

**GENOME-WIDE TRANSLATIONAL CONTROL
IN FISSION YEAST**

Daniel H. Lackner

This dissertation is submitted for the
degree Doctor of Philosophy
September 2007

Clare Hall College
University of Cambridge

The Wellcome Trust Sanger Institute
Hinxton
Cambridge, UK

Declaration

I hereby declare that my dissertation contains material that has not been submitted for a degree or diploma or any other qualification at any other university. This thesis describes my own work and does not include the work that has been done in collaboration, except when specifically indicated in the text.

Daniel H. Lackner

28/09/2007

Acknowledgments

First of all, I would like to thank my supervisor Jürg Bähler for giving me the chance to do my PhD work in his laboratory. He allowed me to pursue my research in an independent way, while his office door was always open for advice and help.

I also want to thank the whole laboratory – especially Juan Mata, Brian Wilhelm and Samuel Marguerat – for invaluable discussions and answers to most of my questions, how trivial or complex they might have been.

Furthermore, special thanks to Sofia Aligianni and Tannia Gracia for bringing female esprit, Greek and Mexican charm, and much laughter into the lab.

Another big thanks goes to the "It's Friday afternoon, let's have a beer!"-folks from the office.

Thanks also to all the Mill Roadies: Susie, Caroline, Anja, Alex and Richard, who by now should know everything about polysome profiling and microarrays.

Thanks also to the Austrian crowd (Alex, Bauze, Geisse, Max, Tom and Toph), who constantly turned visits at home into joyful experiences (and long nights-out!!!).

The last and very special "THANK YOU" goes to my family – Agnes, Erika and Charlie. You have always been – and always will be – there for me with a word of advice, for big decisions and in everyday life.

Abstract

Studies on the regulation of gene expression most often focus on measuring steady-state mRNA levels, especially when using genome-wide approaches. Recently, however, it has become increasingly evident that the expression of genes is frequently also regulated at post-transcriptional levels. I therefore studied both global and mRNA-specific translational regulation and its coordination with other levels of gene expression control in the fission yeast *Schizosaccharomyces pombe*.

To obtain translational profiles for all mRNAs, polysome preparations were separated according to their size using a sucrose gradient, and the mRNAs in each fraction, or pools of fractions, were identified and quantified with DNA microarrays (translational profiling). Starting with exponentially growing cells, I analyzed 12 polysome fractions using DNA microarrays containing elements for all known and predicted genes of fission yeast. This approach provided data for the average number of associated ribosomes for most transcripts. These data were then integrated with other genome-wide data sets such as mRNA steady-state levels, polyadenylation profiles, start-codon sequence context, mRNA half-lives, and RNA polymerase II occupancy. Widespread and unexpected relationships between distinct levels of gene expression were uncovered. Translation and polyadenylation are aligned on a global scale with both the lengths and levels of mRNAs: short and abundant mRNAs have longer poly(A) tails and are more efficiently translated. Transcription and mRNA stability independently contribute to the alignment of mRNA abundance with translation.

Using these data sets as a basis, I then used translational profiling to assess the extent of translational regulation in cells in response to genetic and environmental perturbations. First, translational profiling was used in cells deleted for protein methyltransferase 3 (*rmt3*), and many mRNAs encoding proteins of the small ribosomal subunit were identified to be translationally up-regulated. Furthermore, translation profiling was used in cells exposed to various cellular stresses including heat shock and oxidative stress. Many genes that showed changes in total mRNA levels in these conditions were also regulated translationally. Furthermore, a few genes showed regulation only at the translational level and are good candidates for specific translational regulation. These data provide a comprehensive overview of translational control in fission yeast relative to other aspects of gene expression regulation.

