2012

Autoregulation of the TFC6 promoter and effects of mis-expression of TFC6 protein levels

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AUTOREGULATION OF THE TFC6 PROMOTER AND EFFECTS OF MIS-EXPRESSSION OF TFC6 PROTEIN LEVELS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the requirements for the degree of
Master of Science

in

The Department of Biological Sciences

by

Kimberly Le Blanc
B.S. University of New Orleans, 2008
August 2012
ACKNOWLEDGEMENTS

I would like to thank my parents, Carolyn and Edward Le Blanc, for their unconditional love, support, and prayers in all of my endeavors. Without them molding me into the person that I am and instilling a strong sense of faith in Christ, community, and dedication, I would not be where I am today. I love you guys. I would like to thank my lab mates and graduate school friends for all of their encouragement; especially, Shana Garret and Asa Korde. Shana believed in my dream of attending medical school and never stopped encouraging me; she is a true friend and a very special person who will take the world of Microbiology by storm. Asa helped me become re-acclimated to lab work and laughed with me for no reason at all. Thanks to Richard Kleinschmidt for his help and encouragement with my thesis project. I thank Ms. Prissy, for her support, guidance, patience and prompt attention to every matter that arose. I am sure that without her the department of biological sciences graduate school would be lost.

Thanks to Dr. David Donze, for his support, guidance, honesty and patience. He is a one of a kind advisor and anyone that enters his lab or takes his class can not only learn how to conduct “good” science, but how to be a good son, sibling, friend, father, husband, and advisor. Thanks Dr. Donze! Thanks to my committee members, Dr. Patrick DiMario and Dr. Anne Grove for their encouragement and support.

Special thanks to my best friend & boyfriend Ragav for being a pillar of strength, love and encouragement even from a thousand miles away. He never let me give up or stop believing in myself. I love you.
TABLE OF CONTENTS

ACKNOWLEDGEMENTS................................................................................................. ii
ABSTRACT.................................................................................................................. iv

CHAPTER
  1 INTRODUCTION........................................................................................................ 1
  2 RNA POLYMERASE III COMPLEX ASSEMBLY....................................................... 6
  3 EXTRA TFIIC SITES.................................................................................................... 14
  4 THE EXTRA-TRANSCRIPTIONAL FUNCTIONS OF RNA POLYMERASE III.......................... 18
  5 AUTOREGULATION OF TFC6..................................................................................... 23
  6 EFFECTS OF MIS-EXPRESSION OF TFC6 PROTEIN LEVELS ON THE TFC6 PROMOTER............................................................ 27
  7 MATERIALS AND METHODS................................................................................... 30
  8 DISCUSSION............................................................................................................. 37
  9 REFERENCES............................................................................................................ 40
 10 VITA...................................................................................................................... 45
RNA Polymerase III (Pol III) is best characterized for its transcription of tRNA molecules. Interestingly, Pol III and its associated complexes have been found to be involved in the regulation of transcription of genes transcribed by RNA Polymerase II (Pol II) (Kleinschmidt, LeBlanc et al. 2011). Extra TFIIIC sites (ETC sites) are chromosomally located sites discovered in S. cerevisiae that bind the Pol III transcription factor TFIIIC only through B-box interactions. These chromosomal locations are not normally transcribed (Dieci and Sentenac 1996). One of the subunits of the TFIIIC complex, TFC6 has an ETC site (ETC6) in its promoter region (Simms, Dugas et al. 2008; Kleinschmidt, LeBlanc et al. 2011). The TFIIIC complex has been shown to directly regulate transcription from the TFC6 promoter. This is a unique example of autoregulation of a Pol II transcribed gene by a Pol III transcription factor. Mutating ETC6 in the TFC6 promoter shows that both it and a mutation in TFC3, results in increased transcription of TFC6. Both mutations inhibit TFIIIC binding to ETC6. TFIIIC binding to ETC6 is inversely proportional to TFC6 transcription levels. Thus, when Tfc6p was overexpressed, promoter activity was inhibited. Via stringent control on Tfc6p levels, this autoregulation is hypothesized to be involved in global regulation of tRNA gene expression and on global regulation of translation. The previous results from Kleinschmidt et al. 2011 demonstrated that TFIIIC binding is altered by overexpressing TFC6. To examine whether the varied expression of Tfc6p has a global effect on TFIIIC binding, and possibly Pol III transcription, we created a set of yeast strains with significant variation in Tfc6p expression: wild type, under-expressed, and two levels of over-expression (two-fold and ~12-fold). By performing a genome wide chromatin immunoprecipitation analysis of TFIIIC binding when TFC6 expression is mis-regulated using high throughput sequencing (ChIP-Seq) technology, we expect that different TFIIIC bound loci
will show variations in the ChIP-Seq signals that will differ in magnitude. This analysis will allow us to assess how TFC6 mis-regulation effects tDNA transcription at those loci most sensitive to TFC6 mis-regulation, it may reveal cryptic ETC sites and reveal any changes in the extra-transcriptional functions of Pol III.
CHAPTER 1
INTRODUCTION
Eukaryotic organisms have complex genomes and consist of defined organelles that house the necessary components for gene regulation and gene expression. Due to the complex nature of gene expression patterns, gene regulation is in turn complex. The complexity of the eukaryotic genome contains an additional layer involving the overall packaging and organization of its DNA. Double stranded DNA is wrapped around two copies of the core histone proteins (H2A, H2B, H3 and H4) in 146 base pair (bp) increments to create nucleosomes. The nucleosome is the basic structural component of chromatin. Nucleosomes compact the extended DNA into chromatin, which in turn is condensed to form the full chromosome. To add to the intricacy of gene regulation, eukaryotes must unwind the DNA packaged into nucleosomes during the processes of replication and transcription, which creates yet another additional layer of regulation by chromatin modifying proteins.

The structure of chromatin plays a significant role in gene expression. Chromatin exists in two different forms: Euchromatin and Heterochromatin (Fig. 1). Euchromatin is the transcriptionally active, hyperacetylated, decondensed form of chromatin that replicates early in S phase. This decondensed morphology allows transcription factors and other proteins (replication machinery) easier access to the DNA so that transcription and replication can occur. Heterochromatin is the transcriptionally inactive, hypoacetylated, condensed form of chromatin that replicates late in S-phase. In the condensed form, transcription factors, regulatory elements, and other proteins (replication machinery) are limited in their access to the DNA, as the DNA-protein complex would have to be unwound.

The euchromatic and heterochromatic forms of chromatin persist due to the modification state of the histone proteins, and binding of chromatin proteins. Post – translational modifications of the histone tails (consisting of basic amino acids) are the major contributors of
Figure 1. Chromatin States. Transcriptionally active euchromatin (green nucleosomes) and associated transcription complex and regulatory proteins; transcriptionally inactive heterochromatin (red nucleosomes) with heterochromatin associated proteins in yellow.

the formation of chromatin. Several post-translational modifications can affect the lysine, arginine, and serine residues of the histone tails: methylation (lysine and arginine), acetylation (lysine), phosphorylation (serine), and ubiquitylation (lysine).

