THE PENNSYLVANIA STATE UNIVERSITY
THE SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

ANALYSIS OF A FUNCTIONAL ROLE FOR CONSERVED LYSINE RESIDUES OF THE H4 N-TERMINUS IN PREMATURE mRNA SPlicing EFFICIENCY

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ABSTRACT
Chromatin, repeating units of tightly coiled DNA wrapped around histones, is the most important level of DNA organization. The N-termini of histones are subject to a variety of post-translation modifications that can either promote or discourage vital cell processes, such as DNA replication, transcription, and splicing. Recent studies have established a vital role for histone H3 in splicing, but little investigation concerning H4 has been done. Therefore, this project sought to determine a functional role for lysine residues of the histone H4 in splicing efficiency in S. cerevisiae. Reporter assays revealed that H4 mutants with all native lysine residues of H4 modified to glutamine, a splicing defect was observed. In an H4 mutant with lysine to glutamine point mutations at positions 5, 8, and 12 with a tripeptide insertion, GKG, at position 3, the splicing defect was moderately rescued. Interestingly, when pre-mRNA leakage of each of the aforementioned strains was analyzed, the results were opposite of the expected outcome. The wildtype strain showed the greatest pre-mRNA leakage while the H4 all K→Q strain showed the least. The low β galactosidase activities, however, may partially explain this anomaly. Preliminary qPCR analyses using ECM33 as the gene of interest do not show splicing defects in the H4 mutants compared to wildtype, but this may be attributed to the abundance of the gene. Future investigations and analyses using qPCR are of high priority.
ACKNOWLEDGEMENTS

This thesis would not be possible without the aid, guidance, and patience of Dr. Joseph Reese. The knowledge, commitment, and experience I have gained as a result of his tutelage is invaluable and something I will always treasure. I especially extend my gratitude for helping me with my game plan for the past four years and as a direct result of his help, I am ready to play.

Infinite thanks to the members of the Reese lab for making the work environment feel like home. I have not only found an exceptional coworker in each of them, but also a wonderful friend. Thank you for the support, kindness, and memories.

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INTRODUCTION

Gene regulation is a highly complex and elegantly coordinated process in living organisms that modulates the expression of a given gene at a given point in time. The most important level of DNA organization in eukaryotes is chromatin, structures composed of repeating units of nucleosomes, which are made up of approximately 147 base pairs, wrapped around proteins known as histones (Yang and Arya, 2010). One of the basic mechanisms of gene regulation is accessibility of the gene for transcription by RNA Polymerase II (RNAPII), which is responsible for transcribing DNA into messenger RNA (mRNA) in eukaryotes. The eight core histones, pairs of H2A, H2B, H3, H4, are subject to various post translational modifications, such as acetylation, methylation, and ubiquitylation, which determines the accessibility of the genome for mechanisms such as transcription, repair, and replication (Gunderson and Johnson, 2009).

Past research has shown that the highly charged N-terminal domains of the core histone tails are a prime region of post translational modifications (Glowczewski et al., 2004). Specific modifications of certain residues within the histone tail are associated with various DNA-related processes (Figure 1). For example, methylation of some lysines in the H3 tail are associated with gene repression, while acetylation of some lysines in the H4 tail are associated with gene transcription (Yang and Mizzen, 2009). Further, specific acetylation patterns in the N terminal tails of H3 and H4 histones are necessary for heterochromatin maintenance at telomeres, mating type, and the rRNA gene locus (Glowczewski et al., 2004). The first 20 amino acid residues of the N-terminal tail of S. cerevisiae and mammalian histone H4 are well conserved with 100% sequence identity, which suggests an essential functional role for the histone tails in eukaryotes (Megee et al., 1990).
An exciting and currently intense area of research is investigating chromatin dynamics in transcription and mRNA processing. Eukaryotic transcription results in premature mRNA, or pre-mRNA, which must be processed prior to nuclear export and subsequent translation into protein. The removal of introns from pre-mRNA is termed splicing, and is carried out by the spliceosome, a large nuclear complex comprised of more than 150 proteins. The spliceosome is made up of five small nuclear ribonucleoproteins, or snRNPs: U1, U2, U4, U5, U6, all of which associate with their respective snRNA and other proteins (Figure 2). Splicing of pre-mRNA in eukaryotes is a sequential process involving intron recognition, followed by spliceosome assembly, and finally intron excision. Despite the length of introns, virtually all eukaryotes have three invariant sequences that are essential for proper splicing: the 5' GU, 3' AG, and the branch site adenosine, which is located 18–40 nucleotides upstream of the 3' splice site. The splice sites are recognized by small nuclear ribonucleoproteins (snRNPs) and other auxiliary proteins. Splicing is a 2 step transesterification mechanism; the 2' hydroxyl group of branch site adenosine attacks the phosphate group at the 5' splice site, which releases the 5' exon. The 3' hydroxyl group of the released 5' exon subsequently attacks the phosphate group at the 3' splice site so that
the 5' and 3' exons are joined, forming the mature mRNA. Pre-mRNA splicing occurs cotranscriptionally and the mature mRNA, characterized by the lack of introns, a 5’ cap, and a 3’ poly-A tail, is subsequently exported to the cytoplasm for translation to protein. Evidence strongly suggests that transcription, splicing, and nuclear transport are closely related, often dependent, processes that share common molecular machinery (McKay and Johnson, 2010).