Contents

DECLARATION	II
ACKNOWLEDGMENTS	III
ABSTRACT	IV
CONTENTS	V
LIST OF FIGURES	IX
LIST OF TABLES	XIII

CHAPTER 1

Introduction – Post-transcriptional gene expression regulation	1
An overview	2
Translational regulation	4
After transcription, before translation: RNA processing and export	4
Molecular mechanism of translation initiation in eukaryotes	7
Why translational regulation?	11
Targets for translational regulation: initiation factors, mRNA and the ribosome	12
Classic examples of translational regulation	14
Novel concepts in translational control: P-bodies and microRNAs	22
Functional genomics of post-transcriptional gene expression	31
Genome-wide approaches to identify targets of post-transcriptional gene expression regulation	31
Translation	31
Alternative proteomic approaches to study translational regulation	35
mRNA decay	36
RNA-binding proteins and their target mRNAs	39
Aim of this thesis	43

CHAPTER 2

Materials and methods	44
------------------------------	-----------

<i>S. pombe</i> strains	45
<i>S. pombe</i> growth conditions	45
Translational profiling	46
High-resolution translational profiling	46
Medium-resolution profiling	48
Translational profiling in <i>rmt3Δ</i> cells	50
PASTA analysis of poly(A) tail length distribution	51
LM-PAT assay of poly(A) tail length distribution	53
Determination of mRNA steady-state levels	53
Determination of Pol II occupancy	54
Determination of mRNA half-lives	54
Measurement of changes in total mRNA abundance	55
Northern blotting	56
General microarray protocols	57
Total RNA extraction from cells using the hot-phenol method	57
RNA and DNA labelling	58
Microarray hybridizations and washing	58
Image acquisition and processing	59
Standard normalization protocol and data visualization	59
Statistical analyses	60

CHAPTER 3

From transcription to translation: global translational properties of fission yeast mRNAs and integration with other genome-wide data sets on gene expression	62
Introduction	63
Establishing polysome fractionation	65
Genome-wide translational profiling	67
Global translational properties of mRNAs	71
Short mRNAs are more efficiently translated	76
Genome-wide measurement of poly(A) tail length	79

mRNAs with long poly(A) tails are more efficiently translated	84
Abundant mRNAs are more efficiently translated	85
Stable and highly transcribed mRNAs are more efficiently translated	88
Changes in mRNA polyadenylation in response to transcriptional switch-on	93
Conclusion	95

CHAPTER 4

A translational response in fission yeast cells deleted for the protein arginine methyltransferase 3 (Rmt3p): higher ribosome densities for mRNAs encoding ribosomal proteins of the 40S subunit	97
Introduction	98
No changes in mRNA levels were detected in rmt3Δ cells using DNA microarrays	100
Genome-wide translational profiling in rmt3Δ cells	102
Translational up-regulation of mRNAs encoding 40S ribosomal proteins	107
Conclusion	110

CHAPTER 5

Translational regulation in response to environmental stress	111
Introduction	112
Medium resolution translational profiling	114
Translational profiling in cells exposed to environmental stress	117
Identifying mRNAs with an altered translational status	117
Translationally regulated mRNAs in oxidative and heat stress	119
Coordination between changes in mRNA abundance and translation	131
Regulation of translation under oxidative stress in a time course experiment	137
Conclusion	141

CHAPTER 6

General discussion	142
Global translational profiling and integration with other genome-wide data sets	143
Overview	143
mRNA length and translational efficiency	146
mRNA abundance and translational efficiency	148
Translational control in response to genetic perturbation and environmental stress	151
Translational changes in fission yeast cells deleted for <i>rmt3</i>	151
Translational regulation in response to environmental stress	152
Future work	155
Validation of translationally regulated mRNAs after exposure to stress	155
Translational regulation in response to starvation	155
Alternative methods to measure global translational regulation	156
REFERENCES	157

List of Figures

Figure 1.1	Layers of gene expression regulation	3
Figure 1.2	Molecular mechanisms of translation initiation	8
Figure 1.3	Formation of active ternary complex	9
Figure 1.4	Cis-acting sequence elements that influence translation initiation of specific mRNAs	13
Figure 1.5	Inhibition of global protein synthesis in response to various stress stimuli through phosphorylation of eukaryotic initiation factor-2 α	16
Figure 1.6	Translational regulation of <i>GCN4</i> by upstream open reading frames (uORFs)	17
Figure 1.7	Regulation of translation by the cytoplasmic polyadenylation element (CPE)	19
Figure 1.8	Translational regulation of male-specific-lethal (<i>msl-2</i>) mRNA in <i>Drosophila melanogaster</i> through a multi-step mechanism	21
Figure 1.9	Movement of mRNAs between polysomes and P-bodies	24
Figure 1.10	Biogenesis of miRNAs and siRNAs	25
Figure 1.11	Translational profiling	31
Figure 1.12	Genome-wide measurements of mRNA half-lives	37
Figure 1.13	Genome-wide determination of mRNA targets of RNA-binding proteins (RBPs)	40
Figure 3.1	Polysome profile of ribosomes isolated from <i>S. pombe</i> and resolved by velocity sedimentation through a 5-45% sucrose gradient	62
Figure 3.2	Association of <i>actin</i> mRNA across the polysome profile	63
Figure 3.3	Comparison of polysome profiles obtained using sucrose gradients with different concentrations	64
Figure 3.4	High-resolution polysome profiling	65
Figure 3.5	Average translation profiles for selected groups of RNAs	67
Figure 3.6	Distribution of mRNA levels for protein-coding genes included or excluded from high-confidence translational profiling data	69
Figure 3.7	Correlation between ORF length and mean number of associated ribosomes	70
Figure 3.8.	Correlations of AugCAI values with translation efficiency	72