Histone Modifications

Post-translational modification to the histone tails within chromosomes is known for its role in regulating nuclear function (Strahl and Allis 2000). It is especially important for transcription because of its important role in determining gene expression and (Strahl and Allis 2000; Grant 2001). There are a plethora of post-translational modifications that occur on the tails of histones; these include but are not limited to phosphorylation, ubiquitination, acetylation and methylation (Strahl and Allis 2000; Grant 2001). These modifications can determine how regulatory proteins interact with the histone tails, which means that these same modifications may regulate chromatin structure (Grant 2001).
**Histone Acetylation**

Histone acetylation is the most commonly studied histone modification; acetylation of lysine residues is associated with transcriptional activity and plays an imperative role in transcriptional regulation, at the level of both initiation and elongation (Strahl and Allis 2000; Grant 2001). The main sites of acetylation are the ε-amino groups of lysine residues 9, 14, 18, and 23 on histone H3 and 5, 8, 12, and 16 of histone H4 (Strahl and Allis 2000; Grant 2001). Acetylation can also be linked to nucleosome assembly, DNA replication, higher order chromatin packing, and interaction between non-histone proteins with the nucleosome (Grant 2001). Acetylation is associated with transcriptional activity because when the histone is acetylated, its basic charge is neutralized (Grant 2001). This neutralization of charge is thought to reduce the affinity of histone tails for DNA, and to alter histone interactions between adjacent nucleosomes and various regulatory proteins. These results of acetylation create a chromatin environment more favorable for transcription (Grant 2001).

Important mediators of histone acetylation are the histone acetyltransferases (HATs). HATs are a family of enzymes that are responsible for acetylation of free histones and nucleosome associated histones (Grant 2001). HATs are not only associated with histone transcriptional activity, but some are also considered as transcription factors themselves, as they can also modify other transcription factors (Grant 2001). HATs structurally alter the nucleosome so that transcription factors have increased access to the DNA (Grant 2001). Acting with the opposite function to the HATS, are histone deacetylases (HDACs), which generally repress transcriptional activity. By deacetylating the lysine residues of the histones, heterochromatin formation is enhanced by deacetylated histone interaction with the Sir proteins in yeast (Grant 2001).
**Histone Methylation**
Methylation of histones commonly occurs via histone methyltransferase (HMT) enzymes (Grant 2001). The histone tails of histones H3 and H4 lysine residues 4, 9, 27, and 20 respectively are frequently methylated (Strahl and Allis 2000; Grant 2001). These lysine residues have the potential to be methylated with one, two, or three methyl groups making this post-translational modification more complicated (Strahl and Allis 2000; Grant 2001).

**Histone Phosphorylation**
Not as much is known about histone modifications due to phosphorylation as is known about acetylation and methylation (Grant 2001). Phosphorylation is mainly known for its contribution to DNA repair, cell death, and mitotic chromosome condensation (Grant 2001). Phosphorylation of serine residues (specifically serine 10) has been shown to be associated with gene activation and transcriptional induction (Grant 2001). It appears that the phosphorylation of histone tails (by the addition of negatively charged phosphate groups) neutralizes their basic charge, reducing histone affinity for DNA (Grant 2001).

Saccharomyces cerevisiae, commonly known as baker’s yeast, belongs to the kingdom fungi (ascomycetes). It is now considered a key model system for research in genomics, biochemistry, molecular and cellular biology due to it being a simple, unicellular eukaryote, and is non-pathogenic, among many other desirable features. Its genome contains approximately 12.1Mb of DNA. One of its unique characteristics is that the yeast can exist as either haploid or diploid cell types. The haploid cells exist as MATa or MATα and the diploid cells MATa/α. These different cell types are another advantage to using yeast cells for genetic studies because they arise from various patterns of gene expression from genetically identical cells.
CHAPTER 2
RNA POLYMERASE III COMPLEX ASSEMBLY
Eukaryotic transcription is an important and complex process. It is the process of creating a complementary RNA sequence from a DNA template that may encode either a protein or a non-coding RNA. Retrieving this information stored in the genome of eukaryotes to carry out the process of transcription involves recognizing the various sequences that control the transcription unit (DNA binding proteins, transcription factors and other regulatory proteins), most importantly promoter regions (various sequence sites recognized in order to begin transcription: transcription start sites, transcription factor binding sites, etc.). All of these factors bound to DNA are collectively referred to as the pre-initiation complex and must recognize, and properly assemble on the DNA before transcription can begin. It is important to note that the transcription start site (TSS) is the site where RNA Polymerase binds; that is the site where the RNA product begins.

One of the main players in gene expression/transcription is the RNA polymerase enzyme family. Amongst its many functions, the main functions of RNA polymerases are promoter recognition and gene transcription. These polymerases are guided to their target promoters by the pre-initiation complex. Three enzyme complexes fall under the umbrella of RNA Polymerase in eukaryotic cells, each with specific functions: RNA polymerases I, II, and III. RNA Polymerase I functions to transcribe only those genes encoding ribosomal RNA. RNA Polymerase II transcribes both mRNA and snRNA genes via its multi-component transcription complex.

Here, I will focus on RNA Polymerase III. RNA Polymerase III transcribes RNAs shorter than 400 base pairs that function in basic metabolic processes during protein synthesis. Its main function is expression of the tRNA genes (tDNAs) which mainly function in a structural or catalytic capacity.
RNA Polymerase III has three structurally different promoters that it is recruited to through different combinations of protein-protein and protein-DNA interactions. It has two types of promoter elements located within genes (gene-internal) that may or may not contain TATA boxes, and one outside of genes (genes-external) that contain a TATA box.

**Pol III Complex Assembly**

The assembly of the RNA polymerase III complex is driven by the structure of the promoter. There are 3 promoter types (Fig. 2): type 1 (5S RNA), type 2 (tRNA or Ad2 VAI), and type 3 (Hs U6 snRNA). The intragenic promoters, type 1 and type 2, have a core internal control region (ICR) that consists of an A box, Intermediate element (IE), and a C box. The C box is conserved in the type 1 promoters of different species and is the only required component of the ICR for transcription to occur in Saccharomyces cerevisiae. The first step of beginning transcription of tRNAs from type 2 promoters is dependent on the A and B boxes of the ICR (Schramm and Hernandez 2002; Simms, Dugas et al. 2008). The ICRs A and B box are the binding sites for various transcription factors and some may contain a TATA box. The A and B boxes are highly conserved in genes encoding tRNAs because they are required for tRNA function (they encode tRNA T-loops and D-loops). Type 3 promoters are extragenic and are composed of a proximal sequence element (PSE), and a TATA box. The distal sequence element (DSE), located upstream of the PSE, activates transcription from the core promoter. All type 1 and most type 2 promoters lack a TATA box, but it has been found that the TATA-box-binding-protein (TBP, as part of the TFIIIB complex) is required for tRNA transcription from the type 2 promoter. The TATA box recruits TBP via direct protein-DNA interactions. It has also been shown in previous studies that the TATA box contains all of the information required to assemble the RNA polymerase III transcription initiation complex in the absence of the other
promoter elements in vitro by binding of TFIIIB via TBP. *S. cerevisiae* only has type 1 and 2 promoters; I will focus on the RNA polymerase type 2 intragenic promoter and its assembly on tRNA genes.