**Figure 2.** The spliceosome is a large complex structure and is the site of pre-mRNA splicing. The five snRNPs that comprise the spliceosome are dynamically rearranging and involve several different factors to effectively remove the intron and splice the exons. (From McKay and Gunderson, 2010)

As a general cell surveillance mechanism, intron-containing mRNAs are not exported from the nucleus, but have been observed to "leak" into the cytoplasm in splicing mutants (Bousquet-Antonelli, 2000). Mutations in the nuclear exosome in cells that are defective for splicing results in an accumulation of pre-mRNA, which suggests the existence of a nuclear pre-mRNA turnover pathway, which degrades pre-mRNA, that competes with the splicing machinery (Bousquet-Antonelli, 2000).
Lysine acetylation and deacetylation are catalyzed by lysine acetyltransferases (KAT) and lysine deacetylases (KDAC), respectively. Biochemical studies in mammalian cells have shown that splicing factors that are the targets of acetylation also associate with acetylation/deacetylation machinery. A study done by Choudhary, et al. used mass spectrometry to quantify the changes in acetylation across 1,750 proteins and found that lysine acetylation preferentially targets macromolecular structures that regulate a host of biological functions, including splicing, nuclear transport, and chromatin remodeling (Choudhary et al., 2009). Kuhn et al. found that when specific steps of the spliceosomal pathway were inhibited with small molecules, there was an accumulation of unique non-functional splicing complexes in the absence of proper acetylation or deacetylation. Thus, acetylation and deacetylation of spliceosomal subunits plays an essential role in modulating splicing fidelity via regulation of spliceosomal enzyme activity (Kuhn et al., 2009). More recently, much evidence in support of an essential role for histone tails in the efficient splicing of pre-mRNAs has been reported. Gcn5, a well characterized transcriptional coactivator in S. cerevisiae that encodes the histone acetyltransferase activity of the SAGA complex, was found to play a role in the co-transcriptional association of the U2 snRNP of the spliceosome with the nascent pre-mRNA transcript (Gunderson and Johnson, 2009).

In the eukaryotic H4 N terminus, lysine residues at positions 5, 8, 12, and 16 are subject to reversible acetylation (Davie et al., 1981). During chromatin replication and assembly, new H4 is acetylated at lysines 5 and 12 prior to deposition onto nascent DNA (Benson et al., 2007). Mutants bearing point mutations of lysine to glutamine (K→Q) residues at these positions resulted in an extended G2 + M phase of the cell cycle, mating sterility, temperature sensitive growth, and prolonged period of DNA replication (Megee et al., 1990). Unsurprisingly, H4 tail
deletion mutants have a slower growth rate than the wildtype and K→Q mutants, but are severely impaired in basic cellular functions (Megee et al., 1990).

