 7SK-RNA, MEPCE, LARP7, and HEXIM1. While the majority of P-TEFb is rendered inactive in this complex, it has been found in an active form in two different complexes. Its association with the bromodomain protein BRD4 allows P-TEFb to phosphorylate the Pol II CTD *in vitro* and this association is necessary for HIV transcription.[40,41] P-TEFb has also been identified in its active form in the SEC.[26] Two components of P-TEFb, cyclin-dependent kinase 9 (Cdk9) and cyclin T1 (CycT1), act as the catalytic and regulatory subunits of the complex, respectively. Cdk9 participates in the hyperphosphorylation of the Pol II CTD at the serine 2 residues.[42] In addition, P-TEFb is responsible for phosphorylating negative elongation factors DSIF and NELF.[12–15]

1.2.2 ELL

The eleven-nineteen lysine-rich leukemia (ELL) gene encodes a protein that associates with Pol II during transcriptional elongation.[31] Its presence increases the catalytic rate by releasing Pol II from positions where Pol II transiently pauses because of the underlying sequence of the DNA.[43] There are three ELL family members identified in humans: ELL1, ELL2, and ELL3.[3,44,45] There is only one ELL family member found in Drosophila, dELL, that has been recognized as a Pol II elongation factor.[46,47] There are two identified ELL associated factor (EAF) proteins, EAF1 and EAF2, which colocalize with ELL in the nucleus.[48,49] These proteins are known to interact with

the SEC as positive regulators of ELL elongation activity.[50]

1.2.3 AFF4 and its Drosophila homolog, Lilli

AF4/FMR2 family member 4 (AFF4) is necessary for SEC, as it associates directly with P-TEFb and AF9, providing the scaffold for the complex's formation.[26,37,51] In mammals, there are four identified AFF (AFF1 through AFF4), which contain conserved N- and C-terminal regions, a serine-rich transactivation, and ALF homology regions.[52] This ALF homology domain promotes the protein's degradation by the proteasome by interaction with ubiquitin ligases.[53–60] This allows better regulation and control over AFF stability, turnover, and biological function. AFF2 and AFF3 are both linked to human diseases, but are found in SEC-like complexes that regulate a different subset of genes than the SEC. AFF4 is the AFF family member most commonly associated with the SEC, and research has shown its depletion results in *hsp70* inhibition in humans. The AFF4 *Drosophila* homolog, Lilli, associates with Pol II during elongation on chromatin and is relocalized to *hsp70* loci upon heat shock.[61] Depletion of AFF4 by RNAi, like ELL, prevents proper activation of *hsp70* by heat shock.[26]

1.2.4 AF9

The ALL1-fused gene from chromosome 9 (AF9) is a transcription factor with a serine and proline-rich domain, a nuclear localization signal, and a transcriptional activation sequence.[62] It has homologous regions ENL and yeast ANC1.[63] ENL and ANC1 promote the kinase activity of P-TEFb and histone methyltransferase Dot1L, suggesting similar functions by AF9. ANC1 has not only been identified in association with the Pol II transcription complex, but also the SWI/SNF chromatin remodeling complex. ATP-dependent SWI/SNF is responsible for remodeling chromatin to facilitate transcription activator access.[64–66] Homologous regions to ANC1 indicate that these proteins may associate with a human SWI/SNF complex.[67,68]

1.3 Objectives of the current study

I set out to investigate the roles of SEC components in regulating Pol II at the *hsp70* gene in Drosophila during heat shock. I began by developing an *hsp70* reporter gene assay that could be used to easily screen various RNAi transgenes for their capacity to inhibit expression of the reporter gene in salivary glands. Using this assay, I was able to determine that available RNAi lines that targeted P-TEFb, ELL, and Lilli (AFF4) inhibited *hsp70* expression in glands. The purpose for choosing salivary glands in the first place was so that I could then analyze how depletion of SEC components affected the association of Pol II with the *hsp70* loci of polytene chromosomes. My results show that depletion of ELL or Lilli results in a striking loss of serine 2 phosphorylation on the Pol II, but the overall level of Pol II appears similar to the undepleted specimens. Thus, serine 2 phosphorylation of the CTD may not play a major role in the activation of the paused Pol II.

Chapter 2

Materials and Methods

2.1 Fly lines

Fly lines contained RNAi transgenes, indicated by their names. RNAi fly lines obtained from the Vienna Drosophila Research Center (VDRC) include HSFi (VDRC48691), Cdk9i (VDRC30449), CycT1 short hairpin (BL32976), Lilli RNAi (BL34592), HDAC3i#1 (VDRC-KK 107073), HDAC3i#2 (VDRC20184), SMRTERi(VDRC-KK 106701), Nurf301i (VDRC46645), TFIISi (VDRC22980), and Rpd3i (BL33725). The ELL RNAi fly line was obtained from Ali Shilatifard. The yw;Z243,1824 fly line contained an *hsp70*-beta gal transgene (Z243 provided by John T. Lis) and a transgene expressing Gal4 in salivary glands (1824, BDSC 1824 from the Bloomington stock center) on the same chromosome. The yw fly line served as a control, containing no RNAi transgenes.

2.2 Beta-galactosidase staining assay

The beta-galactosidase staining assay was performed as previously described.[69] I optimized it by modifying the duration of heat shock of yw;Z243,1824 control salivary glands so that the development of blue color did not reach full saturation in the control samples. This was necessary to detect the inhibitory effect of some of the RNAis on expression of the report. Optimal assay conditions were 30 minute heat shock at 36° C followed by a 30 minute recovery at room temperature.

2.3 Polytene chromosome squashing

The polytene chromosome squashing assay was adapted from Lis.[70] Two larvae were placed in a droplet of dissection buffer (130 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$) on a piece of parafilm and incubated for 10 minutes in a 36° C water bath. Larvae were dissected in dissection buffer and transferred to 10 ul of squashing solution (1.85% paraformaldehyde, 45% mM glacial acetic acid) on a siliconized coverslip and incubated for 10-20 minutes. A glass slide was placed over the coverslip and turned over. The coverslip was tapped and gently moved side-to-side in order to spread the chromosomes under the coverslip. Once the chromosomes were spread, the slide was flash frozen in liquid nitrogen. Slides were stored in 95% ethanol overnight.

2.4 Immunofluorescence analysis

Slides were soaked in TBS (150 mM NaCl, 10 mM Tris-Cl pH 7.5) for 5-10 minutes. The slides were transferred to fresh TBS for 5-10 minutes. Slides were blocked with 20 ul of blocking solution (10% Fetal Bovine Serum, 0.5% Sodium azide in TBS) in a humid chamber for one hour. Slides were washed twice in TBS for 5 minutes each. Primary antibodies were diluted 1:100 in blocking solution and 20 ul were used for staining of each slide. Primary antibodies utilized in these assays include: polyclonal Rpb3 and HSF, and monoclonal ARNA3 antibody for detection of Rpb1, 8WG16 antibody for detection of the CTD, H5 antibody for serine 2 phosphorylation, and H14 antibody for serine 5 phosphorylation. Slides were stained with primary antibodies for 2 hours in a humid chamber. Slides were washed twice with TBS for 10 minutes each. Fluorescently tagged secondary antibodies were diluted 1:100 in blocking solution and 20 ul were used for the staining of each slide. Slides were stained with secondary antibodies for one hour in a humid chamber; then washed twice with TBS for 10 minutes each. The slides were washed in TBS containing 1:100,000 Hoechst for 10 minutes and then washed with TBS for 20 minutes. The slides were mounted onto a coverslip with 10 ul of mounting solution (100mM Tris-HCl (pH8.5), 80%

glycerol, 2% n-propylgallate). Chromosome spreads were visualized by fluorescence microscopy.

Chapter 3

Results

3.1 RNAi depletion in salivary glands

Parent *Drosophila* fly lines were established by introducing RNAi transgenes controlled by a UAS promoter. Males of these type were mated with virgin females containing beta-galactosidase reporter genes regulated by an *hsp70* promoter (Z243) and a Gal4 transgene (1824) expressed specifically in the salivary glands. This mating scheme (Figure 3.1) was used in all crosses in order to establish RNAi depletion of target proteins. RNAi depletion was specific to the salivary glands, since the UAS promoter was only induced in these cell types. This allowed further analysis of trancription activity in the salivary glands. The glands could be assayed for beta-galactosidase activity due to activation of the *hsp70* promoter. In addition, the polytene chromosomes could be visualized by squashing salivary glands on slides and immunostaining for Pol II and its various phosphorylation sites.