Adapted from Schramm and Hernandez (2002).

**Figure 2. Various Types of RNA Polymerase III Promoters.** Transcriptional start sites (TSS) are indicated by arrows. Each promoter contains a plus sign and number above the transcription termination sequence (TTTT) denoting the length of the transcript in base pairs. Type 1 promoters (5S) contains an ICR (Internal Control Region) consisting of an intergenic element and A and C boxes. Type 2 promoters (tRNA) contain an ICR consisting of A and B boxes. Type 3 promoters (Hs U6) contain an ICR upstream of the TSS consisting of a Distal Sequence Element (DSE), Proximal Sequence Element (PSE) and a TATA box. The *Saccharomyces cerevisiae* U6 gene promoter (Sc U6) has an ICR consisting of an intragenic A box, a B box downstream of the transcription termination sequence, and a TATA box upstream of the TSS.
RNA Polymerase III Complex Recruitment

RNA polymerase III is recruited to the promoters via different paths and is mediated by the recruitment of various transcription factors (Fig. 3). TFIIIC and TFIIIB are both multi-subunit transcription factors that are required for most Pol III transcription. In the type 2 RNA polymerase promoters (for tRNAs) the conserved A and B boxes are first bound by the transcription factor TFIIIC. Then, TFIIIC recruits TFIIIB, which then recruits RNA Polymerase III. It has been found in yeast that TFIIIB alone is sufficient to recruit RNA polymerase III to the promoter in vitro, as it can drive transcription by directly binding the TATA box via the TBP (directly binding the DNA). This means that TFIIIC is a recruitment factor for TFIIIB, and is not always necessary for the binding of TFIIIB, which can directly recruit Pol III. TFIIIB is composed of TBP, Brf1, and Bdp1 proteins. Interestingly, the Bdp1 subunit binds to DNA at both the promoter region and upstream of the TATA box. The binding of Bdp1 to these regions induces a bend in the DNA that stabilizes the TFIIIB-DNA complex by thermodynamically preventing the DNA from sliding out the transcription complex via Bdp1 kinetic trapping of the DNA (Cloutier, Librizzi et al. 2001).

The process of transcription occurs in three main steps: initiation, elongation, and termination. Normally for Pol II, transcription factors bind to the core promoter, and recruit RNA polymerase dependent on TFIIE and ATP – dependent TFIIH helicase activity (TFIIH unwinds double stranded DNA to form the initial transcription bubble) as a prerequisite for transcription to begin. Transcription mediated by RNA Polymerase III is unique in that after TFIIIB is recruited to the promoter, opening of the transcription bubble by the complex is not ATP – dependent. The Brf1 subunit of TFIIIB plays a major role in unwinding the DNA allowing the transcription bubble to form (Paule and White 2000). TFIIIB consists of the Brf1, Bdp1 and TBP subunits. Chromatin remodeling complexes are also thought to “loosen”
nucleosomes to allow transcription factors access to the chromatin (Grant 2001). Once RNA polymerase III binds, it can move down the gene with the transcription bubble and elongate the newly formed RNA strand (Paule and White 2000).

At TATA box-containing Pol III promoters, TFIIIB can bind the promoter without TFIIIC. This is due to the specific binding of TBP to the TATA box; TBP binds the TATA box, recruits Brf1 which in turn recruits BdpI and the rest of the TFIIIB complex. However, most genes transcribed by RNA Polymerase III do not contain TATA boxes, and TFIIIC is sufficient to begin the association of transcription initiation factors (Kassavetis and Geiduschek 2006). The C-terminal domain of Brf1 interacts with C17 (Ferri, Peyroche et al. 2000) and C34 subunits of RNA polymerase III (these are the subunits required for transcription initiation – (Werner, Chaussivert et al. 1993; Wang, Luo et al. 1997). On genes that do have a TATA box and that do not require TFIIIC, TFIIIB is the only protein required to recruit the initiation complex (Joazeiro, Kassavetis et al. 1994).

Transcription factor TFIIIC binds the A and B boxes of type 2 tRNA RNA polymerase III promoters. TFIIIC in Saccharomyces cerevisiae is made up of two sub-domains, τA (which binds weakly to the A box) and τB (binds to the B box with extremely high affinity). Each sub-domain is of similar size, and is separated by flexible linker subunits that allows it to bind the A and B boxes simultaneously (Marzouki, Camier et al. 1986; Schultz, Marzouki et al. 1989).

TFIIIC in S. cerevisiae consists of 6 essential subunits: Tfc1, Tfc3, Tfc4, Tfc6, Tfc7, and Tfc8. The τB domain is composed of Tfc3 and Tfc6, which recognize the conserved B box of type 2 promoters. Type 2 promoters are the site of transcription of tRNA and rRNA genes. The Tfc3 subunit binds the B box and the Tfc6 subunit binds just downstream of the B box near the tRNA genes. Tfc1, Tfc4, and Tfc7 make up the τA domain, which binds to the A box of the type
I promoter about 30bp downstream of the TSS.

Once bound to both A and B boxes, the TFIIIC complex functions to recruit TFIIIB to the upstream region of tDNAs, and in the process indirectly recruits RNA pol III.

In a combination of protein-protein interactions during transcription initiation, TFIIIC recruits TFIIIB by the TFIIIC subunits Tfc4 and Tfc8 interacting with the Brf1 and Bdp1 and TBP subunits of TFIIIB respectively. Some evidence suggests that TFIIIC directly interacts with RNA polymerase III via its Tfc4 subunit interacting with the C53 and ABC10α subunits of RNA polymerase III, and these additional interactions aid TFIIIB in its recruitment of RNA

Adapted from Schramm and Hernandez (2002).

**Figure 3. Initiation Complex Assembly on Type 2, 1, 3 Promoters and a TATA Box.** Purple arrows denote contacts between RNA polymerase III and the transcription factors; Blue arrows denote interactions between transcription factors; Green arrows denote interactions between DNA binding proteins and the promoter elements. A) Initiation complex on type 2 promoter B) Initiation complex on type 1 promoter C) initiation complex on type 3 promoter D) Initiation complex assembled on artificial promoter consisting of only a TATA box.
polymerase III to the promoter to begin transcription. After transcription initiation has occurred, elongation can proceed.