It has been speculated that post translational modifications can modulate splicing efficiency by a myriad of mechanisms, including, but not limited to, regulating rearrangements of spliceosomal subunits, the timing of rearrangements, or splicing factor activities (McKay and Johnson, 2010). A recent review article proposed that specific histone modifications may facilitate exon recognition by splicing factors as epigenetic markers such as the H4K20me are enriched on exons and increases in accordance with gene expression (Alexander and Beggs, 2010). Previous studies show that H4K20me only occurs in mammalian cells to an appreciable extent; methylation in *S. cerevisiae* occurs at exceptionally low levels (Garcia et al., 2007). Past experimentation by Bellare, et al. demonstrated that ubiquitylation of spliceosomal subunits contributes to splicing efficiency, which further promoted the notion that other post translational modifications may play a role in splicing efficiency as well (Bellare et al., 2008).

Indeed, the role of nucleosomes and histones in pre-mRNA splicing is relatively ambiguous, but several studies have begun to provide a framework of an intricate and complicated process. Histone tail modifications have only recently thought to play more than a passive role in gene regulation; while it has been well established that certain histone modifications modulate active or repressed transcriptional states, its role in pre-mRNA maturation is less understood. Studies by Sims et al. found that CDHI, an RNA Polymerase II elongation and termination factor in yeast and other eukaryotes, is associated with spliceosomal components. The results suggested that CHD1 recognition of H3K4me3 functions to physically bridge spliceosomal components to CHD1 and elegant biochemical analyses provided evidence for the functional association between CHD1 and splicing *in vitro* (Sims et al., 2004, 2007). A
hypothesis proposed by Allemand, et al. known as the Velcro-matin hypothesis, proposes a dual role for nucleosomes and histones in pre-mRNA maturation. The basic histones initially capture the nascent acidic RNA until RNA binding proteins of higher affinity preferentially bind to the RNA. Further, as histone modifications within introns are distinct from those in exons, due to the presence of repeated sequences in introns, altered chromatin structure may be indicative of introns (Fig 3).

**Figure 3.** Velcro-matin hypothesis; the basic histone tails are thought to act as a scaffold for nascent acidic pre-mRNA until other RNA binding proteins bind. (Allemand, et. al, 2006)

Past experimentation suggested a role for the H4 N-terminus conserved lysine residues in splicing, but little primary investigation has been conducted (Fig 4) (Psathas, unpublished data). Based on literature and previous data, this investigation seeks to explore a functional role for the conserved H4 N terminus lysine residues in premature mRNA splicing.
Figure 4. H4 tail is necessary for proper splicing of CYH2 and ACT1. Northern blots of CYH2 (top) and ACT1 (bottom) mRNA are shown, with spliced to unspliced ratios below the blots. (Psathas, unpublished data).
MATERIALS AND METHODS

Yeast Strains

The following yeast strains, with the exception of the wildtype H3, were used for experimentation:

- MSY590 (Wildtype H4)
  SGRGKGKGLGKGGGAKHRKVRDNI
- Wildtype H3
  ARTKQTARKSTGGKAPRKQLATKAARKSAP
- MSY574
  ARTQQTARSGGQAPRQQLAQARQSAP
- MSY535
  SGRGQGGQGLQGGAQRHRVVRDNI
- MSY570
  SGRGQGGQGLQGGAQRHRKVRDNI + GKG after position 3
- MSY400
  SGRGQGGQGLQGGAQRHRKVRDNI
- MSY582
  SGRGKGQGLQGGAQRHRKVRDNI
- MSY711
  SGRGKGKGKGLGKGGGAKHRKVRDNI

Yeast Growth and Harvesting Conditions

Cells were grown in synthetic complete –URA media containing 2% raffinose and 2% glycerol. Triplicates of 6 ml cultures were grown to OD<sub>600</sub> of 0.6-1.0, at which point 300 µl of 40% galactose was added to induce cells. Samples were incubated at 30°C for 2 hours. To harvest the cells, samples were centrifuged and resuspended in breaking buffer (100 mM Tris-Cl, pH 8.0, 20% glycerol) followed by bead beating. Approximately 10 µl of the cell extract was used to carry out a Bradford assay to determine protein concentrations, and the remaining sample was used for the β-galactosidase assay.
**β-galactosidase Assay**

900 µl of cold Z buffer, pH 7.0 (40mM Na_2HPO_4, 60mM NaH_2PO_4, 10mM KCl, 1mM MgSO_4, 50mM β-mercaptoethanol) was added to 100 µl of sample and breaking buffer. The samples were subsequently incubated at 28°C for 5 minutes. 100 µl of artificial substrate, o-nitrophenyl-galactoside (ONPG), was added to the samples and incubated at 28°C until a pale yellow color was apparent. The reaction was then stopped by the addition of 0.5 M NaCO_3. The elapsed time between addition of ONPG and that of NaCO_3 was recorded for analysis of enzyme levels. OD_{420} levels were measured and final β-galactosidase activity levels were measured using the Miller equation.

