TRANSCRIPTIONAL FIDELITY OF RNA POLYMERASE II

A Senior Scholars Thesis

by

ERIN LEE O'BRIEN

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

April 2010

Major: Biochemistry
Genetics

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Research Advisor: David O. Peterson
Associate Dean for Undergraduate Research: Robert C. Webb

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ABSTRACT

Transcriptional Fidelity of RNA Polymerase II. (April 2010)

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This research aims to elucidate possible genes that affect transcriptional fidelity of RNA polymerase II (pol II) and quantify these affects *in vivo*. The main focus of this project is the small nonessential subunit of RNA pol II, Rpb9, which is needed for accurate transcription, and the potential proteins the subunit interacts with. Possible gene candidates were selected based on synthetic lethality when they are simultaneously deleted with *RPB9* or by their ability to suppress mutations in *RPB9*. An *in vivo* assay in *Saccharomyces cerevisiae*, used for this project, takes advantage of the transposable element Ty1. RNA transcribed from Ty1 DNA encodes a reverse transcriptase that copies the RNA into DNA and allows it to randomly insert into a host cell chromosome. Errors that occur during transcription of Ty1 RNA become permanent changes in the inserted chromosomal DNA that can be identified and counted. This research is only a small part of a much larger objective that attempts to explain the molecular mechanisms by which the cell maintains the fidelity of transcription. Maintaining fidelity is critical for cells; with poor fidelity RNA pol II would transcribe a 'faulty' message, leading to the translation of a mutated or nonfunctioning protein which

could disrupt normal cellular functions. This can be seen in transcriptional mutagenesis in which certain types of DNA damage result in misincorporation of nucleotides rather than transcriptional arrest. Another example of the importance of fidelity is molecular misreadings in which transcription errors, particularly insertions that cause frameshifts, have been implicated in age related diseases such as Alzheimer's. The preliminary results of this research have shown that this *in vivo* fidelity assay provides a valid way to quantify the effects of specific gene deletions on transcriptional fidelity. Proteins that were initially investigated were ones associated with RNA pol II: TFIIS (DST1) and the SAGA complex (SPT7). Preliminary experiments strongly suggested that TFIIS was not essential for RNA pol II fidelity. These experiments showed that wild type and *dst1Δ* cells each had indistinguishable error frequencies. These data are consistent with experiments conducted by Dr. Peterson using a different *in vivo* assay; however it contradicts a commonly held notion regarding TFIIS involvement in fidelity. This research is now currently constructing new yeast strains with the correct alleles need for the *in vivo* fidelity assay. Also, a strain containing a 1-59 amino acid deletion on Rpb9 has been created and is almost ready to test in the fidelity assay.

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NOMENCLATURE

COMPASS	*Com*plex of *p*roteins *a*ssociated with *S*et1
CTD	Carboxyl terminal domain
DNA	Deoxyribonucleic acid
HAT	Histone actyltransferases
-*his*	Media without histidine present
mRNA	messenger RNA
RNA pol II	RNA polymerase II
RNA	Ribonucleic acid
SAGA	Spt-Ada-Gcn5-Acetyltransferase
TFIIS	Transcription factor II S
-*trp*	Media without tryptophan present

TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Introduction to transcription

Transcription is an essential process carried out by cell to produce proteins for genes. This first step of gene expression involves copying a RNA message from the DNA that will later be translated for protein formation. Loss of transcriptional fidelity during transcription causes the production of mutated proteins. Thus, excessive transcriptional errors impair cellular function [1]. Transcriptional regulation is important in all eukaryotic organisms for cell growth, development, and responses to environmental changes. *Saccharomyces cerevisiae* has provided a powerful system for genetic analysis of transcriptional regulation, and findings using this model system are broadly applicable to higher organisms. Transcriptional regulation requires the interactions of regulatory proteins with various components of the transcription machinery [2]. Regulation of transcription requires an orchestrated effort to produce a successful RNA message. Transcription can be divided into three phases: initiation, elongation, and termination. Eukaryotes require several proteins for transcription initiation. RNA polymerase II requires a set of core promoters, the minimal set of sequence elements required for

