CONTENTS

List of Abbreviations i
Publication ii
Synopsis iii

Chapter 1: Introduction

1.1 OVERVIEW 2

1.2 RNA POLYMERASE III TRANSCRIPTION AND THE CLASS III GENES 4
   1.2.1 The enzyme RNA polymerase III 4
   1.2.2 Promoters of pol III transcribed genes 6
   1.2.3 Basal transcription apparatus of RNA pol III: transcription initiation factors 7
   1.2.4 Transcription complex assembly on pol III promoters 14
   1.2.5 A minimal RNA pol III transcription system from human cells 16
   1.2.6 Transcription elongation, termination and recycling 16

1.3 THE S. cerevisiae U6 snRNA GENE 18

1.4 TRANSCRIPTION ON CHROMATIN TEMPLATES 20

1.5 CHROMATIN REMODELLING IN EUKARYOTIC GENE EXPRESSION 23
   1.5.1 Chromatin remodeling complexes 23
   1.5.2 The Histone Code Hypothesis 26

1.6 INTERACTIONS BETWEEN TRANSCRIPTION FACTORS AND CHROMATIN REMODELERS 27
   1.6.1 A role for RNA polymerases in chromatin remodeling 28

1.7 AIM OF THE THESIS WORK 30

Chapter 2: Materials and Methods

2.1 MATERIALS 34
   2.1.1 Composition of commonly used buffers 34

2.2 PLASMID TEMPLATES 35
   2.2.1 Structure 35
   2.2.2 Preparation of super coiled plasmid templates 36
2.3 PREPARATION OF *DROSOPHILA* CORE HISTONES  
2.3.1 Preparation of embryonic cell extract and oligonucleosomes  
2.3.2 Hydroxylapatite chromatography  

2.4 PREPARATION OF S-190 EXTRACT FROM *DROSOPHILA* EMBRYOS  
2.4.1 S-190 extract preparation  
2.4.2 Characterization of the S-190 extract  

2.5 CHROMATIN ASSEMBLY ON PLASMID DNA USING S-190 AND *DROSOPHILA* CORE HISTONES  
2.5.1 Protocol for chromatin assembly  
2.5.2 MNase digestion of the assembled chromatin  

2.6 PREPARATION OF TFIIIC FROM YEAST CELLS  
2.6.1 Harvesting and lysis of cells  
2.6.2 35% AmSO4 precipitation of S-100  
2.6.3 70% AmSO4 precipitation of the 35% AmSO4 cut  
2.6.4 Bio-Rex 70 chromatography of the 70% AmSO4 precipitated S-100  
2.6.5 DEAE I Sephadex A-25 chromatography  
2.6.6 Heparin Sepharose column chromatography  
2.6.7 Mono Q column chromatography  
2.6.8 Oligo (box B^+) sepharose chromatography  
2.6.9 TFIIIC Activity assays  
2.6.10 Active molecule calculation of box B^+ affinity column purified TFIIIC  

2.7 PURIFICATION OF TFIIIB COMPONENTS  
2.7.1 TBP  
2.7.2 Brf1  
2.7.3 Bdp1  

2.8 PREPARATION OF TAGGED RNA POLYMERASE III FROM YEAST CELLS  
2.8.1 Harvesting and lysis of cells and the preparation of S100 extract  
2.8.2 70% ammonium sulfate precipitation of the S-100  
2.8.3 Bio-Rex 70, Ni2+-NTA, and Heparin Hi-Trap chromatography  
2.8.4 Activity Assay  

2.9 *IN VITRO* TRANSCRIPTION WITH PURIFIED PROTEINS
Chapter 3: High level transcriptional activation of SNR6 gene in chromatin by TFIIIC

3.1 OVERVIEW

3.2 TFIIIC ACTIVATES U6 TRANSCRIPTION IN A CHROMATIN DEPENDENT MANNER
   3.2.1 Multiple round specific in vitro transcription on chromatin templates
   3.2.2 Control transcription experiments
   3.2.3 A modified multiple round specific in vitro transcription
   3.2.4 Conclusions

3.3 STRUCTURAL ANALYSIS OF THE CHROMATIN TEMPLATE IN THE PRESENCE OF TFIIIC
   3.3.1 TFIIIC can bind box B over chromatin templates
   3.3.2 Gross structural analysis of chromatin by MNase digestion: TFIIIC does not disrupt the periodicity of nucleosomal array
   3.3.3 Indirect end labeling analysis of the chromatin structure on the U6 snRNA gene
   3.3.4 High resolution analysis of the TFIIIC dependent chromatin remodeling

3.4 DISCUSSION

Chapter 4: Characterization of TFIIIC-dependent remodeling activity

4.1 OVERVIEW

4.2 ATP DEPENDENCE OF CHROMATIN REMODELING AND TRANSCRIPTION IN THE PRESENCE OF TFIIIC
   4.2.1 ATP requirement for chromatin remodeling
   4.2.2 ATP requirement for TFIIIC dependent transcription

4.3 DOES THE YEAST TFIIIC HAVE AN ATP ACTIVITY
4.3.1 Salt dilution method for nucleosome reconstitution
4.3.2 ATPase assay for TFIIIC and S-190 proteins
4.3.3 TFIIIC does not have an ATPase activity

4.4 POSSIBLE INTERACTIONS OF YEAST TFIIIC WITH
HISTONE ACETYL TRANSFERASES
4.4.1 The liquid HAT assay
4.4.2 Histone acetyl transferase activity associated with
TFIIIC

4.5 ROLE OF TRANSCRIPTIONAL TERMINATOR ON
ACTIVATION OF CHROMATIN TRANSCRIPTION ON THE
SNR6 GENE

4.6 TRANSCRIPTIONAL TERMINATOR DOES NOT PLAY A
ROLE IN DETERMINING THE POSITION OF THE TFIIIC-
DEPENDENT NUCLEOSOME

4.7 BINDING OF TFIIIC TO SNR6 CHROMAIN MAKES THE
REGION BETWEEN BOX A AND TATA BOX
HYPERSENSITIVE TO MICROCOCCAL NUCLEASE

4.8 DISCUSSION
4.8.1 TFIIIC: A strong transcriptional activator that can
recruit ATP dependent chromatin remodelers in a
gene specific manner in vivo
4.8.2 Interactions of yeast TFIIIC with HAT complexes
4.8.3 Role for transcription terminator in transcription of
SNR6 gene as chromatin

Chapter 5: Mechanism of transcriptional activation of
SNR6 as chromatin templates

5.1 OVERVIEW
5.2 Transcription of SNR6 chromatin templates pre-bound with
TFIIIB
5.3 Mutant lac repressor positions a nucleosome in the gene
region
5.4 Transcription on pU6 LNS templates with R3-dependent positioned nucleosomes
5.5 TFIIC-induced remodeling of the R3 positioned nucleosomes
5.6 TFIIC induced shift of the R3 positioned nucleosome requires the help of a chromatin remodeler
5.7 Effect of dissociation of R3 on TFIIC dependent remodeling
5.8 A model for the TFIIC dependent shift of the R3 positioned nucleosome in the gene region
5.9 Comparison of transcription over templates present in the “intermediate” state and templates in the “remodeled” state
5.10 DISCUSSION

Chapter 6: Conclusions

6.1 SUMMARY
6.2 A model for transcription initiation on SNR6 chromatin templates assembled using S-190 extract
6.3 FUTURE DIRECTIONS

REFERENCES