

Table of Contents

TABLE OF CONTENTS.....	4
ABBREVIATIONS.....	7
1. INTRODUCTION.....	10
1.1. The rDNA transcription unit.....	10
1.2. Chromatin state of rRNA genes.....	11
1.2.1. rRNA gene silencing through DNA methylation.....	13
1.2.2. Histone modifications and heterochromatin formation.....	14
1.2.3. NoRC provides a mechanism for rRNA gene silencing.....	16
1.3. Replication timing and gene expression.....	18
1.4. Epigenetic marking in control of replication time.....	20
1.5. Objective.....	22
2. MATERIALS AND METHODS.....	24
2.1. Materials.....	24
2.1.1. Chemicals.....	24
2.1.2. Enzymes.....	25
2.1.3. Blotting materials.....	26
2.1.4. Solutions.....	26
2.1.5. Plasmids.....	30
2.1.6. Antibodies.....	31
2.1.7. Bacteria.....	31
2.1.8. Cells.....	31
2.2. Methods.....	32
2.2.1. Cell culture techniques.....	32
2.2.1.1. Cell culture.....	32
2.2.1.2. Electroporation of NIH3T3 cells and establishment of stable cell lines.....	32
2.2.1.3. Transient transfection.....	33
2.2.1.4. Establishment of TIP5 stable cell line.....	33
2.2.1.5. Cell synchronization and BrdU labeling.....	33
2.2.1.6. Cell proliferation.....	34
2.2.1.7. Flow cytometry.....	34
2.2.2. DNA analysis.....	35
2.2.2.1. Methylation of plasmid ADPTCBH in vitro.....	35
2.2.2.2. Preparation of genomic DNA.....	35

2.2.2.3. Purification of nascent DNA from bulk DNA.....	35
2.2.2.4. Screening positive colonies by PCR.....	36
2.2.2.5. Methylation-sensitive enzymatic digestion.....	36
2.2.2.6. Southern Blotting.....	37
2.2.2.7. Preparation of ³² P-labeled oligonucleotide probes.....	37
2.2.2.8. Chromatin immunoprecipitation (ChIP) assay and PCR.....	37
2.2.3. RNA analysis.....	39
2.2.3.1. Preparation of total cellular RNA.....	39
2.2.3.2. Northern blot analysis.....	39
2.2.3.3. Preparation of ³² P-UTP labeled RNA probes.....	40
2.2.3.4. RT-PCR.....	40
2.2.3.5. RT- real-time PCR.....	41
2.2.4. Immunoblotting.....	42
2.2.5. Immunofluorescence microscopy.....	42
2.2.6. cDNA Array.....	43
2.2.6.1. cDNA libraries and probe preparation for microarrays.....	43
2.2.6.2. Preparation and post-processing of microarrays.....	44
2.2.6.3. Sample preparation.....	45
2.2.6.4. Microarray hybridisation.....	45
2.2.6.5. Data acquisition, processing and analysis.....	45
3. RESULTS.....	47
3.1. Establishment of stable cell line that harbor artificial ribosomal minigenes.....	47
3.1.1. Generation of stable cell lines that contain artificial ribosomal minigenes.....	47
3.1.2. Identification of stable cell lines which expressed methylated rDNA minigenes.....	49
3.1.3. Methylated rDNA minigenes are transcriptionally active.....	51
3.2. The role of NoRC in the regulation of rDNA transcription.....	53
3.2.1. Overexpression of TIP5 inhibits RNA polymerase I transcription in vivo.....	53
3.2.2. Transcriptional repression by NoRC is mediated by DNA methylation and deacetylation.....	55
3.2.3. NoRC-mediated transcriptional repression requires methylation of CpG-133.....	57
3.3. Generation of stable cell line which overexpressed Flag-tagged TIP5.....	59
3.3.1. Stable overexpression of TIP5 represses rDNA transcription.....	59
3.3.2. Overexpression of TIP5 impairs cell growth and alters cell.....	61
3.3.3. Epigenetic changes at the rDNA promoter in cells overexpressing TIP5.....	63
3.3.4. Overexpression of TIP5 results in methylation of the rDNA promoter.....	65
3.3.5. TIP5-mediated the rDNA methylation is limited to the promoter region.....	67

3.3.6. Overexpression of TIP5 impairs binding of UBF to rDNA promoter and redistribute UBF localization in nucleolus.....	69
3.4. The role of NoRC in the regulation of rDNA replication.....	71
3.4.1. Synchronization of NIH3T3 cells in distinct phase during S phase.....	71
3.4.2. Synchronized NIH3T3 cells reveal characteristic replication pattern.....	71
3.4.3. Biphasic replication of the rRNA genes.....	74
3.4.4. Late replicating ribosomal genes exhibit heterochromatic features.....	74
3.4.5. Late replicating ribosomal genes are silent.....	78
3.4.6. TIP5 is associated with late replicating rDNA.....	80
3.4.7. Overexpression of TIP5 changes the ratio of early to late replicating rRNA genes.....	83
3.5. Analysis of differentially expressed genes in NIH3T3-TIP5 cells by cDNA microarrays.....	85
4. DISCUSSION.....	88
4.1. Molecular mechanisms of NoRC in silencing of Pol I transcription.....	88
4.2. The role of NoRC in the regulation of rDNA replication.....	92
5. SUMMARY.....	97
6. REFERENCES.....	99
7. CURRICULUM VITAE.....	109
8. PUBLICATIONS.....	110
9. ACKNOWLEDGEMENTS.....	112