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This thesis follows the style of *Proceeding of the National Academy of Science.*

accurate transcription initiation [3]. Transcription factors bind to these promoter sequences to signal where pol II should initiate transcription. The transcription factors must bind in a specific order to recruit pol II. Also, repressor proteins that prevent polymerase from binding must be removed, and activator proteins that contact polymerase and increase its affinity for the promoter or stimulate the transition from a closed to an open polymerase-promoter complex must bind [4]. In addition, histone actyltransferases and chromatin remolding complexes are then recruited by the transcription factors to help open the DNA. After pol II is bound to in the initiation complex, several more proteins are still required. The mediator complex associates with pol II and other DNA bound activators. The Mediator protein functions as a bridge between the regulatory proteins and the basal pol II transcription machinery and directly interacts with activators [5]. Once pol II has cleared the initiation complex, elongation has begun.

The elongation phase requires a new set of proteins including elongation factors and enzymes required for RNA processing. Various proteins thought to stimulate elongation are recruited to pol II and are controlled by phosphorylation of the CTD tail of pol II. Phosphorylations of the CTD tail leads to an exchange of initiation factors for elongation factors. [3] RNA processing enzymes prepare the RNA for export from the nucleus to be translated in the cytoplasm. These processing events included the following: capping of the 5’ end of the RNA, splicing, and polyadenylation of the 3’ end of the RNA. The addition of the poly A tail triggers the final stage of transcription, termination.

Termination depends on the conformational change in the elongating polymerase, which reduces the processivity of the enzyme leading to spontaneous termination. [3]

Genes of interest

A small subunit of RNA pol II, Rpb9 and the potential proteins the subunit interacts with are needed for accurate transcription. Possible gene candidates for this research were selected based on synthetic lethality when they are simultaneously deleted with *RPB9* or by their ability to suppress mutations in *RPB9*. These candidates include: *ADA2*, *CCR4*, *DST1*, *ELP3*, *GCN5*, *PAF1*, *SOH1*, *SPT7*, and *SRB5*.

The SAGA complex

The SAGA complex is a multifunctional coavtivator that regulates transcription by RNA pol II [6]. The SAGA complex consists of at least 20 subunits that acetylate histones throughout the genome and at specific genes. SAGA is recruited to specific gene promoter to induce transcription. The subunit Gcn5, encoded by *GCN5*, is the enzymatic activity in SAGA that preferentially acetylates histone H3 at and histone H2B. Ada2, another subunit of SAGA encoded by *ADA2*, functions as a coactivator with Gcn5 to increase HAT activity [7]. Another important subunit of SAGA is Spt7, encoded by *SPT7*. Spt7 functions as a SAGA core component and maintains the integrity of the

complex. Previous studies suggest that Spt7 may act dynamically to regulate SAGA function [8].

The CCR4-NOT complex

The Ccr4-Not complex is the major deadenylase responsible for initiating mRNA degradation. The Ccr4-Not complex is conserved throughout evolution and in yeast consists of nine defined subunits, one of which is Ccr4, encoded by *CCR4*. Ccr4 is the deadenylase catalytic subunit and contains a 3'→5' exonuclease domain at its C terminus. Ccr4-Not also functions in the initiation and elongation phases of RNA pol II dependent transcription [9].

The Elongator complex

Elongator is a complex associated with the phosphorylated form of RNA pol II. Elongator is made up of several subcomplexes, including the Elp3-containing core complex. The subunit, Elp3 encoded by *ELP3*, is a highly conserved HAT and required for Elongator to properly function [10].

The Paf1 complex

The Paf1complex, encoded by *PAF1,* associates with the elongating RNA pol II. The presence of the Paf1 complex is need for COMPASS and RNA pol II to associate. Compass is a histone methyltransferase that is required for silencing of expression of

genes located near chromosome telomeres. These histone modifying proteins are necessary to clear the DNA of histones and allow RNA pol II to continue transcribing.