3.2 Optimization of beta-galactosidase assay

The beta-galactosidase assay was used as a measure of *hsp70* expression after heat shock activation. Using yw;Z243,1824 third instar control larvae, optimization of this assay was done by modifying the recovery time after heat shock activation (Figure 3.2). Salivary glands were heat

shocked for 30 minutes. Recovery at room temperature allowed time for activation, transcription, and translation of hsp70-beta-galactosidase to occur. When dissection and staining occurred after 15 minutes of recovery (Figure 3.2A), there was less blue color produced than when the larvae were allowed to recover for 30 minutes (Figure 3.2B). Glands that were allowed to recover for 60 minutes showed staining intensity that was similar to the 30 minute recovery, so I decided to use 30 inutes for all subsequent experiments.

3.3 Beta-galactosidase screen for RNAi transgenes that impair hsp70 expression

Transcription of *hsp70* is induced by heat shock. This promoter drives the transcription of a beta-galactosidase reporter gene. Incubation with the X-gal substrate caused blue pigmentation in the glands. Higher amounts of blue color are an indirect indication of increased *hsp70* induced transcription. The yw mated with Z243, 1824 served as a negative control, since no RNAi transgene was present (Figure 3.3A). This beta-galactosidase screen identified factors that contribute to *hsp70* expression. As expected, RNAi depletion of HSF completely inhibited expression of the *hsp70* reporter gene (Figure 3.3B). Depleting the components of P-TEFb resulted in very low promoter activity, comparable to the level of activity in HSFi salivary glands (Figures 3.3C-D). There was a reduced level of activity upon RNAi depletion of ELL or Lilli in comparison to the yw control (Figure 3.3E-F). This lower level of blue pigment indicate that ELL and Lilli play a role in *hsp70* promoter activity and contribute to expression.

In order to verify that the blue staining results in this assay are a valid analysis of *hsp70* expression, reverse transcription qPCR analysis was used to show that lower levels of beta-galactosidase are indicative of reduced *hsp70* activation.[69] Histone deacetylases (HDACs) are generally thought to repress transcriptional activity.[71] Upon depletion of HDAC3 and its co-repressor protein, SMRTER, there were lower levels of beta-galactosidase (Figure 3.4). Two different HDAC3 RNAi fly lines were used, and both had reduced amounts of beta-galactosidase (Figure 3.4A-B). Depletion

of SMRTER by RNAi also showed lower activation of the *hsp70*-beta-galactosidase reporter gene (Figure 3.4C). Reverse transcription qPCR showed that mRNA levels of both hsp70 and hsp70-beta-galactosidase were reduced in the transgenic fly lines (Figure 3.4D-E). This quantification shows that the inhibition of *hsp70* expression is occurring at the transcription level. The decrease in levels of beta-galactosidase appears to be much greater than the decrease in mRNA between control and HDAC3 depleted samples. This indicates that there may be inhibition at the translational level or during RNA processing.

3.4 RNAi-mediated depletion of HSF from glands prevents Pol II accumulation at hsp70 loci of polytene chromosomes during heat shock

Immunofluorescence analysis of Pol II on polytene chromosomes provides a way to assess whether RNAi-mediated depletion of a protein affects the interaction of Pol II with the *hsp70* gene. To validate this approach I analyzed the effect of depleting HSF on the association of Pol II with *hsp70* during heat shock. During heat shock, Pol II and HSF accumulate at puffs corresponding to the *hsp70* loci (compare Figure 3.5A and 3.5B). In contrast, RNAi-mediated depletion of HSF results in the loss of both HSF and Pol II from *hsp70* (compare Figure 3.5C and 3.5D).

3.5 Detection of serine 2 phosphorylation and serine 5 phosphorylation in the CTD of Pol II on polytene chromosomes

Several antibodies are available to characterize Pol II on polytene chromosomes using immunofluorescence microscopy. The ARNA3 and Rpb3 antibodies monitor parts of Pol II that are expected to be unaffected by post-translational modifications and therefore should monitor all states of Pol II. Antibodies against different phosphorylated residues of the CTD could be indicative of the tran-

scriptional status of the Pol II. Ser5 phosphorylation but not Ser2 phosphorylation is associated with the paused Pol II near the transcription start site, whereas Ser2 phosphorylation is associated with actively elongating Pol II.

To test the various antibodies, I began by using the various antibodies to detect on polytene chromosomes from heat shocked, control cells. All of the antibodies detected Pol II at the hsp70 loci, but there were intriguing differences in the pattern of fluorescence. The staining by the ARNA3 antibody that targets the body of Rpb1 and the Rpb3 antibody gave largely overlapping patterns of fluorescence (Figure 3.6C). In contrast, Antibodies against phosphorylated serine 2 or serine 5 of CTD displayed staining the extended beyond the periphery of the region of staining from the Rpb3 antibody (Figure 3.6F and 3.6I). These modifications are located at opposite ends of the transcription unit so significance of this pattern of staining is unclear.

3.6 ELL depletion reduces Serine 2 phosphorylation

Depletion of ELL by RNAi inhibited expression of the *hsp70* beta-galactosidase reporter gene (Figure 3.3E). In order to determine if this might be due to a defect in transcription, I stained polytene chromosomes from heat shocked, ELL-depleted glands with antibodies against Rpb3 and CTD phosphorylation on serine 2. Serine 2 phosphorylation was chosen because it had been previously shown that this was reduced at heat shock puffs upon depletion of ELL, but the researchers never looked to see if this loss was due to the absence of Pol II or simply the absence of the modification.[72] In agreement with this previous report, serine 2 phosphorylation staining was absent from the *hsp70* heat shock puffs (Figure 3.7C). However, this absence appears to be due solely to the loss of the modification because antibody to Rpb3 indicates that Pol II still accumulates at the puffs. Moreover, since there is still an accumulation of Pol II at the puffs upon heat shock, it appears that the paused Pol II must become active in the absence of serine 2 phosphorylation so that additional Pol II molecules can accumulate on the gene.

3.7 Lilli depletion reduces Serine 2 phosphorylation

Heat shock loci were also visualized upon heat shock activation in the absence of Lilli. Lilli, the *Drosophila* homolog to AFF4, is the scaffold protein that stabilizes the SEC. Polytene chromosome squashes were prepared and immunostained for Rpb3 and serine 2 phosphorylations on the CTD (Figure 3.8). As in RNAi depletion of ELL, serine 2 phosphorylation staining was absent from the *hsp70* heat shock puffs. The Rpb3 antibody indicates that Pol II still accumulates at the puffs upon heat shock. This data validates the results seen upon ELL depletion; paused Pol II is still activatedin the absence of serine 2 phosphorylation.

3.8 Screening for other players in *hsp70* expression

ELL, Lilli, CycT1, and Cdk9 were analyzed more closely because of their association within the SEC, but other proteins associated with transcription were also analyzed. The beta-galactosidase assay was used as a screen to search for other proteins that may play a role in regulating transcription. By performing the beta-galactosidase assay on different transgenic RNAi lines, reduction in blue color was used as an indication of proteins that promote *hsp70* expression upon heat shock. Complete elimination of blue color, comparable to HSFi/yw;Z243,1824 (Figure 3.3B), would indicate that the protein depleted by RNAi was essential to *hsp70* activation. By screening the transgenic RNAi lines available with this assay, reduced levels of blue color and beta-galactosidase raises interest in how that particular protein effects transcription that results in lower levels of *hsp70* expression.

Two candidates found in this screening were Nurf301 and TFIIS. RNAi lines of these two proteins crossed with yw;Z243,1824 were found to have reduced beta-galactosidase levels after heat shock activation (Figure 3.9). This assay implicated these transcription factors as candidates for further analysis of heat shock-induced *hsp70* expression. Nucleosome remodeling factor, Nurf, has been recognized to facilitate transcription activation in vitro by working with transcription

factors to mobilize nucleosomes at promoter regions.[?,?] The Nurf complex is made up of four proteins: Nurf140, Nurf55, Nurf38, and Nurf301.[73–76] Nurf301 is the largest subunit and is related to the chromatin remodeling and assembly complex, ACF1/WCRF1.[77] Nurf301 is involved in transcription activation in vivo and proper induction of heat shock genes.[78] While studies have indicated that Nurf301 is necessary for proper induction of *hsp70* genes, there is still evidence of expression when it is depleted (Figure 3.9A), suggesting that Nurf301 is a good candidate for further study of Pol II activity at heat shock loci. TFIIS is known as the transcript cleavage factor, and functions to rescue Pol II that undergoes reverse translocation along the DNA.[79–81] This movement misaligns the transcribed mRNA within the Pol II active site, and TFIIS is responsible for inducing cleavage of the mRNA to create a 3' end that is properly aligned for catalysis.[82–84] TFIIS has been shown to not only decrease pausing of Pol II by facilitating this cleavage, but also stimulate promoter escape and transcription.[85,86] TFIIS would be a good candidate for studying *hsp70* expression, since it has been reported to increase the catalytic rate of escape of Pol II from the promoter-proximal region.[87] The depletion of Nurf301 and TFIIS result in lower levels of beta-galactosidase (Figure 3.9), and they would be a good candidate for furthering the study on *hsp70* expression regulatory mechanisms that do not include SEC components. Depletion of Rpd3 (HDAC1) does not inhibit induction of the *hsp70* reporter (Figure 3.9). This negative control shows that not all RNAi knockdowns inhibit *hsp70* expression.