**Plasmids**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Feature</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLSDG5</td>
<td>Intron-less plasmid</td>
<td>Legrain and Rosbash, 1989</td>
</tr>
<tr>
<td>pJCR1</td>
<td>Leakage reporter</td>
<td>Legrain and Rosbash, 1989</td>
</tr>
<tr>
<td>pJCR51</td>
<td>Splicing reporter</td>
<td>Legrain and Rosbash, 1989</td>
</tr>
</tbody>
</table>

**qPCR analysis**

RNA extraction and Real-Time PCR were performed as previously described by Gunderson and Johnson, 2009. Total RNA was extracted by hot phenol-chloroform extraction and DNase I treated. cDNA was synthesized from 1 µg of DNase-treated RNA in a 20 µl reaction mixture containing 10 mM dNTP, 2U RNasin, 1 µM random primer. cDNA was diluted 1:30 and 1 µl of this was used in a 25 µl reaction volume. Reactions consist of 12.5 µl SYBR GREEN Master Mix and 1 µM Primers. For quantification, standard curves were generated for each primer set, and to calculate ratio of precursor to total RNA, amount of unspliced (precursor) transcript was divided by the total amount of transcript (both spliced and unspliced).

**Primers used in qPCR analysis**

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECM33 I260F</td>
<td>TCTCGTTGAGATGGTTTTGG</td>
</tr>
<tr>
<td>ECM33 E2_515R</td>
<td>CACCGGTGATGGTCAAGTTAC</td>
</tr>
<tr>
<td>ECM33 E2_435F</td>
<td>CTGCCACTGCTACTGCTCAA</td>
</tr>
<tr>
<td>Rpl18bIntronF2</td>
<td>GCGGACCAACTTATTAACGTGAC</td>
</tr>
<tr>
<td>Rpl18bIntronR2</td>
<td>TGGTATTCTCGATAGCGAC</td>
</tr>
<tr>
<td>Rpl18bExon2F1</td>
<td>ACAAGGGTAAAGCTCCAAGAT</td>
</tr>
<tr>
<td>Rpl18bExon2R1</td>
<td>TGAAAACAGGAAAAAGGAACA</td>
</tr>
<tr>
<td>Scs22IntronF3</td>
<td>AGTGCCAGAAAAGCTGCTGT</td>
</tr>
<tr>
<td>Scs22IntronR3</td>
<td>CTGTTGCATCGCACA</td>
</tr>
<tr>
<td>Scs22Exon2-2</td>
<td>GGTAACGGGCAAAGTCTGAG</td>
</tr>
<tr>
<td>Scs223’UTRR-2</td>
<td>CAGTAGTATATCCAGCCGAC</td>
</tr>
</tbody>
</table>
RESULTS

H4 all K→Q mutants display splicing defect compared to wild-type

To determine the splicing efficiency of the yeast strains of interest, each strain was transformed with a control plasmid (pLGSD5) and a splicing reporter (pJCR51) (figure 5). The reporters are under the control of the GAL1 promoter, which is induced in the presence of galactose. The splicing reporter, pJCR51, contains an intron that disrupts the reading frame of the LacZ coding sequence, which encodes for β galactosidase. Splicing activity is measured by the production of functional enzyme, which is only generated when proper splicing occurs. Splicing efficiencies measured for wildtype, H3 all K→Q, a negative control, and H4 K→Q show that compared to the wildtype, the H4 tail mutant shows reduced splicing efficiency.
Figure 5. (A) A splicing reporter, pJCR51, measures splicing activity via production of β galactosidase. (B) Pre-mRNA splicing efficiencies of wildtype (MSY590), H3 all K→Q (MSY574), and H4 all K→Q (MSY535), determined by β galactosidase assays.
**H4 tail mutants may show decreased pre-mRNA leakage compared to wild-type.**