During elongation, RNA polymerase III may transiently displace some transcription factors to allow it to transcribe along the gene, but during this process those displaced factors remain associated with the complex (Paule and White 2000). After the intended RNA strand has been fully transcribed, termination sequences are recognized by RNA Polymerase III and this process seems to be only orientation dependent (Paule and White 2000). After termination is complete, the transcription complex can be recycled to begin the next round of transcription. RNA Polymerase III is recycled for the next round of transcription while remaining associated with the DNA template and is not released after termination (Paule and White 2000). The main transcription factors TFIIIA, TFIIIB, and TFIIIC induce bends in the DNA which may allow room for the polymerase to remain associated with the DNA (Paule and White 2000).
CHAPTER 3
EXTRA TFIIIC SITES
Extra TFIIIC sites (ETC sites) are chromosomally located sites discovered in S. cerevisiae that bind TFIIIC only through B-box interactions. These chromosomal locations are not normally transcribed, indicating that RNA polymerase III and its components are not recruited in vivo (Dieci and Sentenac 1996). ETC sites are preferentially found in intergenic regions of divergently transcribed RNA polymerase II genes and suggest a function in gene demarcation. Before recent evidence by Simms et al. 2008., these ETC sites were not demonstrated to have a defined function in Saccharomyces, but in Schizosaccharomyces pombe they act as heterochromatin barriers and are referred to as Chromosome-organizing clamps (COC). In S. pombe, there are 23 COC sites that have TFIIIC bound (Noma, et al., 2006). In S. cerevisiae, these sites can function as chromatin boundary elements by blocking the spread of heterochromatic silencing and chromatin insulation (Simms, Dugas et al. 2008).

It is already known that tRNAs and bound TFIIIC can block the spread of heterochromatic silencing by acting as barriers. Simms et al. 2008. showed that in S. cerevisiae, the RNA polymerase III complex can act as a chromosomal barrier to the spreading of heterochromatin, and both tDNAs and ETC sites can act as insulators by inhibiting gene activation when located between promoters and upstream activating sequences (UAS). ETC sites therefore function as insulators to gene activation and also barriers to heterochromatic spreading. These findings suggest an expanded role for the RNA Polymerase III complex, and TFIIIC in particular, in genome organization, and regulation of neighboring genes transcribed by RNA Polymerase II.

Specifically, Simms et al. 2008. showed that tDNAs can act as insulators by inserting the TRT2 tDNA (previously suggested to act as a barrier at its native locus) between the UASG and the GAL1-10 promoters to see if it blocked the activation of the GAL gene. They found that
cloning the TRT2 tRNA between the UASG and the promoters in either orientation completely abolished Gal4 mediated activation of transcription, in effect insulating the GAL promoter from being activated by its UAS. If the tDNA is inserted upstream of the GAL10 UAS, the tDNA will have no insulating effect on GAL10, but does result in blockage of enhancer activity/function of the neighboring gene GAL1. Clear insulator activity occurred only when the tDNA was cloned between the transcription factor binding site (UAS) and the promoter.

Simms et al. 2008. 2008 also showed that ETC sites function as insulators to gene expression and barriers to gene repression. When their sequences are aligned, the B-box sequences between ETC sites and tDNAs are highly conserved. ETC sites have been well characterized to be sites that bind only TFIIIC without the rest of the RNA pol III complex, and in yeast only where box B sequences are located. In humans cells, however, TFIIIC has been found at non-B-box sites also in genome-wide ChIP-Seq experiments (Moqtaderi, Wang et al. 2010; Oler, Alla et al. 2010; Raha, Wang et al. 2010). Simms et al. 2008. 2008 defined the ability of ETC sites to also act as insulators between regulatory elements of divergently transcribed gene promoters. They showed that by cloning the ETC4 at the GAL10 locus between the UASG and the GAL10 promoter, growth of the cells was inhibited when grown with galactose a the sole carbon source. This result indicated the ETC4, which binds TFIIIC, can also function as an insulator to gene activation. When the mutated etc4 box b was cloned at the same locus, gene activation by the UASG was restored, as TFIIIC is not recruited to the mutated etc4 site.

Similarly Simms et al. 2008. 2008 showed that the ETC4 site can act not only as an insulator to gene activation but also as a barrier to heterochromatic silencing of an ADE2 reporter gene mediated by the SIR proteins. This was found by mutating the natural tDNA barrier to heterochromatic silencing and finding that in its absence, silencing spread downstream,
inhibiting expression of an ADE2 marker gene and yielding a phenotype characteristic of Ade-strains (pink colonies). When this mutated site was replaced with ETC4, silencing was blocked and a normal white colony phenotype was observed indicating expression of ADE2.

It is well documented that COC and ETC sites bind TFIIIC. Simms et al. 2008. 2008 showed that the above described boundary activities (insulator and barrier) of ETC4 not only binds TFIIIC, but requires the binding of TFIIIC to act as a boundary element, but does not require TFIIIB. They showed this by FLAG-epitope tagging the protein subunits Tfc1 and Brf1 of TFIIIC and TFIIIB respectively with the anti-FLAG antibody to them via chromatin immunoprecipitation. In the case of ETC4 they found that Tfc1p was enriched when compared with the control strains at the GAL locus, but Brf1p was not. Further more, when ETC4 was mutated at the conserved box B sequence preventing TFIIIC binding, insulator activity was not observed. This is evidence that ETC4 binds only TFIIIC and that TFIIIC but not TFIIIB is required for insulator activity of ETC sites.
CHAPTER 4
THE EXTRA-TRANSCRIPTIONAL FUNCTIONS OF RNA POLYMERASE III
RNA polymerase III-transcribed genes can be involved in several extra-transcriptional functions, as tDNAs are known to influence the following: sister chromatid cohesion, recruitment of condensin complex to chromatin, creating pause sites for replication fork progression, direction of yeast and Dictyostelium retrotransposon integration, overriding of nucleosome positioning sequences, repressing transcription from RNA Polymerase II promoters and protecting Polymerase II genes from repression by transcriptional repressors including heterochromatin (Simms, Dugas et al. 2008). It has also been found that partial RNA polymerase III complexes at ETC sites are sufficient to impart some of these extra-transcriptional effects. These extra-transcriptional effects are described in more detail below.

**Ty Element Targeting**

Transposon integration is directed by tDNAs and the interaction of retrotransposons with tDNAs is one of the first described examples of RNA polymerase III associated extra-transcriptional activity (Chalker and Sandmeyer 1992). Experiments were done by Chalker et al. to tag the Ty3 transposon in yeast to see where it inserts relative to tDNAs. They found that all insertion points were immediately upstream of the tDNA TSS. The integration of Ty3 upstream of tDNAs is inhibited by RNA polymerase III but requires the binding of TFIIC and TFIIIB, interacting directly with the Brf1 and Bdp1 subunits of the latter (Kirchner, Connolly et al. 1995; Connolly and Sandmeyer 1997). Integration also requires the Set3C histone deacetylase complex, which is also present at tDNAs. More evidence shows that the retrotransposon Ty1 inserts upstream of tDNA TSSs, but over a larger area than the Ty3 transposons (Ji, Moore et al. 1993; Devine and Boeke 1996). Like Ty3, Ty1 requires binding of TFIIIB to integrate into the DNA, and sometimes it also requires the ISW2 chromatin
remodeling complex (Yieh, Kassavetis et al. 2000; Yieh, Hatzis et al. 2002; Bachman, Gelbart et al. 2005).