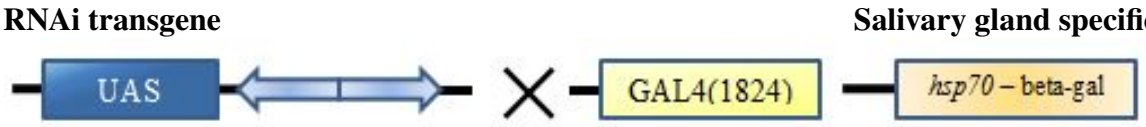

Figure 3.1: **Mating schematic for RNAi depletion**
Males containing the UAS-RNAi transgene were crossed with females containing the salivary gland-specific GAL4 driver and *hsp70*-beta-galactosidase genes in order to produce the desired genotype. RNAi depletion was specific to the salivary glands containing polytene chromosomes.

Figure 3.2: **Optimization of beta-galactosidase assay**
yw/yw;Z243,1824 third instar larvae were incubated at 36 ° C for 30 minutes for heat shock activation of *hsp70* genes. A recovery time period allowed transcription and translation of the hsp70-beta-galactosidase reporter. X-gal staining indicated the amount of beta-galactosidase present after recovery. Larvae were heat shocked for 30 minutes, then allowed to recover for: A) 15 minutes B) 30 minutes C) 60 minutes. A 30 minute recovery time was selected because it represented the time when color development just began to saturate and was consistent from experiment to experiment

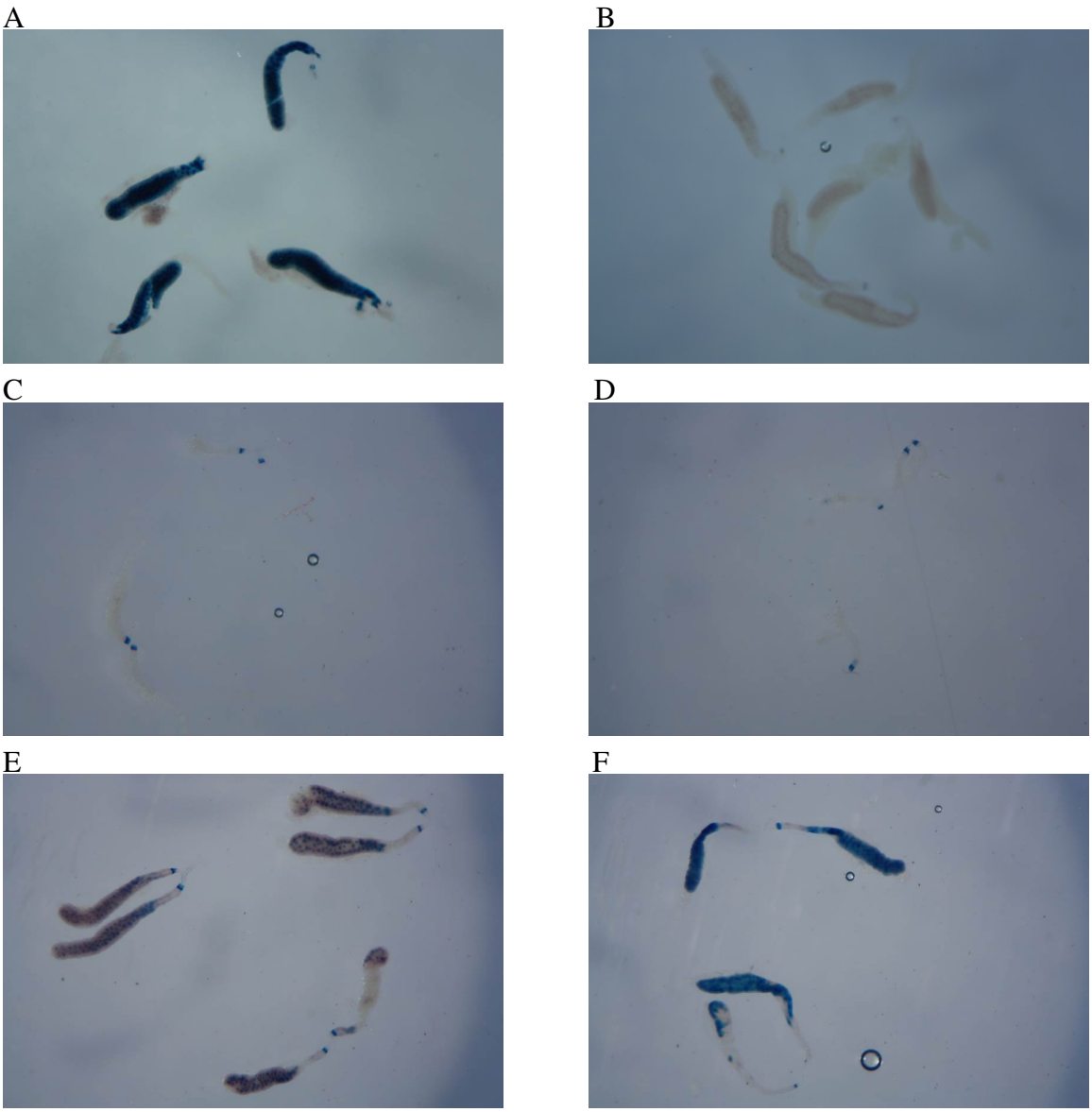

Figure 3.3: **Beta-galactosidase reporter activity upon depletion of SEC components**

Third instar larvae were incubated at 36° C for 30 minutes and recovered at room temperature for 30 minutes. The salivary glands were dissected and incubated in X-gal solution for 2 hours. (A-F) show X-gal stained salivary glands of larvae from: A)yw/yw;Z243,1824 control B)HSFi (VDRC48691)/yw;Z243,1824 C)Cdk9i(VDRC30449)/yw;Z243,1824 D)CycT1 short hairpin (BL32976)/yw;Z243,1824 E)ELLi/yw;Z243,1824 F)Lilli RNAi (BL34592)/yw;Z243,1824

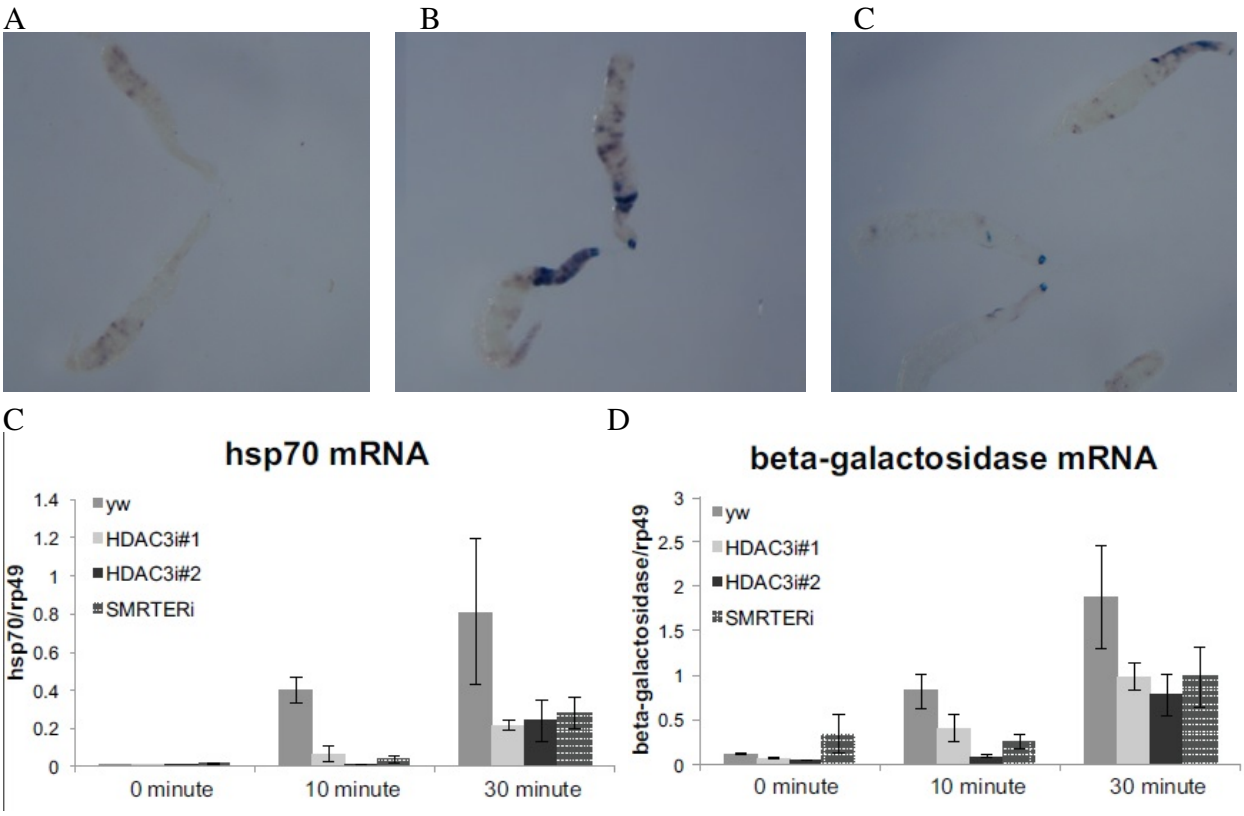

Figure 3.4: **Reduction in beta-galactosidase at the protein and mRNA level by RNAi depletion**