TFIIS

TFIIS, encoded by *DST1*, promotes the reactivation of the RNA pol II at arrest sites and induces endonucleolytic cleavage. The arrest of RNA pol II occurs after backtracking and extrusion of the 3' end of the RNA from the catalytic center, and the reactivation of RNA pol II involves the stimulation by TFIIS of the intrinsic RNA cleave activity of the polymerase [11]. This proofreading function of TFIIS is the reason many researcher believe that TFIIS is responsible for maintaining fidelity of RNA pol II.

Mediator

The most universal cofactor that serves to transducer regulator information between gene-specific transcription factors and the core RNA pol II machinery is Mediator. Specific subunits interact directly with diverse transcriptional regulatory proteins, such as transcription factors. Yeast Mediator comprises at least 20 subunits, including Srb proteins, and Med proteins [12]. *SRB5* encodes the protein Med18, which interacts with the C-terminal of RNA pol II subunit, Rbp11 [13]. Another component of Mediator, Med31 encoded by *SOH1*, is linked to transcription elongation [14]. There is little research published on the Med31 and its interaction with RNA pol II.

1-59 Rpb9 allele

This 1-59 Rpb9 protein contains only the first 59 amino acids of the subunit . Previous research indicates that a deletion of the C-terminal half of Rpb9 is phenotypically similar to a wildtype subunit *in vivo* [15]. However, deletion of the N-terminus results in a phenotype similar to a complete Rpb9 deletion. [15] However, no previous research has looked at the effect of this deletion on fidelity.

Project overview

Previous *in vivo* experiments demonstrated that Rpb9, a small nonessential subunit of RNA pol II is required for accurate transcription. This result led to many more questions about Rpb9 involvement in fidelity and its exact mechanism. Proteins that were initially investigated were ones associated with RNA pol II: TFIIS (DST1) and the SAGA complex (SPT7). Preliminary experiments strongly suggested that TFIIS was not essential for RNA pol II fidelity. These experiments showed that wild type and *dst1Δ* cells each had indistinguishable error frequencies. These data are consistent with experiments conducted by Dr. Peterson using a different *in vivo* assay; however it contradicts a commonly held notion regarding TFIIS involvement in fidelity.
These preliminary experiments were important because they showed that this *in vivo* assay was reproducible. However, the published protocol is not appropriate for all of the

experiments that need to be performed. The published version of this assay used *spt3Δ* to suppress transcription of endogenous Ty elements and thus prevent the expression of potentially error-prone reverse transcriptase, which could generate a high background of errors not due to pol II. Unfortunately for this project, this presents a major problem, as *spt3Δ* is a synthetic lethal with *rpb9Δ*. A new approach was needed. Transcription of endogenous Ty elements can also be suppressed in diploid cells by thea1/α1 repressor protein. This allows the *in vivo* assay to be conducted in diploids.

CHAPTER II

METHODS

Strain construction

Sporulation

This project began by creating yeast strains that contain the correct combination of alleles as well as the necessary gene deletion. The Peterson yeast strain library contained α and *a* strains consisting of the following deletions; *spt7*Δ, *rpb4*Δ, *dst1*Δ, *ada2*Δ, and *gcn5*Δ. These deletion genotypes are listed in Table 1.

Table 1: Strain Genotypes

Yeast Strain	Genotype	Mating Type
Y32	dst1Δ::KANMX4his3Δ1leu2Δ0lys2Δ0trp1ΔFAura3Δ0	alpha
Y58	gcn5Δ:: KANMX4his3Δ1leu2Δ0lys2Δ0trp1ΔFAura3Δ0	alpha
Y63	can1-100his3Δ1leu2Δ0lys2Δ0spt7Δ::KANMX4trp1ΔFAura3Δ0	alpha
Y119	his3Δ1leu2Δ0lys2Δ0rpb4Δ::KANMX4trp1ΔFAura3Δ0	alpha
Y155	ada2Δ:: KANMX4his3Δ1leu2Δ0lys2Δ0trp1ΔFAura3Δ0	alpha
Y230	his3Δ200	a
Y235	his3Δ200lys2Δ0rpb9Δ::KANMX4trp1ΔFAura3Δ0	a
Y236	his3Δ200leu2Δ0rpb9Δ:: KANMX4trp1ΔFAura3Δ0	alpha

