<u>INDEX</u>

ABBREVIATIONS	25
INTRODUCTION	29
SACCHAROMYCES CEREVISIAE AS A MODEL ORGANISM	31
GENOME ORGANIZATION IN S. CEREVISIAE	32
CHROMATIN ORGANIZATION	33
POST-TRANSCRIPTIONAL MODIFICATIONS	35
HISTONE ACETYLATION	38
HISTONE METHYLATION	39
HISTONE UBIQUITINATION AND HISTONE "CROSS-TALK"	41
HISTONES CHAPERONES	46
THE REGULATION OF GENE EXPRESSION BY RNA POL II IN S. CEREVISIAE	48
TRANSCRIPTION INITIATION	48
SAGA CO-ACTIVATOR COMPLEX AS A MODULATOR OF TRANSCRIPTION REGULATION	52
DUBM FUNCTION AND STRUCTURE	55
TRANSCRIPTION ELONGATION	59
COUPLING TRANSCRIPTION TO MRNA EXPORT	61
THE TREX-2 COMPLEX	64
SAGA ACTIVITY IS LINKED TO TREX-2 COMPLEX	66
SAGA AND TREX COMPLEXES IN NUCLEAR PERIPHERY-GENE TETHERING	68
PROTEIN TRAFFICKING; MOG1 PROTEIN AND ITS ROLE IN RAN GTP/GDP CYCLE	70
SPINOCEREBELLAR ATAXIA TYPE 7 (SCA7) DISEASE	74

MATERIALS AND METHODS	79
MATERIALS	81
YEAST STRAINS	81
Primers	85
Commercial Kits	88
Antibodies	88
PRIMARY ANTIBODIES	89
Secondary Antibodies	89
PLASMIDS	90
HUMAN CELL LINES	91
MICE	91
RADIOACTIVITY	92
Methods	92
SACCHAROMYCES CEREVISIAE	92
YEAST CULTURES AND CELL GROWTH ASSAY	92
GENE DELETION AND PROTEIN TAGGING	93
GENOMIC DNA ISOLATION	94
PLASMID DNA ISOLATION FROM E. COLI	94
RNA ISOLATION	95
Reverse Transcription or cDNA synthesis	96
TRANSFORMATION OF YEAST CELLS	97
PCR (POLYMERASE CHAIN REACTION)	98
QUANTITATIVE-PCR	99
NUCLEIC ACIDS SEPARATION BY ELECTROPHORESIS	100
PROTEIN EXTRACTS FOR WESTERN BLOT	100
PROTEIN SEPARATION BY ELECTROPHORESIS	101

PROTEIN TRANSFERENCE AND IMMUNODETECTION	102	
PROTEIN PURIFICATION: THE TAP TECHNIQUE	103	
PROTEIN IDENTIFICATION BY MASS SPECTROMETRY	105	
Colloidal coomassie blue staining	105	
TOTAL HISTONE EXTRACTION	106	
PROTEIN IMMUNOPRECIPITATION (IP)	106	
CHROMATIN IMMUNOPRECIPITATION (CHIP)	107	
METABOLITE EXTRACTION AND ANALYSIS	109	
MICROSCOPIC TECHNIQUES	112	
In vivo protein localization	112	
In Situ Hybridization (FISH)	112	
IMAGE ANALYSIS	113	
GENOMIC RUN-ON (GRO) AND MEASUREMENT OF MRNA LEVELS	113	
HUMAN CELLS LINES 293T (HEK293T)	120	
HEK293T GROW CONDITIONS	120	
IN VITRO DUB ASSAY USING HEK293T CELLS	120	
COLUMN FRACTIONATION AND ANALYSIS	121	
MICE MODEL	123	
MOUSE CEREBELLUM EXTRACTION AND RNA ISOLATION	123	
OBJECTIVES	125	
RESULTS AND DISCUSSION	129	
CHAPTER 1	131	
FUNCTIONAL CHARACTERIZATION OF THE NOVEL FACTORS ASF1 AND MOG1 INVOLVED IN THE		
UBIQUITINATION/DEUBIQUITINATION OF HISTONE H2B	131	

1. DECIPHERING THE ROLE OF ASF1 IN TRANSCRIPTION	131
ASF1 IS DISPENSABLE FOR SUS1 RECRUITMENT TO GAL1 GENE, ITS INDUCTION AND MRNA	
EXPORT	131
ABSENCE OF ASF1 REDUCES GLOBAL LEVELS OF H2B UBIQUITINATION AND COUNTERACTS T	ΉE
EFFECTS OF <i>SUS1</i> DELETION	135
2. ROLE OF MOG1P IN THE CONTROL OF GENE EXPRESSION	137
SUS1 INTERACTS GENETICALLY WITH MOG1	137
<i>MOG1</i> INTERACTS GENETICALLY WITH FACTORS INVOLVED IN HISTONE H2B	
UBI/DEUBIQUITINATION	139
Absence of <i>MOG1</i> reduces global levels of H2Bub ¹ <i>in vivo</i>	142
UBP 8 preserves the association with its protein partners in $mog1\Delta$ cells	144
Mog1 does not affect the localization of the DUB module and UBP8 recruitment to	
ACTIVELY TRANSCRIBED GENES	146
Chromatin recruitment of Rad6 is decreased in $mog1\Delta$ cells	149
Mog1 interacts genetically with the methyltransferases and affects ${\rm H3K4me}^{3}$	
LEVELS	151
Set1 recruitment to chromatin is decreased in absence of $MOG1$	154
Mog1 associates with active genes	156
GENOME-WIDE ANALYSIS LINKS <i>MOG1</i> ABSENCE WITH DECREASED LEVELS OF TRANSCRIPTION	
AND MRNA CONCENTRATION.	157
Mog1 interacts genetically with TREX-2 components and it is involved in $mRNA$	۱.
EXPORT	167
Levels of Swd2 are reduced in absence of <i>MOG1</i>	171
Mog1 co-purifies with components of the transcription machinery	172
Discussion	177
CHAPTER 2	187

${\bf Molecular\ mechanisms\ of\ the\ DUBm\ related\ disease\ Spinocerebellar\ Ataxia\ type\ 7}$	
(SCA7)	187
METABOLIC PROFILE OF WT AND POLYQ-ATXN7	187
EXPRESSION LEVELS OF MITOCHONDRIAL AND RIBOSOMAL GENES ARE NOT AFFECTED IN SCA	7
MICE CEREBELLUM	190
POLYQ REPETITIONS IN ATXN7 PROTEIN PRESERVES DUB ACTIVITY OF SAGA COMPLEX IN	
HUMAN CELL LINES	193
POLYQ-ATXN7 SEEM TO AFFECT THE ASSOCIATION BETWEEN ENY2 AND TREX-2 COMPLEX	196
Discussion	198
FUTURE PERSPECTIVES	<u>207</u>
CONCLUSIONS	<u>213</u>
ANNEXES	<u>217</u>
BIBLIOGRAPHY	221