Third instar larvae were incubated at 36° C for 30 minutes and recovered at room temperature for 30 minutes. Salivary glands were dissected and incubated in X-gal solution for 2 hours: A) HDAC3#1i (VDRC-KK 107073)/yw;Z243,1824 B) HDAC3#2i (VDRC 20184)/yw;Z243,1824 C)SMRTERi (VDRC-KK 106701)/yw;Z243,1824 D-E) Reverse transcriptase-qPCR analysis of *hsp70* mRNA and *hsp70*-beta-galactosidase mRNA levels in salivary glands from the indicated RNAi lines targeting HDAC3, SMRTER, or YW larvae (Methods and results are from Achary[69]). mRNA levels were detected by qPCR and expressed as a ratio relative to a control gene, RP49, whose expression was unaffected by heat shock. Results are from three independent experiments and error bars represent SEM.

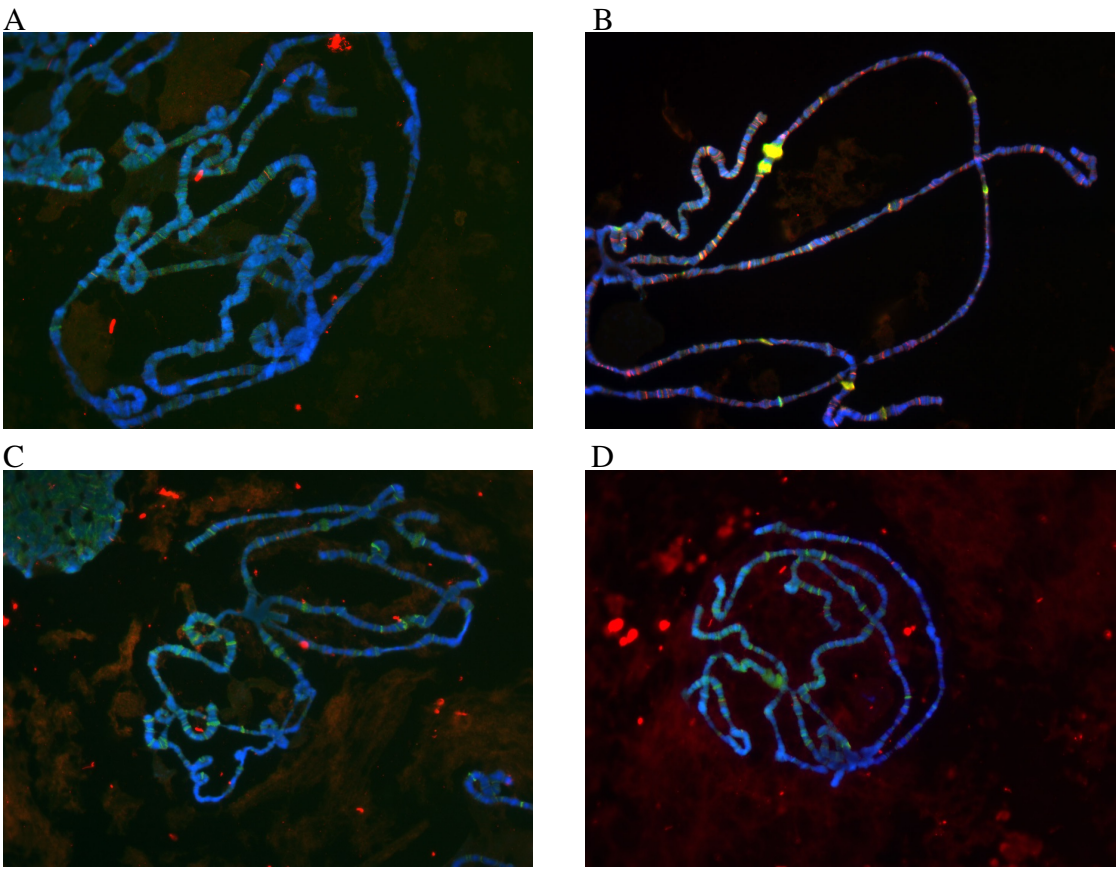

Figure 3.5: **Heat shock and HSF are necessary for *hsp70* induction**
Polytene *Drosophila* chromosome squashes were immunostained with ARNA3 for Pol II (green) and anti-HSF Ab for HSF (red). A)YW/yw;Z243,1824 control under non-heat shock conditions. B)YW/yw;Z243,1824 control third instar larvae were incubated at 36degC for 10 minutes. Their salivary glands were dissected and were squashed. C) HSFi(VDRC48691)/yw;Z243,1824 under non-heat shock conditions. D) HSFi(VDRC48691)/yw;Z243,1824 under heat shock conditions.

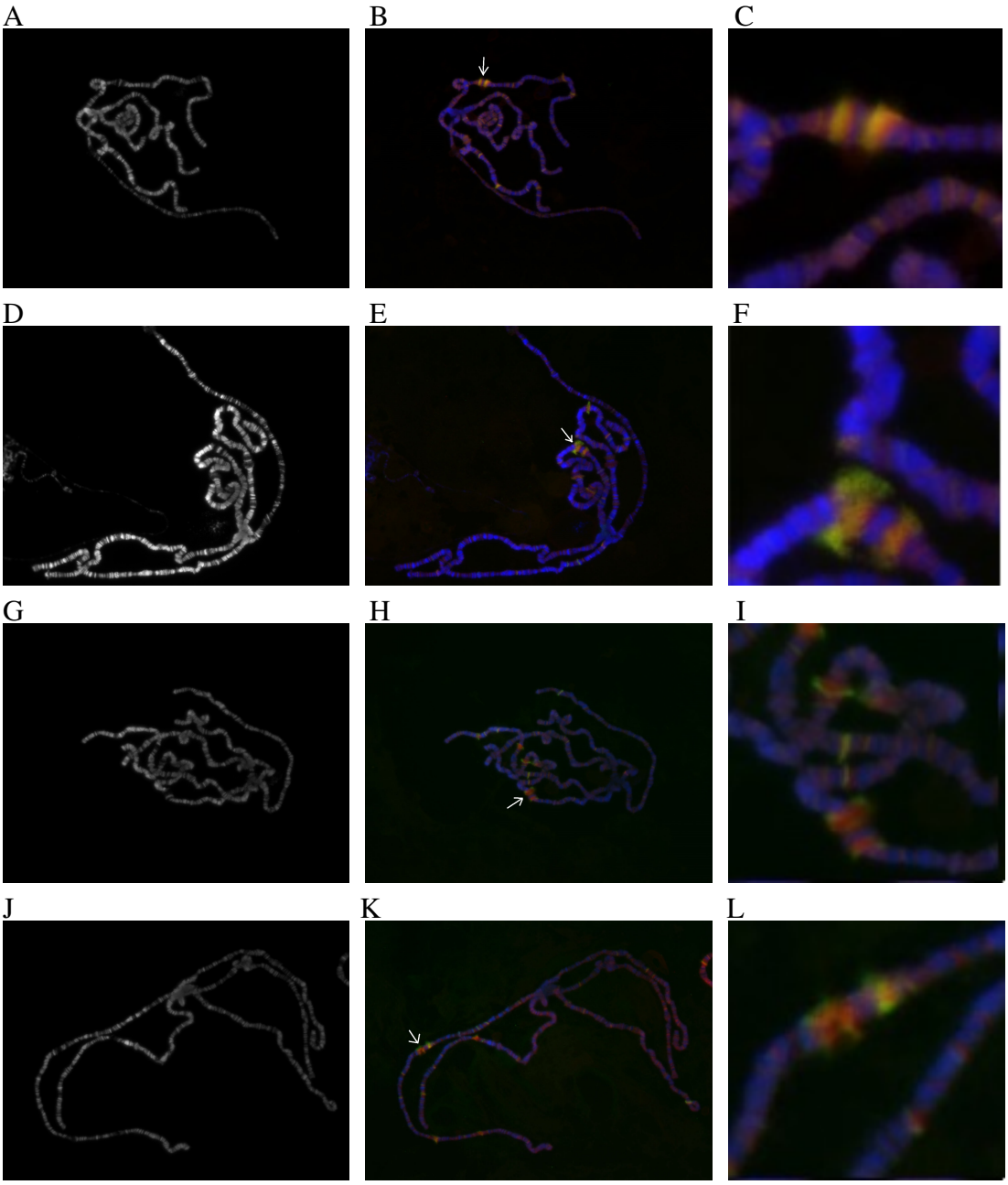

Figure 3.6: **RNA polymerase II localization at *hsp70* loci**

Control yw male flies were crossed with yw; Z243, 1824 virgin females. Salivary glands were dissected from heat-shocked third instar larvae and squashed on slides. RNA polymerase II was immunostained for Rpb3 (red) and either Rpb1 (ARNA3 antibody, B, C, green), the CTD (8WG16 antibody, E, F, green), phosphorylated serine 5 (H14 antibody, H, I, green), or phosphorylated serine 2(H2 antibody, K, L, green). DNA was detected with Hoechst stain (blue).