Yeast strains from the Peterson strain library were used to engineer ideal strains for the *in vivo* fidelity assay. The goal was to mate the deletion alpha strain with an *a* strain that contained the correct *his3* allele (Y230). However, all the other alleles need to remain the same. Taking advantage of genetic recombination, diploids were allowed to sporulate and hopefully find an *a* and an α spore with the correct combination of alleles would be found in the spores. After crosses were made, the mated cells were checked for diploids on lawns of mating tester strains. After diploids were verified, the cells were grown overnight in a liquid culture of YEPD. The overnight culture was then spun down for 2 minutes in a micro-centrifuge. The pellet was resuspended in sterile 1% potassium acetate. After this preparation, the cells incubated at room temperature on a wheel for 4-6 days to promote sporulation. After this time period, sporulation was verified by looking at the cultures under the microscope. Successful sporulation was determined by the presence of tetrads. 10% sporulation was ideal. After sporulation was verified, the sporulated cells were spun down in a sterile microfuge tube for 1 minute. Then the pellet was resuspended with sterile distilled water and spun down again. The pellet was resuspended once more in a 100 µg/mL of Zymolyase 100T solution. After a 30 minute incubation at 37 °C, the cells were spun down for 30 seconds and resuspended in sterile water and spun down again. The pellet was resuspended in 100 µL of sterile distilled water and vigorously mixed at top speed continuously for 2 min. The spores stick to the walls of the microfuge tube. All liquid was removed and a 1 mL of sterile distilled water to the 'empty' tube and vortex for a couple of seconds and the liquid was removed. This was repeated 3 additional times. After the four washes, sterile 0.01% NP-40 was added

and vortex at top speed for 2 minutes. The detergent removed the spores from the wall of the microfuge tube. This suspension is then diluted and plated on YEPD plates and allowed to grow for 2-3 days.

Replica plating screening

After colonies appeared, the cells were patched onto another YEPD plate. The spores were then screened through replica plating onto a series of plates. This included: growth on a G418 YEPD plates verifying the KANMX4 plasmid, no growth on a SC-trp plate verifying the trp1ΔFA allele, no growth on a SC-leu plate verifying the leu2Δ0 allele, no growth on a SC-lys plate verifying the lys2Δ0 allele, and no growth on a SC-ura plate verifying the ura3Δ0 allele. To determine mating type, the mating tester strains were spread onto a YEPD plate and then the patched template plate was replica plated onto it. After a day of incubation, the YEPD mating tester plate was replica plated onto a SM plate where only a diploid cell could grow. Hopefully after screening, there will be a colony that grows on the alpha strain lawn (an *a* mating type) and another colony that grows on an *a* strain lawn (an alpha mating type). Table 2 shows graphically the desired combination of allele.

Table 2: The Desired Combination of Alleles.

Replica Plate Selection						
G418	SC-ura	SC-leu	SC-lys	SC-trp	Y7	Y8
+	-	-	-	-	-	+
+	-	-	-	-	+	-

Confirmation of his3Δ200 allele

Confirmation of the *his3Δ200* involved lys-n-go PCR reactions. The PCR product was run on a 1% agarose gel to determine which *his* allele was present.

pRS41N strain construction plasmid

Plasmid yeast transformation

To transform the pRS415 plasmid in yeast, an overnight culture of dop Y9 was grown up. (dop Y9 has a genotype of his3Δ1leu2Δ0lys2Δ0trp1ΔFAura3Δ0). The overnight was diluted 50x and allowed to grow in warm YEPD for 4-5 hours at 30 °C. After the long incubation, the cells were spun down and then washed with sterile water. The cells were then resuspended and transferred to a microfuge tube. The cells were spun down and washed again with sterile water. The resuspended cells were then alloquoted into six tubes and spun for 30 s in the centrifuge. The supernatant was removed and a transformation mix was added to the cells. The transformation mix consisted of 50% PEG 3500, 1 M Lithium Acetate, 2 mg/mL carrier salmon sperm DNA, and 0.5 µg of the plasmid DNA. The cells were then incubated for 40 min at 42 °C. After 40 min, the cells were added to YEPD media and allowed to grow for several hours. This incubation allowed time for recombination and gave a greater yield of cells with successful plasmid transformation. The transformed cells were then plated onto cloNAT YEPD plates to

select for the plasmid in the transformed cells. Once colonies appeared, they were patched onto cloNAT plates.