**Replication Fork Pause Site**
Recent studies have shown that tDNAs are one of the causes of replication pause sites (RFP sites) (Deshpande and Newlon 1996). This pausing at RFP sites is dependent on the binding of TFIIIC (Ivessa, Lenzmeier et al. 2003). It was recently found that most of the tDNAs present and a few Pol II genes colocalized with RFP sites in a genome wide chromatin immunoprecipitation study done to track RFP sites (Sekedat, Fenyo et al. 2010). It was further found by a different group that RNA Polymerase III is globally present at RFP sites.

**Position Effect on RNA Polymerase II promoter**
It has been found that components of the RNA polymerase III transcription unit has effects on transcription of neighboring RNA polymerase II genes, specifically transcription directed by the Ty promoter (Kinsey and Sandmeyer 1991). When TFIIIC binding is inactivated and the RNA Polymerase III complex does not assemble, transcription of Ty3 elements is significantly increased, meaning that the functional tDNA inhibits transcription from the Ty element RNA Polymerase II promoter (Kinsey and Sandmeyer 1991). This effect of tDNAs on the Pol II promoter has been called tRNA gene mediated gene silencing or tRNA position effect (Hull, Erickson et al. 1994; Bolton and Boeke 2003; Simms, Miller et al. 2004). The exact mechanism of tRNA position effects depends on the system.

As discussed previously, the assembled RNA polymerase III complex appears to function as an insulator by the activation of a given promoter’s UAS under certain conditions (Simms, Dugas et al. 2008). When the RNA Polymerase III complex near other Pol II promoters was prevented from fully assembling, Pol II promoter transcriptional activity increased. In certain instances, it was shown that the tDNA prevented the activation of another gene promoter’s UAS.
from activating the Pol II promoter, as when the UAS itself was also deleted, RNA Pol II transcriptional activity decreased (Simms, Miller et al. 2004; Simms, Dugas et al. 2008).

**Overriding Nucleosome Positioning**

Certain extra-transcriptional functions of the pre-initiation complex are due to nucleosome free regions of DNA that are created by the TFIIIC nucleoprotein complex bound to DNA (Mavrich, Ioshikhes et al. 2008; Xu, Wei et al. 2009). The position of nucleosomes in the eukaryotic genome can be dependent on what is called a nucleosome positioning sequence, which preferentially associates with nucleosomes; this positioning can be affected by adjacent tDNAs (Morse, Roth et al. 1992). Nucleosome position at such sequences can be displaced by inserting a tDNA adjacent to a nucleosome positioning sequence. This result, along with the presence of ETC sites in areas where there are no nucleosomes show that the binding of TFIIIC and PolIII to the DNA supercedes that of nucleosome formation (Mavrich, Ioshikhes et al. 2008; Xu, Wei et al. 2009).

**Chromatin Boundaries**

Gene regulation can also be dependent on a class of DNA sequences called chromatin boundary elements. These elements prevent inappropriate regulation of a given gene by regulatory elements (enhancers and silencers) of neighboring genes (Donze, Adams et al. 1999; Simms, Dugas et al. 2008). Insulators and barriers make up the chromatin boundary elements. Insulators or enhancer-blockers are DNA sequences and their associated protein components located between an enhancer and a promoter that prevent the improper activation of transcription of non-target promoters. Barrier elements prevent the spread of heterochromatic transcriptional silencing. Heterochromatic silencing can silence one gene or a group of genes by propagating
histone modifications, specifically deacetylation in S. cerevisiae. tDNAs function as boundary elements in S. cerevisiae by blocking this heterochromatic silencing.

Silencing propagates down the chromosome in a stepwise fashion along nucleosomes. In the previous section the nucleosome-free region of DNA was discussed and how the Pol III complex appears to bind instead of the nucleosome. In various studies, tDNAs appear to be important in insuring that genes adjacent to transcriptionally repressed areas due to silencing, maintain normal transcriptional levels by acting as a barrier to heterochromatic silencing. At the yeast mating locus, HMR, a tDNA has been shown to function as a barrier to heterochromatic silencing (Donze, Adams et al. 1999; Simms, Dugas et al. 2008). When this tDNA is deleted, an inserted downstream marker gene is transcriptionally repressed, allowing silencing to spread (Donze and Kamakaka 2001). At the STE6 locus, another tDNA, named TRT2, has two functions: it acts as a barrier to repression in MATa cells and as an insulator in MATa cells (Simms, Dugas et al. 2008). It functions as an insulator by inhibiting Mcm1p from inappropriately activating the CBT1 promoter (Mcm1p functions to activate STE6).

Furthermore, there is evidence that not only do tDNAs act as an insulators, but that insertion of an ETC site (which only binds TFIIIC), can also act as a barrier (Simms, Dugas et al. 2008).

**Cohesin, Condensin, Clustering and Genome-Wide Organization**

Chromosomal segregation, chromatid cohesion, and chromatin organization require the Structural Maintenance of Chromosomes (Smc) essential protein complexes cohesins and condensins. If the cohesin subunits Smc1 or Smc3 are mutated, a loss of barrier function of the HMR tDNA is observed in S. cerevisiae (Donze, Adams et al. 1999).
CHAPTER 5
AUTOREGULATION OF TFC6
RNA Polymerase III is known for its transcription of several RNA molecules, the predominant function is the transcription of tRNA molecules. RNA Polymerase II transcribes heterogenous RNAs (mainly protein coding mRNAs). Even though the major function of RNA Polymerase III and its associated complexes have been well studied, it is becoming more clear that these complexes of RNA Polymerase III are additionally involved in the regulation of transcription of genes transcribed by RNA Polymerase II, in some cases directly affecting RNA Polymerase II promoters (Kleinschmidt, LeBlanc et al. 2011).