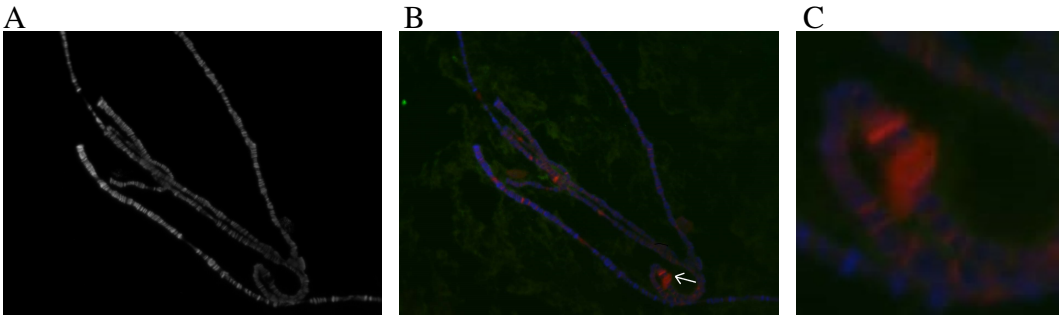

Figure 3.7: **Serine 2 phosphorylation at heat shock loci upon ELL depletion**
ELLi transgenic male flies were crossed with yw; Z243, 1824 virgin females. Salivary glands were dissected from heat-shocked third instar larvae and squashed on slides. RNA polymerase II was immunostained for Rpb3 (red) and phosphorylated serine 2 (green). DNA was detected with Hoechst stain (blue).

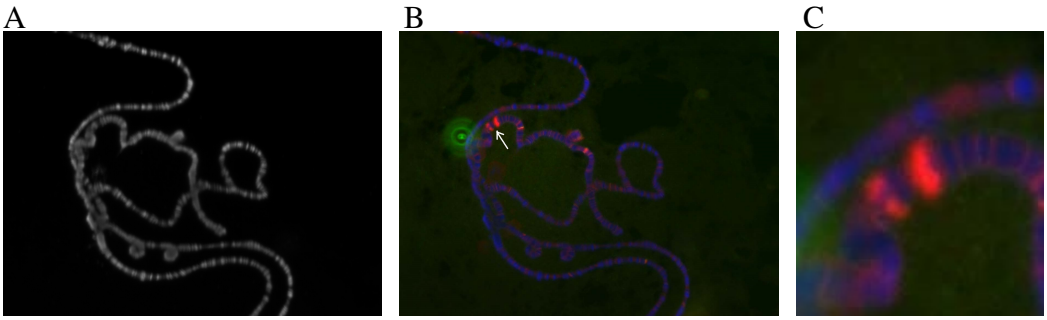

Figure 3.8: **Serine 2 phosphorylation at heat shock loci upon Lilli depletion**
Lilli RNAi (BL34592) transgenic male flies were crossed with yw; Z243, 1824 virgin females. Salivary glands were dissected from heat-shocked third instar larvae and squashed on slides. RNA polymerase II was immunostained for Rpb3 (red) and phosphorylated serine 2 (green). DNA was detected with Hoechst stain (blue).

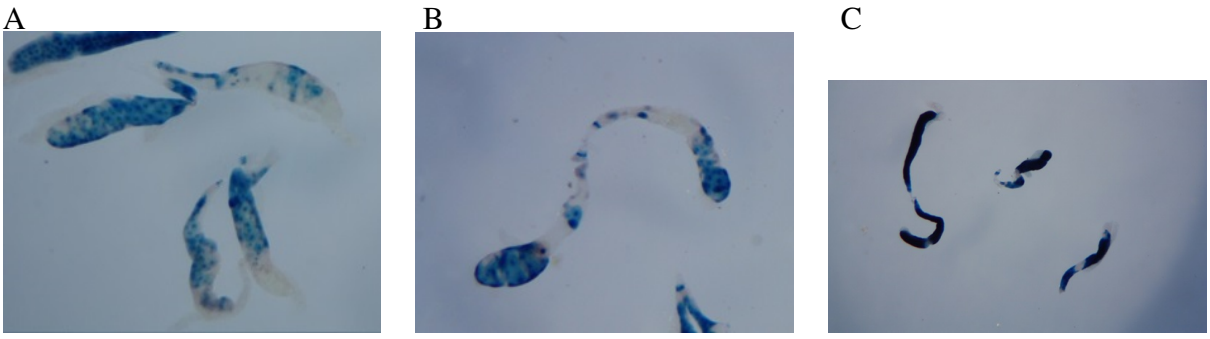

Figure 3.9: **Nurf301 and TFIIS play a role in regulating *hsp70* expression**
A) Nurf301i(VDRC46645)/yw;Z243,1824 and B) TFIISi (VDRC22980) showed a reduction in beta-galactosidase staining after 30 minutes of heat shock activation and 30 minutes of recovery. Lower levels of expression indicate their role in regulation of *hsp70* expression upon heat shock. C)Rpd3i (BL33725)/yw;Z243,1824 does not show inhibition of *hsp70* upon heat shock.

Chapter 4

Discussion

In order to investigate the role of SEC subunits in heat shock induction of *hsp70* in *Drosophila*, a reporter gene assay was developed. GAL4 activation of a UAS-RNAi transgene caused depletion of target genes. The simplicity of this screen allows beta-galactosidase staining to show whether *hsp70* expression is being inhibited. Its limitation is that it does not provide a direct measure of transcription, so the outcome may also be influenced by the quality of the mRNA produced by the reporter and the translation of this mRNA. To verify that levels of beta-galactosidase seen in the salivary glands are an indication of *hsp70* inhibition, reverse transcriptase and qPCR can be used to monitor hsp70 and beta-galactosidase mRNA levels. This reporter gene assay was validated by HSF depletion. HSF activates transcription of *hsp70* and causes a dramatic increase in the level of Pol II at the *hsp70* loci on polytene chromosomes. Immunostaining showed that HSF colocalizes with Pol II at heat shock puffs and that the levels of both proteins are severely diminished when HSF has been depleted with RNAi.

The SEC is proposed to facilitate Pol II's passage through the transcription elongation control checkpoint that occurs in the promoter proximal region. The kinase activity in the Cdk9 subunit of P-TEFb is responsible for the phospho-modifications that release Pol II from its paused state. Depletion of either Cdk9 or its regulatory subunit CycT1 inhibited induction of the *hsp70*-beta-galactosidase reporter gene. When elongation factor ELL or scaffold protein Lilli was depleted,

there were reduced levels of beta-galactosidase, but depletion of P-TEFb subunits might have more severe effects. This may be because it has multiple roles in releasing paused Pol II after initiation. Negative elongation factor NELF and DSIF associate with Pol II in its paused state. Phosphorylation of these subunits by P-TEFb dissociates NELF from Pol II and converts DSIF into a positive elongation factor. Upon association with the other components of the SEC, P-TEFb is responsible for the hyperphosphorylation of serine 2 on the carboxyl terminal domain of Pol II. When ELL or Lilli are depleted, there are still low levels of reporter expression. Since P-TEFb is still present in these two cases, the phosphorylation of NELF and DSIF may still occur. This activity may not be dependent upon the entire SEC. My results indicate that serine 2 phosphorylation by P-TEFb is not necessary for elongation to occur, but it would be interesting to determine if phosphorylation of NELF or DSIF is diminished when ELL or Lilli are depleted. This would allow us to speculate why Rpb3 still accumulates on the puffs under these conditions.