Yeast miniprep

After a successful plasmid transformation into Y9, a DNA miniprep was used to rescue the plasmid from the yeast cells so that it could be transformed into *E.coli*. The transformed colonies were grown in an overnight in YEPD. The cells were centrifuged and the supernatant was removed. The pellet was resuspended in a STET solution consisting of 8% sucrose, 50 mM TRIS, 50 mM EDTA, and 5% Triton-X-100. After the addition of 0.45 mm sterile glass beads, the cells were vigorously vortex for 5 min. Another 100 μL of STET was added to the cells and were briefly mixed before being placed at 100 °C for 3 min. The cells were then placed on ice to allow a precipitate to form. The precipitate solution was centrifuged and the supernatant was removed and added to a microfuge tube of 7.5 M ammonium acetate. The solution was placed at -20 °C. After an hour, the solution was centrifuged at full speed for 10 min at 4 °C. The supernatant was then added to ice cold ethanol and the DNA was recovered by centrifugation at 4 °C. The pellet was washed with 70% ethanol and allowed to dry. The pellet was then resuspended in sterile water.

Bacterial transformation

After the plasmid was recovered from yeast, it was ready to be transformed into bacteria, specifically into the *E. coli* strain XL-1 Blue. The XL-1 Blue cells were thawed on ice

before beginning the bacterial transformation protocol. Once thawed, 0.1 µg of DNA was added and the cells were placed on ice for 30 min. After the incubation on ice, the cells were heat shocked at 43 °C for 2-2.5 min. Then the cells were diluted 50 fold in warm LB and incubated at 37 °C for no longer than 2 hours. The transformed cells were then plated and selected for the pRS425 plasmid on LB AMP plates.

Plasmid purification from E.coli

To purify plasmid from *E.coli* the Qiagen QIAprep Miniprep Kit was used. To verify that the plasmid was present, the DNA was run on a 1% agarose gel. A 5,014 kb band confirmed that the pRS415 plasmid was purified. For further confirmation, the purified DNA was digested with the enzymes Bgl1, Xma1, and Xho1. If the pRS415 plasmid was present, Bgl1 would cut the DNA into three segments of 1,975 kb, 1691 kb, and 1,339 kb. Xho1 would cut the DNA into two segments at 1,605 kb and 3,409 kb. Xma1 would cut the DNA into two segments at 1,030 kb and 3,984 kb.

Manipulation of the pRS41N to Create Different Rpb9 Partial Allele Deletions

The goal of working with the pRS41N plasmid is to create strains with different alleles of Rpb9. This includes strains with larger pieces of Rpb9 missing, such as deleting amino acids 1-59, or single point mutations. A 1-59 Rpb9 deletion strain was created through site directed mutagenesis**.**

In vivo fidelity assay

After the strain construction is complete, the cells are now ready for the *in vivo* fidelity experiment. Each diploid strain was transformed with the pJS366 plasmid. This plasmid has several key features that this *in vivo* fidelity assay is based on. Pol II transcription of the Ty1-*TRP1*-*his3AI* transcription unit is induced by the presence of galactose. This transcript will be spliced to remove the artificial intron with *his3-AI* and translated to produce reverse transcriptase from Ty. Reverse transcriptase converts the single-stranded RNA into double-stranded DNA, which will be randomly inserted into host chromosomes. Only cells that have undergone a transposition event will grow without histidine (the artificial intron prevents the plasmid from making a functional *HIS3* mRNA). This involves placing the diploid cells in a galactose medium at 25 °C for 7-10 days. After induction, the diploid strains were plated and then patched onto plates lacking histidine and then replica plated onto plates lacking tryptophan. Transcriptional fidelity was assessed by determining *his+* colonies that are also *trp-*. These colonies identify pol II errors made while transcribing the *TRP1* gene during transposition; such errors generate a defective *TRP1* gene in the transposed DNA.