One of the subunits of the TFIIIC complex, TFC6, is a 75 kDa protein. The gene that encodes TFC6 has an ETC site (ETC6) in its promoter region (Simms, Dugas et al. 2008; Kleinschmidt, LeBlanc et al. 2011). Because TFC6 is a subunit of the TFIIIC complex, it was hypothesized that the Tfc6 protein autoregulates its own promoter by binding to the ETC6 site as part of TFIIIC. Mutational analysis confirmed that the B-box, located within the ETC6 site, functions as a regulatory element in the TFC6 promoter mediating autoregulation of the promoter. Tfc6 protein (Tfc6p) levels and the binding of the TFIIIC complex effect the regulation of the TFC6 promoter (Kleinschmidt, LeBlanc et al. 2011). This indicates that an essential component of the RNA Polymerase III transcription complex, a TFIIIC subunit, directly regulates an RNA Polymerase II promoter (Kleinschmidt, LeBlanc et al. 2011). Thus a stringent control on Tfc6p levels, this autoregulation is hypothesized to be involved in global regulation of tRNA gene expression and overall on global regulation of translation.

Four parameters were examined by Kleinschmidt et al. 2011 to confirm TFC6 autoregulation: identification of TFC6 upstream promoter elements, analysis of how TFIIIC associates with ETC6 and its effect on TFC6 transcript levels, effects of overexpressing TFC6, and TFIIIC association with B-boxes when TFC6 is overexpressed.
Identification of TFC6 Upstream Promoter Elements

Promoter elements upstream of the TFC6 gene were identified using a combination of sequence analysis and site-directed mutagenesis (Kleinschmidt, LeBlanc et al. 2011). Seven 12-base pair mutations were created at TFC6 promoter regions most highly conserved between five species of yeast; the 12bp regions of each site were mutated on plasmids, reintegrated into the yeast genome, and TFC6 mRNA levels examined via Northern blot analysis (Kleinschmidt, LeBlanc et al. 2011). The most significant effects were seen in mutants 3, 4, and 5 (Fig. 4).

Adapted from Kleinschmidt, et al. 2011.

**Figure 4. Identification of TFC6 Upstream Promoter Elements.** Promoter regions of highest homology upstream of the Transcriptional Start Site (TSS) across five different budding yeast species were designated as promoter boxes 1 – 7. The most significant effects of these mutations were seen in mutants 3, 4, and 5.

Mutant 3 appears to affect a key transcription factor binding site, as it results in weakened TFC6 transcription and a slow growth phenotype. Mutants 4 and 5 spanned the ETC6 site and increased mRNA expression approximately two-fold, indicating that the ETC6 B-box is involved in negative regulation of the TFC6 promoter. Mutations in RNA Polymerase III and the TFIIIC complex show evidence that TFC6 regulation is directly mediated by binding of TFIIIC, and is not due to RNA Polymerase III activity. Chromatin immunoprecipitation (ChIP) analysis
confirmed that TFIIIC binding was inhibited in strains containing mutations in ETC6 or TFC3, and that reduced complex binding correlated with increased TFC6 transcription.

**Effects of Overexpressing TFC6**

Overexpression of TFC6 followed by ChIP analysis using an antibody against TFC1-3xFLAG showed an increased association of the entire TFIIIC complex at ETC6, which also correlated with an apparent decrease in activity of the TFC6 promoter. Neither of these effects were seen upon overexpression of other TFIIIC subunits. This appeared to be in part caused by decreased binding of TBP to the TFC6 promoter shown by ChIP experiments. Other ETC sites and tDNAs were examined for increased TFIIIC association after overexpression of TFC6, with each site analyzed showing varying degrees of increased TFIIIC binding. This result suggests that global TFIIIC binding is sensitive to TFC6 levels, suggesting a potential for TFC6 levels in global Pol III transcriptional regulation.
CHAPTER 6
EFFECTS OF MIS-EXPRESSION OF TFC6 PROTEIN LEVELS ON THE TFC6 PROMOTER
The TFIIIC complex has been shown to directly regulate transcription from the TFC6 promoter, which encodes one of the TFIIIC subunits. This indicates regulation of an RNA Polymerase II transcribed gene by an RNA Polymerase III transcription factor. Mutating ETC6 in the TFC6 promoter, shows that both it and a temperature sensitive mutation in TFC3, results in increased transcription levels of TFC6. Both mutations inhibit TFIIIC binding to ETC6; TFIIIC binding to ETC6 upstream of the TFC6 promoter is inversely related to TFC6 transcription levels.

Also, in previous experiments it was shown that TFIIIC binding was increased at ETC6 and tDNA sites when Tfc6p was overexpressed indicating that Tfc6p expression affects TFIIIC binding globally, which potentially might lead to global effects on translational gene regulation (Kleinschmidt, LeBlanc et al. 2011).

Based on the result found by examining the ETC6 site that TFIIIC binding was increased, further ChIP experiments were done to examine TFIIIC enrichment at multiple tDNA and ETC loci when Tfc6p was over expressed. All TFIIIC binding sites tested are sensitive to increased levels of Tfc6 protein, showing an increased ChIP signal. (Fig 5; (Kleinschmidt, LeBlanc et al. 2011). At all loci tested, TFIIIC binding was enriched from 1.2 fold to over 2 fold (Kleinschmidt, LeBlanc et al. 2011).

These results are consistent with Tfc6 being the most limiting component of the TFIIIC complex. These differences in TFIIIC binding did not correlate with any altered levels of Pol III transcription at these few selected loci. Due to the differences in binding of the TFIIIC complex, we can ask the question “is this increase in binding global?”

To examine whether the varied expression of Tfc6p has a global effect on TFIIIC binding, and possibly Pol III transcription, we created a set of yeast strains with significant
variation in Tfc6p expression: wild type, under-expressed, and two levels of over-expression (two-fold and ~12-fold, Fig. 3.2; Table 2). These strains were created from strains used to test how overexpression of Tfc6p altered TFIIIC binding in studies done by Kleinschmidt et al. 2011 (Table 1).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chr.</th>
<th>Tfc1 ChIP</th>
<th>Fold Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>tR(CCG)L</td>
<td>XII</td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>tF(GAA)P2</td>
<td>XVI</td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>tK(CUU)G1</td>
<td>VII</td>
<td></td>
<td>1.6</td>
</tr>
<tr>
<td>ZODI</td>
<td>XIII</td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td>ETC4</td>
<td>VII</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>ETC5</td>
<td>XIII</td>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td>TFC6 overexpression</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Kleinschmidt et al. 2011.

**Figure 5. Overexpression of TFC6 Increases TFIIIC Binding at Multiple Genomic Loci.** Tfc6p was overexpressed at various tDNA and ETC loci at representative locations across the *Saccharomyces cerevisiae* genome to assess TFIIIC binding upon overexpression of TFC6 via ChIP. At each site, TFIIIC enrichment increased. Each result was confirmed by conducting assays in triplicate.
CHAPTER 7
MATERIALS AND METHODS
Yeast strains 1, 2, 3, 4, and 5 (Table 1) from the Donze Lab yeast collection were streaked for isolation on YPD plates and grown at 30°C for 2-3 days. Strains 2, 3, 4, and 5 and were mated to strain 1 (Table 1) by overlaying single colonies of each to a single colony of strain 1, smeared in to small patches on YPD and incubated at 30°C overnight. After mating the cells, they were streaked for isolation and selection of diploids on selective media (selecting for Adenine and Uracil, or Adenine, Uracil, and Leucine prototrophy) and incubated at 30°C for 3-4 days. Single colonies from the selective media for each cross were patched to YPD and incubated at 30°C overnight. YPD grown cells were transferred on to sporulation media in dime sized patches and incubated at 30°C for 3-5 days.