Immunofluorescence microscopy showed that the depletion of ELL or Lilli affected Pol II's association with heat shock loci. Staining for the Rpb3 subunit of Pol II indicated its presence at *hsp70* genes, but there was minimal serine 2 phosphorylation at these loci. Since depletion of ELL or Lilli results in the loss of serine 2 phosphorylation, immunostaining for Rpb3 indicates that the serine 2 phosphorylation is not essential for activating paused Pol II.

Another is that residual P-TEFb activity phosphorylates a subset of heptads and this is sufficient to reactivate Pol II, but is not detectable in our immunofluorescence assay. Another possibility is that since P-TEFb is still present but not necessarily associated with the rest of the SEC, it may phosphorylate NELF and DSIF. Further studies can be done in order to see if depletion of ELL or Lilli affect the levels of NELF and DSIF phosphorylation.

My results are an important addition to previous studies showing that ELL contributes to heat shock induction of *hsp70*. Previous studies have indicated that ELL is involved in activating paused Pol II on *hsp70*.[72] These experiments utilized RNAi depletion of ELL and immunostained chromosomes for serine 2 phosphorylation and ELL individually. These results indicate that ELL is localized at the heat shock puffs under normal conditions. Their results also show a much lower

level of serine 2 phosphorylation upon RNAi depletion of ELL. In this paper, the authors do not monitor Rpb3 levels or any information on the levels of Pol II in ELLi conditions. As a result, they report that the presence of ELL is required at heat shock loci for proper heat shock gene expression. The presence of Rpb3 at these loci indicates that transcription is still occurring upon heat shock induction.

Although there appear to be high levels of Rpb3 associated with *hsp70* loci, the levels of beta-galactosidase seen in the salivary glands do not seem to correlate. This disconnect between transcription and expression of beta-galactosidase is likely to be due to defects in the quality of the mRNA. Previous studies have shown that serine 2 phosphorylation is required for proper polyadenylation of the mRNA.[88] The absence of a poly-A tail destabilizes the mRNA leading to its destruction before it can be translated. This could be determined by quantifying mRNA levels by reverse transcriptase and qPCR. In addition, it would be good to quantify more precisely the levels of Pol II associated with *hsp70* in the control and depleted samples, since it is difficult to precisely quantify differences in immunofluorescence signals detected on separate samples. Chromatin immunoprecipitation or permanganate footprinting could track the movement of Pol II along the gene as well as the relative levels of Pol II in control and depleted samples.

The RNAi screen that I have prefected as proven to be a facile way to identify candidates involved in regulating the expression of *hsp70*. Thousands of transgenic lines targeting virtually all known genes in *Drosophila* are available from public stock centers, so a systematic screen of all known transcription factors should be feasible.

Bibliography

[1] J. W. Conaway, A. Shilatifard, A. Dvir, and R. C. Conaway. Control of elongation by RNA polymerase II. *Trends Biochem. Sci.*, 25:375–380, 2000.

[2] D. Reines, J. W. Conaway, and R. C. Conaway. The RNA polymerase II general elongation factors. *Trends Biochem. Sci.*, 21:351–355, 1996.

[3] A. Shilatifard, R. C. Conaway, and J. W. Conaway. The RNA polymerase II elongation complex. *Annu. Rev. Biochem.*, 72:693–715, 2003.

[4] M. G. Guenther, S. S. Levine, L. A. Boyer, R. Jaenisch, and R. A. Young. A chromatin landmark and transcription initiation at most promoters in human cells. *Cell*, 130:77–88, 2007.

[5] G. W. Muse, D. A. Gilchrist, S. Nechaev, J. S. Parker R. Shah, S. F. Grissom, J. Zeitlinger, and K. Adelman. RNA polymerase is poised for activation across the genome. *Nat Genet*, 39:1507–1511, 2007.

[6] M. Levine. Paused RNA polymerase II as a developmental checkpoint. *Cell*, 145:502–511, 2011.

[7] E. Smith, C. Lin, and A. Shilatifard. The super elongation complex (SEC) and MLL in development and disease. *Genes Dev.*, 25:661–672, 2011.

[8] A. N. Boettiger and M. Levine. Synchronous and stochastic patterns of gene activation in the Drosophila embryo. *Science*, 325:471–473, 2009.

[9] J. Zeitlinger, A. Stark, M. Kellis, J. W. Hong, S. Nechaev, K. Adelman, M. Levine, and R. A. Young. RNA polymerase stalling at developmental control genes in the Drosophila melanogaster embryo. *Nat. Genet.*, 39:1512–1516, 2007.

[10] T. Wada, T. Takagi, Y. Yamaguchi, A. Ferdous, T. Imai, S. Hirose, S. Sugimoto, K. Yano, G. A. Hartzog, F. Winston, S. Buratowski, and H. Handa. DSIF, a novel transcription elongation factor that regulates RNA polymerase II processivity, is composed of human Spt4 and Spt5 homologs. *Genes Dev.*, 12:343–356, 1998.

[11] Y. Yamaguchi, T. Takagi, T. Wada, K. Yano, A. Furuya, S. Sugimoto, J. Hasegawa, and H. Handa. NELF, a multisubunit complex containing RD, cooperates with DSIF to repress RNA polymerase II elongation. *Cell*, 19:535–545, 1999.

[12] N. J. Fuda, M. B. Ardehali, and J. T. Lis. Defining mechanisms that regulate RNA polymerase II transcription in vivo. *Nature*, 461:186–192, 2009.

[13] K. A. Jones and B. M. Peterlin. Control of RNA initiation and elongation at the HIV-1 promoter. *Annu. Rev. Biochem.*, 63:717–743, 1994.

[14] J. T. Lis, P. Mason, J. Peng, D. H. Price, and J. Werner. P-TEFb kinase recruitment and function at heat shock loci. *Genes Dev.*, 14:792–803, 2000.

[15] B. M. Peterlin and D. H. Price. Controlling the elongation phase of transcription with P-TEFb. *Mol Cell*, 23:297–305, 2006.

[16] C. H. Wu, Y. Yamaguchi, L. R. Benjamin, M. Horvat-Gordon, J. Washinsky, E. Enerly, J. Larsson, A. Lamertsson, H. Handa, and D. S. Gilmour. NELF and DSIF cause promoter proximal pausing on the hsp70 promoter in Drosophila. *Genes Dev.*, 17:1402–1414, 2003.

[17] T. Yamada, Y. Yamaguchi, N. Inukai, S. Okamoto, T. Mura, and H. Handa. P-TEFb-mediated phosphorylation of hSpt5 C-terminal repeats is critical for processive transcription elongation. *Mol. Cell*, 21:227–237, 2006.

[18] J. Lis and C. Wu. Protein traffic on the heat shock promoter: parking, stalling, and trucking along. *Cell*, 1993.

[19] E. D. Andrulis, E. Guzman, P. Doring, J. Werner, and J. T. Lis. High-resolution localization of Drosophila Spt5 and Spt6 at heat shock genes in vivo: roles in promoter proximal pausing and transcription elongation. *Genes Dev.*, 14:2635–2649, 2000.

[20] J. M. Park, J. Werner, J. M. Kim, J. T. Lis, and Y. J. Kim. Mediator, not holoenzyme, is directly recruited to the heat shock promoter by HSF upon heat shock. *Mol. Cell*, 8:9–19, 2001.

[21] S. Buratowski. The CTD code. *Nat Struct Biol*, 10:679–680, 2003.

[22] G. Orphanides, T. Lagrange, and D. Reinberg. The general transcription factors of RNA polymerase II. *Genes Dev*, 10:2657–2683, 1996.

[23] S. Akoulitchev, T. P. Makela, R. A. Weinberg R. A, and D. Reinberg. Requirement for TFIIH kinase activity in transcription by RNA polymerase II. *Nature*, 377:557–560, 1995.

[24] P. C. Holstege, P. C. van der Vliet, and H. T. Timmera. Opening of an RNA polymerase II promoter occurs in two distinct steps and requires the basal transcription factors IIE and IIH. *EMBO J*, 15, 1996.

[25] A. Dvir, R. C. Conaway, and J. W. Conaway. A role for TFIIH in controlling the activity of early RNA polymerase II elongation complexes. *Proc Natl Acad Sci*, 94, 1997.

[26] C. Lin, A. Shilatifard, E. R. Smith, H. Takahashi, K. C. Lai, and S. Martin-Brown. AFF4, a component of the ELL/P-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia. *Mol. Cell*, 37:429–437, 2010.

[27] N. He, M. Liu, J. Hsu, Y. Xue, S. Chou, A. Burlingame, N. J. Krogan, T. Alber, and Q. Zhou. HIV-1 Tat and host AFF4 recruit two transcription elongation factors into a bifunctional complex for coordinated activation of HIV-1 transcription. *Mol. Cell*, 38:428–438, 2010.

[28] B. Sobhian, N. Laquette, A. Yatim, M. Nakamura, Y. Levy, R. Kiernan, and M. Benkirane. HIV-1 Tat assembles a multifunctional transcription elongation complex and stably associates with the 7SK snrnP. *Mol. Cell*, 38:439–451, 2010.