To measure pre-mRNA leakage of the yeast strains of interest, each strain was transformed with a control plasmid (pLGSD5) and a leakage reporter (pJCR1) (figure 6). The reporters are under the control of the GAL1 promoter, which is induced in the presence of galactose. The leakage reporter, pJCR1, contains an intron that, when spliced, disrupts the reading frame of the LacZ coding sequence, which encodes for β galactosidase. Transformants with the leakage reporter only produced functional enzyme in the absence of splicing. The intron-less plasmid β galactosidase levels were used to normalize the extent of splicing. Pre-mRNA leakage was measured for wildtype, H3 all K→Q, a negative control, and H4 K→Q and, curiously, the wildtype samples showed the highest leakage activity. However, these findings may be arguable as the β galactosidase assay values were very low for the plasmids, perhaps due to a low gene copy number.
Figure 6. (A) A leakage reporter, pJCR1, measures pre-mRNA leakage via production of β galactosidase. (B) Pre-mRNA leakage of wildtype (MSY590), H3 all K→Q (MSY574), and H4 all K→Q (MSY535), determined by β galactosidase assays.
**H4 K5, 8, 12Q + K3 displays a splicing defect compared to wildtype, but improved phenotype than H4 all K→Q.**

The H4 K5, 8, 12Q + K3 mutant displayed a splicing defect when compared to the wildtype and the negative control, but improved splicing efficiency when compared to the H4 all K→Q. As shown in Figure 7, the H4 K5, 8, 12Q + K3 mutant (MSY570) showed greater splicing efficiency than the H4 all K→Q mutant, but is still appreciably impaired compared to the wildtype. The use of multiple graphs to illustrate this is to impart the consistency of these findings. While the wildtype and the negative control were relatively similar in terms of splicing efficiencies, it is important to note that the H4 K5, 8, 12Q + K3 mutant displayed twice the splicing efficiency in one trial compared to another, which may be a point of concern since the beta galactosidase levels are low.
Figure 7. Both graphs illustrate that the H4 K5, 8, 12Q + K3 mutant (MSY570) displays a splicing defect compared to wildtype (MSY590), but improved phenotype than H4 all K→Q (MSY535)
H4 K5, 8, 12Q + K3 mutant shows higher pre-mRNA leakage phenotype as H4 all K→Q mutant, but less than wildtype.

The H4 K5, 8, 12Q + K3 mutant showed less pre-mRNA leakage than the wildtype strain, but more than the H4 all K→Q mutant. These results are contrary to the expected outcome, especially because the H4 K5, 8, 12Q + K3 mutant appeared to partially rescue the splicing defect seen in the H4 all K→Q mutant. It is difficult to explain why the H4 all K→Q shows the least pre-mRNA leakage and the wildtype shows the greatest, but it may, once again, be partially explained by the low levels of protein.
Figure 8. The pre-mRNA leakage phenotype of the H4 K5, 8, 12Q + K3 mutant (MSY570) is improved compared to wildtype (MSY590), but not compared to H4 all K→Q mutant (MSY535).
H4 tail mutants do not display splicing defect in ECM33

As described previously by Gunderson, et al., intron- and exon-specific primers were designed for ECM33, which has been described to be a gene to which splicing factors are co-transcriptionally recruited (Gunderson and Johnson 2009). RNA from all H4 strains of interest (see materials and methods) were used in the initial qPCR analysis, but none showed a splicing defect in ECM33 when compared to wildtype, as the spliced/unspliced ratios were not significantly different.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Splicing Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSY590</td>
<td>0.9142</td>
</tr>
<tr>
<td>MSY574</td>
<td>0.9235</td>
</tr>
<tr>
<td>MSY535</td>
<td>0.9194</td>
</tr>
<tr>
<td>MSY570</td>
<td>0.9012</td>
</tr>
<tr>
<td>MSY400</td>
<td>0.9263</td>
</tr>
<tr>
<td>MSY582</td>
<td>0.9110</td>
</tr>
<tr>
<td>MSY711</td>
<td>0.9124</td>
</tr>
</tbody>
</table>

Table 1. Preliminary splicing ratios of ECM33 derived from qPCR analysis.
DISCUSSION

Although histone modifications, transcription, and splicing have long been thought to be dynamically interrelated, only recently has solid evidence been presented to support this. A study done by Gunderson and Johnson provided strong evidence for the close association between histone modifications and splicing, which was a long-standing gap in our understanding of gene regulation dynamics. They demonstrated that the SAGA complex of *S. cerevisiae* plays a dual role of acetylating histones as well as interacting with specific components of the splicing machinery. Since much of this investigation has been observed only in H3, the possible role of H4 in splicing has been largely abandoned. This study sought to provide some insight on the role of histone modifications in H4 in splicing based on the studies done in H3.