After incubating on the sporulation medium, a pinhead amount of cells was resuspended in 6uL of Zymolyase (100T, 1 mg/ml) enzyme solution in an eppendorf tube using a sterile 0.1ul pipette tip. The cell suspension was Incubated 2 minutes at room temperature. 300uL of water was added. Then, 12ul of the cell suspension was plated across a line on a YPD plate about an inch from the circumference. The plates were incubated at 30°C for 30 minutes. After incubating, each spore was dissected using an Olympus BX41 microscope and incubated at 30°C for 2 days. Each dissected spore was then re-patched to a YPD plate an

<table>
<thead>
<tr>
<th>Table 1. Yeast Strains used to make Constructs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strain #</strong></td>
</tr>
<tr>
<td>1. DDY4715  MATα ade2 his3 leu2 lys2 trp1 ura3 etc6Δ::URA3 GAL4-FLAG-KanMX</td>
</tr>
<tr>
<td>2. DDY4717  MATα ADE2 his3 leu2 lys2 trp1 ura3 TFC1:3XFLAG::KanMX ADH1 Promote- TFC6-LEU2</td>
</tr>
<tr>
<td>3. DDY4549  MATα ADE2 his3 leu2 lys2 trp1 ura3 TFC1:3XFLAG::KanMX TFC6</td>
</tr>
<tr>
<td>4. DDY4074  MATα ADE2 his3 leu2 lys2 trp1 ura3 TFC1:3XFLAG::KanMX</td>
</tr>
<tr>
<td>5. DDY4077  MATα ADE2 his3-11 leu2-3, 112 lys2 trp1-1 ura3-1 etc6 boxB TFC1-3XFLAG::KanMX</td>
</tr>
</tbody>
</table>

Most strains are isogenic to S. cerevisiae W-303. Most experiments were confirmed with at least three independent isolates.
incubated at 30°C overnight. The cells were then replica–plated to minimal media lacking each essential amino acid (minus adenine, histidine, leucine, lysine, tryptophan, uracil and including kanamycin) to determine which had the desired genotype. After confirming the genotypes, those with the correct genotype were prepared to be frozen as stock cultures by re-patching them to YPD plates and incubating them at 30°C overnight. The cells were scraped from the plate and resuspended in 2ml of YPD plus 7% of DMSO. This suspension was then aliquoted as 950uL aliquots in cryo-preservation tubes and frozen at -80°C. All of the strains were created with TFC1-FLAG and GAL4-FLAG to normalize ChIP-Seq results (Table 2). At least 3 isolates of each genetic cross were frozen as stock cultures.

The strains created to test varying levels of Tfc6p had TFC6 integrated at the chromosome and were created to verify the effects of mis-regulation of the Tfc6p. The significant characteristics of the strains are a normal wild type strain, promoter mutant #3 (underexpressed), ETC6 B-box mutant (2X overexpressed), and an ADH1 promoter-TFC6 cassette inserted at LEU2 (~10-20X overexpressed) (Table 2, Figure 3.2). Each strain was also engineered to contain an epitope Flag tagged Tfc1 subunit of the TFIIIC complex to confirm TFIIIC binding.

Table 2. Yeast Constructs

<table>
<thead>
<tr>
<th>Strain #</th>
<th>Strain</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. DDY4778</td>
<td>MATa ADE2 his3 leu2 LYS2 trp1 ura3 TFC1:3XFLAG:KanMX ADH1 Promoter-TFC6-LEU2 GAL4-FLAG-KanMX</td>
<td>Donze Lab</td>
</tr>
<tr>
<td>2. DDY4780</td>
<td>MATa ADE2 his3 leu2 LYS2 trp1 ura3 TFC1:3XFLAG:KanMX Promoter mutant #39 GAL4-FLAG-KanMX</td>
<td>Donze Lab</td>
</tr>
<tr>
<td>3. DDY478</td>
<td>MATa ADE2 his3 leu2 LYS2 trp1 ura3 etc6 C&gt;G TFC1:3XFLAG:KanMX GAL4-FLAG-KanMX</td>
<td>Donze Lab</td>
</tr>
<tr>
<td>4. DDY4786</td>
<td>MATa ADE2 his3 leu2 LYS2 trp1 ura3 TFC1:3XFLAG:KanMX GAL4-FLAG-KanMX</td>
<td>Donze Lab</td>
</tr>
</tbody>
</table>

Most strains are isogenic to *S. cerevisiae* W-303. Most experiments were confirmed with at least three independent isolates.
The above strains were cultured in YPD media to an optical density of approximately 1.5 at 600 nm in a 120ml culture. The cells were cross linked with 37% formaldehyde (3.25mL) and nutated at room temperature for 20 minutes to crosslink the DNA to the proteins. The cells were harvested via centrifugation at 4°C. Cell pellets were washed in 1X Phosphate Buffered Saline (PBS) (pH 7.4). At this point the washed pellets can be stored at -80°C until all samples are prepared for lysis.

To lyse the cells, pellets were thawed resuspended them in lysis buffer (50 mM HEPES-KOH, pH 7.5, 140 mM NaCL, 1% Triton X-100 and 0.1% Sodium deoxycholate) containing the protease inhibitors pepstatin A (1 ug/mL), luepeptin (1 ug/mL) and PMSF (100 mM). The cell suspension was then transferred to 1.5mL microfuge tubes and 300ul of acid-washed glass beads were added to each tube and vortexed at 4°C for 40 minutes to lyse the cells. The lysate was collected by puncturing a hole at the bottom and top of each tube using a 0.5 gauge needle and placing it inside of a 1.5 mL microfuge tube containing 10uL PMSF. The tubes were spun for 4-5 seconds to separate lysate from glass beads and incubated on ice.

The cell lysate was sonicated six times each for 10 sec using a Branson sonifier output with 0.9 sec intermittent pulses for a total of 10 sec. The lysates were incubated on ice between sonication steps. The sonicated lysates were then centrifuged at 13,200 rpm for 10 minutes at 4°C to pellet and remove any cellular debris. The cells were spun again to ensure removal of cellular debris. The supernatant was collected as whole cell extract (WCE) and transferred to a new 1.5mL microfuge tube for further analysis; this WCE can be stored at -80°C until needed.