[29] J. D. Rowley. The critical role of chromosome translocations in human leukemias. *Annu. Rev. Genet.*, 32:495–519, 1998.

[30] K. Tenney and A. Shilatifard. A COMPASS in the voyage of defining the role of trithorax/MLL-containing complexes: linking leukemogenesis to covalent modifications of chromatin. *J. Cell. Biochem.*, 95:11970–11974, 2005.

[31] M. J. Thirman, D. A. Levitan, H. Kobayashi, M. C. Simon, and J. D. Rowley. Cloning of ELL, a gene that fuses to MLL in a t(11;19)(q23;p13.1) in acute myeloid leukemia. *Proc. Natl. Acad. Sci. USA*, 91:12110–12114, 1994.

[32] C. Lin, A. S. Garrett, B. De Kumar, E. R. Smith, M. Gogol, C. Seidel, R. Krumlauf, and A. Shilatifard. Dynamic transcriptional events in embryonic stem cells mediated by the super elongation complex (SEC). *Genes Dev.*, 25:1486–1498, 2011.

[33] M. Mohan, C. Lin, E. Guest, and A. Shilatifard. Licensed to elongate: a molecular mechanism for MLL-based leukaemogenesis. *Nature Rev. Cancer*, 10:721–728, 2010.

[34] E. Bitoun, P. L. Oliver, and K. E. Davies. The mixed-lineage leukemia fusion partner AF4 stimulates RNA polymerase II transcriptional elongation and mediates coordinated chromatin remodeling. *Hum. Mol. Genet.*, 16:92–106, 2007.

[35] A. V. Krivtsov, Z. Feng, M. E. Lemieux, J. Faber, S. Vempati, A. U. Sinha, X. Xia, J. Jesneck, A. P. Bracken, and L. B. Silverman. H3K79 methylation profiles define murine and humanMLL-AF4 leukemias. *Cancer Cell*, 14:355–368, 2008.

[36] D. Mueller, C. Bach, D. Zeisig, M. P. Garcia-Cuellar, S. Monroe, A. Sreekumar, R. Zhou, Z. Nesvizhskii, A. Cinnaiyan, and J. L. Hess. A role for the MLL fusion protein ENL in transcriptional elongation and chromatin modification. *Blood*, 110:4445–4454, 2007.

[37] D. Mueller, M. P. Garcia-Cuellar, C. Bach, S. Buhl, E. Maethner, and R. K. Slany. Misguided transcriptional elongation causes mixed lineage leukemia. *PLos Biol.*, 7:e1000249. 10.1371/journal.pbio.1000249, 2009.

[38] Y. Okada, Q. Feng, Y. Lin, Q. Jiang, Y. Li, V. M. Coffield, L. Su, G. Xu, and Y. Zhang. hDOT1L links histone methylation to leukemogenesis. *Cell*, 121:167–178, 2005.

[39] W. Zhang, X. Xia, M. R. Reisenauer, C. S. Hemenway, and B. C. Kone. Dot1a-AF9 complex mediates histone H3 Lys-79 hypermethylation and repression of ENaCalpha in an aldosterone-sensitive manner. *J. Biol. Chem.*, 281:18059–18068, 2006.

[40] M. K. Jang, K. Mochizuki, M. Zhou, H. S. Jeong, J. N. Brady, and K. Ozato. The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. *Mol. Cell*, 19:523–534, 2005.

[41] Z. Yang, J. . Yik, R. Chen, N. He, M. K. Jang, K. Ozato, and Q. Zhou. Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. *Mol. Cell*, 19:535–545, 2005.

[42] A. K. Boehm, A. Saunders, J. Werner, and J. T. Lis. Transcription factor and polymerase recruitment, modification, and movement on dhsp70 in vivo in the minutes following heat shock. *Mol Cell Biol*, 23:7628–737, 2003.

[43] A. Shilatifard, W. S. Lane, K. W. Jackson, R. C. Conaway, and J. W. Conaway. An RNA polymerase II elongation factor encoded by the human ELL gene. *Science*, 271:1873–1876, 1996.

[44] T. Miller, K. Williams, R. W. Johnstone, and A. Shilatifard. Identification, cloning, expression, and biochemical characterization of the testis-specific RNA polymerase II elongation factor ELL3. *J. Biol. Chem.*, 275:32052–32056, 2000.

[45] A. Shilatifard, D. R. Duan, D. Haque, C. Florence, W. H. Schubach, J. W. Conaway, and R. C. Conaway. ELL2, a new member of an ELL family of RNA polymeraseII elongation factors. *Proc. Natl. Acad. Sci. USA*, 94:3639–3643, 1997.

[46] J. C. Eissenberg, J. Ma, M. A. Gerber, A. Christensen, J. A. Kennison, and A. Shilatifard. dELL is an essential RNA polymerase II elongation factor with a general role in development. *Proc. Natl. Acad. Sci. USA*, 99:9894–9899, 2002.

[47] M. Gerber, J. Ma, K. Dean, J. C. Eissenberg, and A. Shilatifard. Drosophila *ELL* is associated with actively elongating RNA polymerase II on transcriptionally active sites in vivo. *EMBO*, 21:6104–6114, 2001.

[48] F. Simone, P. E. Polak, J. J. Kaberlein, R. T. Luo, D. A. Levitan, and M. J. Thirman. EAF1, a novel ELL-associated factor that is delocalized by expression of the MLL-ELL fusion protein. *Blood*, 98:201–209, 2001.

[49] F. Simone, R. T. Luo, P. E. Polak, J. J. Kaberlein, and M. J. Thirman. ELL-associated factor 2 (EAF2), a functional homolog of EAF1 with alternative ELL binding properties. *Blood*, 101:2355–2362, 2003.

[50] S. E. Kong, C. A. Banks, A. Shilatifard, J. W. Conaway, and R. C. Conaway. ELL-associated factors 1 and 2 are positive regulators of RNA polymerase II elongation factor ELL. *Proc. Natl. Acad. Sci. USA*, 102:10094–10098, 2005.

[51] N. He, C. K. Chan, B. Sobhian, S. Chou, Y. Xue, M. Liu, T. Alber, M. Benkirane, and Q. Zhou. Human polymerase-associated factor complex (PAFc) connects the super elongation complex (SEC) to RNA polymerase II on chromatin. *Proc. Natl. Acad. Sci. USA*, 108:E636–645, 2011.

[52] E. Bitoun and K. E. Davies. The robotic mouse: unravelling the function of AF4 in the cerebellum. *Cerebellum*, 4:250–260, 2005.

[53] I. Nilson, M. Reichel, M. G. Ennas, R. Greim, C. Knorr, G. Siegler, J. Greil, G. H. Fey, and R. Marschalek. Exon/intron structure of the human AF-4 gene, a member of the AF-4/LAF-4/FMR-2 gene family coding for a nuclear protein with structural alterations in acute leukaemia. *Br. J. Haematol.*, 98:157–169, 1997.

[54] R. Prasad, T. Yano, C. Sorio, T. Nakamura, R. Rallapalli, Y. Gu, D. Leshkowitz, C. M. Croce, and E. Canaani. Domains with transcriptional regulatory activity within the ALL1 and AF4 proteins involved in acute leukemia. *Proc. Natl Acad. Sci. USA*, 92:12160–12164, 1995.

[55] C. Ma and L. M. Staudt. LAF-4 encodes a lymphoid nuclear protein with transactivation potential that is homologous to AF-4, the gene fused to MLL in t(4;11) leukemias. *Blood*, 87:734–745, 1996.

[56] K. Baskaran, F. Erfurth, G. Taborn, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, P. M. Iannaccone, and P. H. Domer. Cloning and developmental expression of the murine homolog of the acute leukemia proto-oncogene AF4. *Oncogene*, 15:1967–1978, 1997.

[57] M. A. Hillman and J. Gecz. Fragile XE-associated familial mental retardation protein 2 (FMR2) acts as a potent transcription activator. *J. Hum. Genet.*, 46:251–259, 2001.

[58] M. C. Estable, M. H. Naghavi, H. Kato, H. Xiao, J. Qin, A. Vahlne, and R. G. Roeder. MCEF, the newest member of the AF4 family of transcription factors involved in leukemia, is a positive transcription elongation factor-b-associated protein. *J. Biomed. Sci.*, 9:234–245, 2002.

[59] A. Bursen, S. Moritz, A. Gaussmann, T. Dingermann, and R. Marschalek. Interaction of AF4 wild-type and AF4-MLL fusion protein with SIAH proteins: indication for t(4;11) pathobiology. *Oncogene*, 23:6237–6249, 2004.