Previous research has suggested a role for H4 in several cell processes, including transcription, DNA replication, and splicing. The most compelling evidence for the involvement of the H4 N-terminus in splicing stemmed from data that suggested a splicing defect in mutants that had glutamine substitutions for all of the lysine residues of H4. Skewed splicing ratios of the mutant strongly suggested a specific role for the lysine residues (Psathas, unpublished data). Therefore, this study sought to investigate a functional role for conserved lysine residues in the H4 N-terminus of *S. cerevisiae* in pre-mRNA splicing efficiency.

Conversion of lysine residues to glutamine residues mimics acetylated lysines, which suggests that the observed phenotypes are representative of lysine acetylation, and not other post-translational modifications. In the H4 all K→Q mutant, which contains point mutations of lysine to glutamine at all 5 native lysine residues, a splicing defect is observed when compared to the wildtype. In the case of H4 K5, 8, 12Q + K3, a splicing defect was observed, but not as severe as the H4 all K→Q mutant. This suggests the possibility that not all lysines within the H4 tail are
acetylated for proper splicing; in fact, it is likely that the acetylation of all lysines is detrimental to proper pre-mRNA processing.

Megee, et al generated several of the yeast strains of interest and published their characteristic phenotypes. The H4 K5, 8, 12, 16Q mutant grew significantly slower than wildtype due to increased time spent traversing G2 +M. However, a single functional lysine residue of the four was sufficient to induce similar growth patterns in comparison to wildtype. In fact, when a tripeptide, GKG, was inserted after the third residue of the H4 K5, 8, 12Q mutant, the phenotype was rescued such that the G2 + M phase was reverted to wildtype (Megee et al., 1995). This mutant, MSY570, was found to display a splicing defect when compared to wildtype, but displayed an improved splicing efficiency compared to the H4 all K→Q mutant. This may imply that although the H4 K5, 8, 12Q mutant displayed wildtype growth pattern with the GKG insert at K3, splicing efficiency was not rescued. Therefore, even though growth and DNA replication may only require a single functional lysine residue anywhere within the first sixteen residues of H4, proper splicing may be dependent on the presence of specific lysine residues. Future investigations can explore several different mutants with combinations of mutations in the five native lysine residues of H4.

For reasons that are unclear, the results in this study found that the leakage of pre-mRNAs from the nucleus to the cytoplasm is highest in the wildtype strain, as opposed to the H4 mutants. One contributing factor to these unexpected results may be due to the very low protein levels for the yeast strains transformed with the leakage reporter, which led to very low beta galactosidase activity levels as well. Additionally, the values were variable, which resulted in slightly high error bars. Based on the H4 all K→Q splicing data, it was expected that the pre-mRNA accumulation and leakage would be higher than the wildtype, since there is an obvious
defect in splicing efficiency. However, this was not observed in the leakage assay, which is difficult to explain, but may suggest unexpected underlying complexities. In past studies, a splicing defect was often accompanied by higher pre-mRNA leakage, which is expected as inhibition of splicing results in 20 to 50-fold pre-mRNA accumulation as well as increased mRNA production (Bousquet-Antonelli et al, 2000). The most plausible explanation for the results observed from this study is that the low levels of protein may have prevented accurate retention values. All the normalized beta galactosidase values were very low, making it difficult to accurately extrapolate a concrete conclusion, which warrants the need for further experimentation.