100uL of WCE was mixed with 300uL of lysis buffer containing protease inhibitors and incubated in protein A sepharose beads (Amersham Biosciences). Before use, the protein beads were equilibrated by washing them thrice with lysis buffer containing protease inhibitors and
resuspended in lysis buffer containing 200 ug/mL of salmon sperm DNA, 500 ug/mL BSA, and protease inhibitors; this mixture was incubated on ice for 30 min. 30uL of the equilibrated beads were added to each sample and incubated with 5uL of the anti-FLAG antibody overnight at 4°C with gentle rocking. The antibody reactions were spun and the supernatant transferred to new tubes. 30uL of equilibrated beads were added to each sample and incubated at 4°C 1 hour with gentle rocking to bind the cross linked DNA. The samples were spun as above and supernatants transferred to tubes as unbound fractions. The beads were washed 5 min each with 1mL each of lysis buffer containing protease inhibitors, wash buffer I (lysis buffer + 500 mM NaCl), wash buffer II (10 mM Tris 8.0, 250 mM LiCl, 0.5% Sodium Deoxycholate, 1 mM EDTA), and 1X TE. After the TE wash the samples were spun again to remove any residual liquid. The immunoprecipitated DNA was isolated by eluting each bead pellet twice in 250uL IP elution buffer. 20uL of 5M NaCl was added to each sample, and incubated at 65°C for 4 hours to reverse the crosslinks. 10µl of the whole cell extract was mixed with 475µl of Elution buffer (1% SDS, 0.1M NaHCO3), and 20µl of 5M NaCl to reverse crosslinks for input controls. 20µl of 1M Tris pH8.0, 10µl of 0.5M EDTA and 1µl of Roche Proteinase K (20mg/ml) was added to each tube and incubated at 55°C for 30 min. Each sample was extracted with Phenol Chloroform; 50µl of NaOAc, 1µl of glycojen (Roche, 20mg/ml stock), and 1ml of absolute ethanol was added to each sample to precipitate at -20°C overnight. The samples were spun at 4°C for 10minutes and the pellets washed with 300uL of 70% ethanol, air dried and redissolved in 200µl TE + RNase A (60µg/ml).
Figure 6. Yeast Constructs Exhibiting Differential Expression of Tfc6p Levels. Wildtype strain expressing normal levels of TFC6. Promoter mutant number 3 contains a mutation upstream of the ETC site at the UAS of the TFC6 promoter and expresses TFC6 at levels 50% reduced from wildtype. Etc6 B-box mutant contains a mutation in the ETC site, directly inhibiting the binding of TFIIIC resulting in a 2-fold increase in TFC6 expression. LEU2 ADH1-promoter - TFC6 mutant drives expression of TFC6 from a stronger promoter on an additional copy of the gene to increase TFC6 expression levels 12-fold.

Before preparing selected isolates differentially expressing TFC6 to perform the ChIP-Seq experiments, the genotypes of each strain was confirmed in triplicate (Table 2) and cell extracts prepared and frozen at -80°C.

ChIP Seq Binding
The first main step in ChIP – Seq is preparation of the genomic library containing DNA fragments from 100 bp to 2 kb. For the purposes of our study, we are using DNA fragments ~200 bp in size. The DNA is to then be sheared an purified, the ends repaired, the DNA purified again, and DNA adaptor sequences ligated to the end-repaired DNA. The samples are then run on a PAGE gel, and the appropriate sized fragments excised from the gel after destaining it. The
library is then extracted from the gel slice and amplified. Next, the library is to be purified and quantitated.

Once analysis of the ChIP-Seq data is complete, future work will be done to assess the effects of TFC6 mis-expression and identify those loci and tDNAs whose transcription is affected. The results will also identify cryptic ETC sites and reveal any changes in the extra-transcriptional functions of Pol III.
CHAPTER 8
DISCUSSION
The main function of the eukaryotic RNA polymerase III is to transcribe RNAs less than 400 bp, like the tRNA and a few other RNAs. It is becoming more evident that the entire Pol III complex and its associated components perform extra-transcriptional functions throughout the genome: sister chromatid cohesion, recruitment of condensing complex to chromatin, creating pause sites for replication fork progression, direction of Ty element and Dictyostelium retrotransposon integration, overriding of nucleosome positioning sequences, repression of transcription from RNA Polymerase II promoters and protecting Polymerase II genes from repression by transcriptional repressors including heterochromatin. It has also been found that partial RNA polymerase III complexes are sufficient to impart some of these extratranscriptional effects.

Evidence is growing that one such partial Pol III complex, TFIIIC, may exert some of these extra-transcription effects by binding or not binding to ETC sites. ETC sites bind only the TFIIIC component of the Pol III complex. Evidence has already been collected that one such ETC site, located between divergently transcribed genes, acts as insulator to gene expression in S. cerevisiae (Simms, Dugas et al. 2008). More recent results show that an ETC site lying in the promoter region of the TFC6 gene (encoding one of the TFIIIC subunits) autoregulates expression of TFC6, limiting its transcription (Kleinschmidt, LeBlanc et al. 2011). Overall results by Kleinschmidt et al. 2011 show that inhibiting TFIIIC binding to ETC6 results in increased TFC6 transcript levels, that there is an inverse correlation between TFIIIC association and TFC6 transcript levels, and over-expressing TFC6 inhibits expression from its own promoter. Therefore, the autoregulation of TFC6 is ETC6 site B-box dependent and Tfc6 protein specific.
The regulation mechanism appears to be due to either TFIIIC binding to ETC6 (functioning as an insulator) or competitive binding of transcription factors at an UAS. While previous results from Kleinschmidt et al. 2011, demonstrated that TFIIIC binding is affected at several loci by overexpressing TFC6, it did not demonstrate how Pol III transcription is affected when TFC6 expression is mis-regulated. That is, when it is normally expressed, over-expressed, or under-expressed.

By performing a genome wide analysis of TFIIIC binding when TFC6 expression is mis-regulated via chromatin immunoprecipitation and subsequent high throughput sequencing (ChIP-Seq), we expect that different TFIIIC bound loci will show variations in the ChIP-Seq signals that will differ in magnitude. Using this method will not only reveal those tDNA sites whose expression is altered by TFC6 overexpression, but also those that respond to TFC6 under-expression.

We hypothesize that once we identify those sites most sensitive to altered TFC6 expression, that those tDNAs bound to those loci will most likely have their transcript levels affected. This analysis will allow us to assess how TFC6 mis-regulation affects tDNA transcription at those loci most sensitive to TFC6 mis-regulation, it may reveal cryptic ETC sites and reveal any changes in the extra-transcriptional functions of Pol III.


VITA

Kimberly LeBlanc was born in New Orleans, Louisiana to Carolyn and Edward Le Blanc. She was home educated by her parents from kindergarten to her senior year in high school, after which she enrolled at the University of New Orleans (UNO) to obtain her Bachelor of Science with a major in Biology and minor in Chemistry. While enrolled at UNO, Kimberly worked as a C-Print Captionist and then a Research Assistant. She is now in pursuit of her Master of Science at Louisiana State University. She has been accepted to the Louisiana State University Medical School and will begin in August 2012.