[60] P. L. Oliver, E. Bitoun, J. Clark, E. L. Jones, and K. E. Davies. Mediation of Af4 protein function in the cerebellum by Siah proteins. *Proc. Natl Acad. Sci. USA*, 101:14901–14906, 2004.

[61] Z. Luo, C. Lin, E. Guest, A. Garrett, N. Moaghegh, S. Swanson, S. Marshall, L. Florens, M. Washburn, and A. Silatifard. The super elongation complex family of RNA polymerase II elongation factors: gene target specificity and transcriptional output. *Mol. Cell. Biol.*, 32:2608–2617, 2012.

[62] S. Iida, M. Seto, K. Yamamoto, H. Komatsu, A. Tojo, S. Asano, N. Kamada, Y. Ariyoshi, T. Takahashi, and R. Ueda. MLLT3 gene on 9p22 in t(9;11) leukemia encodes a serine/proline rich protein homologous to MLLT1 on 19p13. *Oncogene*, 8:3085–3092, 1993.

[63] M. D. Welch and D. G. Drubin. A nuclear protein with sequence similarity to proteins implicated in human acute leukemias is important for cellular morphogenesis and actin cytoskeletal function in *saccharomyces cerevisiae*. *Mol. Biol. Cell*, 5:617–632, 1994.

[64] M. Carlson and B. C. Laurent. The SNF/SWI family of global transcriptional activators. *Curr. Opin. Cell Biol.*, 6:396–402, 1994.

[65] J. Cote, J. Quinn, J. L. Workman, and C. L. Peterson. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science*, 265:53–60, 1994.

[66] C. L. Peterson, A. Dingwall, and M. P. Scott. Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. *Proc. Natl. Acad. Sci. USA*, 91:2905–2908, 1994.

[67] B. R. Cairns, N. L. Henry, and R. D. Kornberg. TFG3/TAF30/ANC1, a component of the yeast SW1/SNF complex that is similar to the leukemogenic proteins ENL and AF-9. *Mol. Cell. Biol.*, 16:3308–3316, 1996.

[68] B. R. Cairns, Y. J. Kim, M. H. Sayre, B. C. Laurent, and R. D. Kornberg. A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. *Proc. Natl. Acad. Sci. USA*, 91:1950–1954, 1994.

[69] B. G. Achary, K. M. Campbell, I. S. Co, and D. S. Gilmour. RNAi screen in Drosophila larvae identifies histone deacetylase 3 as a positive regulator of the hsp70 heat shock gene expression during heat shock. *Biochim. Biophys. Acta.*, 14:50–59, 2014.

[70] B. E. Schwartz, J. K. Werner, and J. T. Lis. Indirect immunofluorescent labeling of Drosophila polytene chromosomes: Visualizing protein interactions with chromatin in vivo. *Methods in Enzymology*, 376, 2004.

[71] X. Yang and E. Seto. Collaborative spirit of histone deacetylases in regulating chromatin structure and gene expression. *Curr. Opin. Genet. Dev.*, 13:143–153, 2003.

[72] E. R. Smith, B. Winter, J. C. Eissenberg, and A. Shilatifard. Regulation of the transcriptional activity of poised RNA polymerase II by the elongation factor ELL. *Proc. Natl. Acad. Sci. USA*, 25:8575–8579, 2008.

[73] T. Tsukiyama, C. Daniel, J. Tamkun, and C. Wu. ISWI, a member of the SW12 SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. *Cell*, 83:1021–1026, 1995.

[74] M. A. Martinez-Balbas, T. Tsukiyama, D. Gdula, and C. Wu. Drosophila NURF-55, a WD repeat protein involved in histone metabolism. *Proc. Natl. Acad. Sci.*, 95:132–137, 1998.

[75] D. A. Gdula, R. Sandaltzopoulos, T. Tsukiyama, V. Ossipow, and C. Wu. Inorganic pyrophosphatase is a component of the Drosophila nucleosome remodeling factor complex. *Genes Dev.*, 12:3206–3216, 1998.

[76] H. Xiao, R. Sandaltzopoulos, H. M. Wang, A. Hamiche, R. Ranallo, K. M. Lee, D. G. Fu, and C. Wu. Dual functions of largest NURF subunit NURF301 in nucleosome sliding and transcription factor interactions. *Mol. Cell*, 8:531–543, 2001.

[77] T. Ito, M. E. Levenstein, D. V. Fyodorov, A. K. Kutach, R. Kobayashi, and J. T. Kadonaga. ACF consists of two subunits, Acf1 and ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. *Genes Dev.*, 13:1529–1539, 1999.

[78] P. Badenhorst, M. Voas, I. Rebay, and C. Wu. Biological functions of the ISWI chromatin remodeling complex NURF. *Genes Dev.*, 16:3186–3198, 2002.

[79] S. Borukhov, V. Sagitov, and A. Goldfarb. Transcript cleavage factors from E. coli. *Cell*, 72:459–466, 1993.

[80] R. N. Fish and C. M. Kane. Promoting elongtaion with transcript cleavage stimulatory factors. *Biochim. Biophys. Acta.*, 1577:287–307, 2002.

[81] M. Wind and D. Reines. Transcription elongation factor SII. *Bioessays*, 22:327–336, 2000.

[82] N. Komissarova and M. Kashlev. Transcriptional arrest: Escherichia coli RNA polymerase translocates backward, leaving the 3' end of the RNA intact and extruded. *Proc. Natl. Acad. Sci. USA*, 94:1755–1760, 1997.

[83] M. G. Izban and D. S. Luse. The RNA polymerase II ternary complex cleaves the nascent transcript in a 3'-5' direction in the presence of elongation factor SII. *Genes Dev.*, 6:1342–1356, 1992.

[84] D. Reines. Elongation factor-dependent transcript shortening by template-engaged RNA polymerase II. *J. Biol. Chem.*, 267:3795–3800, 1992.

[85] H. Guo and D. H. Price. Mechanism of DmS-II-mediated pause suppression by Drosophila RNA polymerase II. *J. Biol. Chem.*, 268:18762–18770, 1993.

[86] L. M. Hsu, N. V. Vo, and M. J. Chamberlin. Escherichia coli transcript cleavage factors GreA and GreB stimulate promoter escape and gene expression in vivo and in vitro. *Proc. Natl. Acad. Sci. USA*, 92:11588–11592, 1995.

[87] K. Adelman, M. T. Marr, J. Werner, A. Saunders, Z. Ni, E. D. Andrulis, and J. T. Lis. Efficient release from the promoter-proximal stall sites requires transcript cleavage factorTFIIS. *Mol. Cell*, 17:103–112, 2005.

[88] N. Zhuoyu, A. Saunders, N. Fuda, J. Yao, J. R. Suarez, W. W. Webb, and J. T. Lis. P-TEFb is critical for the maturation of RNA polymerase II into productive elongating in vivo. *Mol. Cell Biol.*, 28:1161–1170, 2008.

ACADEMIC VITA

SCHOOL ADDRESS
672 East Prospect Ave, Apt B
State College, PA 16801

Katie Campbell
kmc5655@psu.edu
(610)781-6864

HOME ADDRESS
216 West Penn Ave
Wernersville, PA 19565

EDUCATION

The Pennsylvania State University — University Park, PA
Schreyer Honors College — Graduation: May 2014
Bachelor of Science in Biochemistry

PROFESSIONAL EXPERIENCE

The Pennsylvania State University — University Park, PA
Undergraduate Research in Biochemistry and Molecular Biology — September 2011-present
I am currently researching gene regulation in *Drosophila* and RNA polymerase activity. Techniques include performing DNA/RNA isolation, agarose gel electrophoresis, polytene chromosome squashing, immunofluorescent imaging, and immunostaining.

The Pennsylvania State University- Berks Campus — Reading, PA
Undergraduate Research in Microbiology and Food Science — May 2011-August 2011
I investigated the effects of yogurt probiotics on pathogens. I also collaborated with Giorgio's mushroom farms to quantify the shelf-life of mushrooms. Techniques learned include media preparation, culture growth and maintenance, and bacterial isolation.

PROFESSIONAL PRESENTATIONS

Eberly College of Science — Spring 2013, 2014
Undergraduate Research Exhibition

PUBLICATIONS

Achary B. G., Campbell K. M., Co I. S., and Gilmour D. S. RNAi screen in Drosophila larvae identifies histone deacetylase 3 as a positive regulator of the hsp70 heat shock gene expression during heat shock. *BBA-Gene Regulatory Mechanisms* (2014) 1839. Web.

ACTIVITIES

Penn State Athletics — April 2012-present
Sports Marketing Intern
Letterman's Club Intern
Schreyer Honors College Student Council — January 2011-present
American Cancer Society Relay for Life — April 2011-present
Student Government Academic Affairs Committee — August 2011-May 2012

HONORS

Dean's List- 5 semesters
Schreyer Academic Excellence Scholarship
Exide Technologies Scholarship Endowment