Quantitative PCR is an exceptionally useful tool for measuring mRNA levels in vivo and provides a more sensitive quantification of transcription products. Based on H3 studies by Gunderson and Johnson, intron- and exon-specific primers were designed for different genes of interest. The most useful data was extrapolated from ECM33 as the gene of interest. For various reasons, the other gene-specific primers did not provide usable data for calculating splicing ratios. However, the calculated splicing ratios were very similar for all H4 mutants and wildtype, indicating that there were no splicing defects. Although the qPCR technique is an invaluable tool, the ECM33 results derived from this experiment may not be completely accurate. It was thought that the results from the qPCR analysis would corroborate the reporter assay results, but this was not the case. The H4 all K→Q and H4 K5, 8, 12Q + K3 mutants were expected to have markedly reduced splicing ratios, but instead showed comparable values. The most surprising factor was that the H4 tail deletion mutant showed a similar splicing ratio as the wildtype, which was not expected as it was too deficient, in terms of protein production, to even yield results in the reporter assay. There were also several other strains included in the qPCR analysis that were
not yet evaluated in the reporter assay, and thus it was not known where the splicing ratios would fall relative to the wildtype. Although these results were unexpected, there is a possibility that technique and gene-specific differences between the reporter assay and qPCR can at least partially explain them. In addition to further experimentation with ECM33, it would be worthwhile to examine other genes, preferably those known to be modulated by H4 modifications. There are multiple possibilities as to why this data does not correspond to that of the reporter assay. One important point to consider is that the gene of interest may not be impacted by H4 histone modifications. Unlike the reporter assay, where the gene of interest was artificially inserted, ECM33 is inherent to the genome, which can lead to a more complex system. Initially, a random primer was used to generate cDNA from RNA, but in optimizing the procedure, a gene-specific primer was found to be more efficient and can serve to better compensate for low abundance genes or gene products with high turnover rates. Further, RNA extraction and Real-Time PCR techniques have been better optimized for future qPCR analyses.

Future work to better understand the role of lysine residues in the H4 terminus can be done using the reporter assays and qPCR reported here. Using more yeast strains with various point mutations of interest can provide more insight into which residues specifically confer normal splicing efficiencies. Further, more trials should be conducted to investigate pre-mRNA leakage phenotypes to determine whether H4 lysine residues play a role in regulating gene product translocation. It would also be interesting to investigate PTMs apart from acetylation that may be essential in proper splicing. Successful qPCR analysis will be important for substantiating the results of the reporter assay as well as providing reliable measurements of splicing ratios. Thus far, the data from the previously mentioned experiments have demonstrated that lysine residues are essential for proper splicing, most likely in the context of acetylation.
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Position: Howard Hughes Student Scientist in Immunology and Virology Division
Examined the influence of cytoskeletal inhibitors on measles virus spread in primary hippocampal neurons

Wistar Institute, University of Pennsylvania, Philadelphia, PA
June 2006- May 2007 (PI: Dr. Carlo Maley)
Position: Student Intern
Screened and examined mutations in Barrett's esophageal and squamous cell samples

The Pennsylvania State University, University Park, PA
October 2007-May 2011 (PI: Dr. Joseph Reese)
Position: Student Researcher
Examined the functional roles of lysine residues in premature mRNA splicing efficiency in S. cerevisiae

The Scripps Research Institute, La Jolla, CA
June 2010- August 2010 (PI: Dr. Luc Teyton)
Position: Summer Intern
Worked on developing a strategy of a soluble TCR-CD3 complex via sortases

RESEARCH SKILLS

PCR, gel electrophoresis, cell culture, RNA/DNA isolation, plaque assay, immunohistochemistry, immunofluorescence, cDNA synthesis, gel purification and extraction, RT-PCR, gene sequencing, Molecular cloning, Ni-NTA Protein purification, siRNA transfection in cell culture, yeast cloning, SDS PAGE gel, Western blot, bacterial transformation, plasmid preps, β galactosidase assay
WORK/VOLUNTEER EXPERIENCE
Science-U Summer Camps, University Park, PA
June 2009-August 2009, Curriculum Mentor
-Mentored and instructed children aged 9-13 in science camp
-Designed experiments for the children
-Aided in science experimentation.

Sears, Roebuck & Co., Philadelphia, PA
May 2007- June 2010
-Managed customer sales and service
-Handled up to $10,000 daily

International Language Partnership, University Park, PA
August 2009-October 2010, Mentor
-Met on weekly basis with international students and aided in teaching English.

The Navigators Christian Fellowship, University Park, PA
August 2007-May 2011, Member
August 2009- May 2010, Bible Study Leader

AWARDS AND HONORS
Schreyer Academic Scholarship
Terry Lewis Scholarship
Robert C. Byrd Scholarship
Women in Science, Engineering, and Research (WISER) Internship