CHAPTER III

RESULTS

There were two important goals for this project. The first was to create diploid strains of homozygous deletions of the genes of interest. The second was to create Rpb9 deletion alleles. All of these strains were to be tested in the in vivo fidelity assay to better understand how transcriptional fidelity is maintained in a eukaryotic system.

Preliminary *in vivo* fidelity assay results

The first important result that came from this research was that the in vivo fidelity assay was reproducible. The relative frequency of error for the wildtype strain was equal to previously published error rates for wildtype RNA Pol II [16]. This relative error rate was 2%. Along with the construction of a wildtype strain, *rpb9Δ*, *dst1Δ*, and *spt7Δ* strains were constructed. It was also possible to run the in vivo fidelity assay on the *dst1*Δ strain. The relative rate of error for *dst1Δ* cells was found to also be similar to the wildtype relative error rate. The average relative error rate was 2.4% error (table 3). This result confirms the in vitro canavanine assay that a TFIIS deletion has minimal effect on transcriptional fidelity.

Table 3: Fidelity Assay Results of *dst1Δ*

Plate	Number of Colonies on –his	Number of –trp Mistakes	Relative Error Percentage
I	74	2	2.7%
II	72	2	2.7%
III	55	1	1.8%

The canavanine assay is another in vivo assay used to assess fidelity of RNA pol II. Transcriptional mistakes are recorded through an increased sensitivity to the toxic arginine analog canavanine [1] .Unfortunately, attempts to construct the *rpb9Δ*::natMX4 and *spt7Δ*::natMX4 strains were repeatedly unsuccessful. After generating a natMX4 PCR product with the appropriate flanking DNA, (*SPT*, *DST1*, or *RPB9*), it was not possible to recover a successful site-specific recombination of the *rpb9Δ*::natMX4 and *spt7Δ*::natMX4 segments into a yeast strain. There was little growth after the transformation, and the colony PCR conducted on the cells that did grow did not confirm that site-specific recombination had occurred at the desired locus. It was assumed that these cloNAT-resistant cells were from recombination at another site within the yeast genome. The source of this nonsuccess was later understood to be the fault of the yeast strain used. The Strathern yeast strain that was used for this *in vivo* assay was engineered to turn off all endrogenous Ty elements. This would prevent these endogenous Ty elements from interfering with the Ty1 element used in the in vivo assay. This was

achieved by including a deletion of SPT3, which encodes a subunit of the SAGA complex. A double deletion of *spt3Δ* and *rpb9Δ* is a synthetic lethal and a double deletion of *spt3Δ* and *spt7Δ* yields extreme slow growers. Thus despite, the exciting results for *dst1Δ*, it was necessary to try an alternate approach to construct the desired deletion strains.

Construction of *Rpb9* homozygous diploid strain

All endogenous Ty elements are repressed in diploid cells. This repression in diploid cells is due to the presence of an *a*1/α1 repressor complex binding site within their transcriptional regulatory sequences [17]. In lieu of the nonsuccess with the Strathern yeast strain, a homozygous diploid *rpb9Δ* strain was created. However, it was not possible to test this strain in the *in vivo* assay without a control wildtype diploid strain. Theoretically, the desired wildtype *a* and α strains should have been generated in this cross, but then were not isolated after screening approximately 1600 haploid strains derived from the cross.

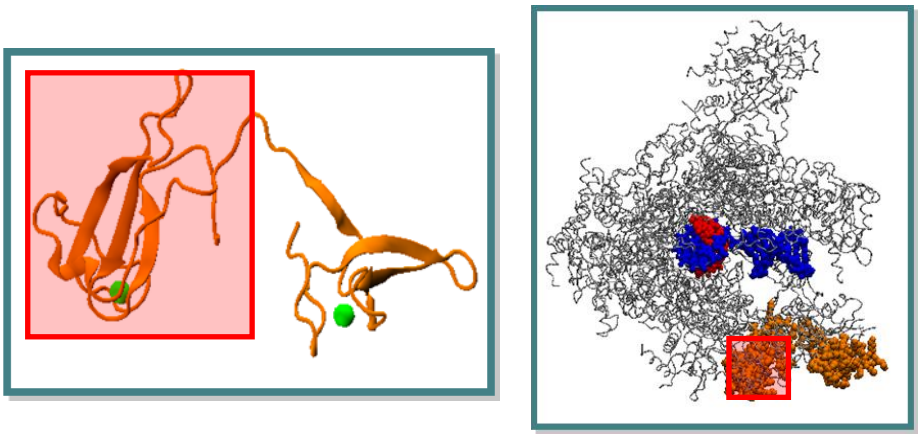

Figure 1: 1-59 Rpb9 Allele. Crystal structures of Rpb9 and of RNA pol II. The red box indicates the piece of the subunit that is missing with a 1-59 Rpb9 allele.

Construction of a pRS41N strain for different alleles of Rpb9

The pRS41N plasmid was created from the pRS416 plasmid through gap repair. The pRS416 plasmid has a *URA3* gene that was cut with a restriction enzyme Stu1. This cut causes the plasmid to linearize and yeast are unable to efficiently ligate the cut DNA to repair the gap. It is possible to introduce the cloNAT gene as the new selectable marker by repairing the gap through recombination. Using site directed mutagenesis, a 1-59 amino acid Rpb9 deletion plasmid was created. This plasmid encodes a partial Rpb9 protein. This 1-59 Rpb9 protein contains only the first 59 amino acids of the subunit (figure 1). Previous research indicates that a deletion of the C-terminal half of Rpb9 is phenotypically similar to a wildtype subunit *in vivo* [18]. However, deletion of the N-terminus results in a phenotype similar to a complete Rpb9 deletion. [17] This plasmid was then cut using restriction enzymes. The pRS41N plasmid was digested with Sac1

and Sal1 to cut out a fragment of about 80 base pairs. The pRS415-Rpb9 1-59 plasmid and pRS415 wildtype plasmid was also cut with Sac1 and Sal1, and the *RPB9*-containing fragment about 1100 base pairs and 1300 base pairs, respectively, were isolated. The wildtype and Rpb9 deletions digestion fragments were then ligated into the digested pRS41N plasmid to create plasmids ready for the fidelity assay. The ligation was proved successful through sequencing reactions. These strains are now ready for the *in vivo* fidelity assay. However, this assay was not completed by the time this thesis was written.

CHAPTER IV

SUMMARY AND CONCLUSIONS

This research has provided another method to quantitate the fidelity of RNA pol II *in vivo*. This *in vivo* fidelity assay has proved to be reproducible. The fidelity results of the *dst1*Δ show that this assay can be used to elucidate interesting information about RNA pol II's interaction with the transcription machinery. Also the Rpb9 deletion allele in the fidelity assay will be useful to better understand Rpb9's role as a subunit and how it affects fidelity.

Future directions

The construction of diploid strains with homozygous deletions of the genes of interest proved to be a daunting task. It was difficult to find yeast strains that, after sporulation, had the desired collection of alleles for both the *a* and α mating type. However, this process can be made simpler by taking advantage of the HO gene in yeast. In yeast strains carrying the HO gene, mating type of a haploid cell is unstable and can change from *a* to alpha or from alpha to *a* nearly every cell division [19]. For most laboratory strains, the HO gene is inactivated so that the mating type stays the same after every cell division. Using a plasmid with an active HO gene, it is possible to switch the mating

type of one of the screened colonies. This would allow the construction of a diploid with a homozygous deletion much easier.

The 1-59 allele of Rpb9 will be tested in the *in vivo* fidelity assay as well as the canavanine assay. There will also be other Rpb9 alleles created to further understand Rpb9's role in transcription.

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