Figure 3.9	Inverse correlation between ribosome density and ORF length	74
Figure 3.10	Overestimation of ribosome number for fraction 12 does not affect negative correlation between ribosome density and ORF length	75
Figure 3.11	Correlations between ORF length/ribosome density and protein level	76
Figure 3.12	Experimental layout for polyadenylation state array (PASTA)	77
Figure 3.13	mRNAs fractionated using poly(U)-sepharose chromatography	78
Figure 3.14	Experimental layout of LM-PAT assay	79
Figure 3.15	Examples of poly(A) tail length determination by LM-PAT assays and PASTA analysis	80
Figure 3.16	Poly(A) tail profiles for mitochondrially encoded mRNAs determined by PASTA analysis	80
Figure 3.17	Correlations between ORF length and ribosome density and poly(A) tail length	82
Figure 3.18	No correlation between mRNA levels and ORF length	83
Figure 3.19	Correlations between mRNA level and poly(A) tail length and ribosome occupancy	84
Figure 3.20	Determination of mRNAs with short and long half-lives	86
Figure 3.21	Correlations between mRNA half-lives and other gene expression properties	87
Figure 3.22	Experimental layout for estimating Pol II occupancy on a genome-wide scale	88
Figure 3.23	Correlations between Pol II occupancy and other gene expression properties	89
Figure 3.24	No changes in poly(A) tail length for mRNAs induced in expression using <i>nmt1</i> promoters with long induction time	90
Figure 3.25	Transient changes in poly(A) tail length for mRNAs induced in expression using a promoter with short induction time	91
Figure 4.1	Imbalance in free 40S:60S ratio in <i>rmt3Δ</i> cells	96
Figure 4.2	Genome-wide mRNA profiling comparing <i>rmt3Δ</i> and wt cells	98
Figure 4.3	Experimental layout for translational profiling comparing monosomal and polysomal fractions between <i>rmt3Δ</i> and wt cells	100
Figure 4.4	Translational changes in <i>rmt3Δ</i> cells	101

Figure 4.5	Polysomal and monosomal ratios for mRNAs encoding ribosomal proteins	105
Figure 4.6	Gene expression changes of mRNAs encoding ribosomal proteins in <i>rmt3Δ</i> cells	106
Figure 5.1	Experimental layout for medium resolution translational profiling under stress conditions	111
Figure 5.2	Comparison of the distribution of mRNAs with high and low ribosome occupancy between medium- and high-resolution translational profiling	112
Figure 5.3	Outline of data analysis to define translationally regulated mRNAs	114
Figure 5.4	Sum of total difference between the translational profile in the stress conditions and in the control	116
Figure 5.5	Translationally up-regulated mRNAs under heat and oxidative stress	117
Figure 5.6	Translationally down-regulated mRNAs under heat and oxidative stress	118
Figure 5.7	Average translation profiles for mRNAs translationally regulated under oxidative stress	124
Figure 5.8	Average translation profiles for mRNAs translationally regulated under heat stress	125
Figure 5.9	Changes in total mRNA levels for translationally regulated mRNAs in stress conditions	126
Figure 5.10	Example profiles of mRNAs that show translational regulation under oxidative stress, but are not regulated at the level of total mRNA abundance	130
Figure 5.11	Example profiles of mRNAs that show translational regulation under heat stress, but are not regulated at the level of total mRNA abundance	131
Figure 5.12	Translation profiles of down-regulated mRNAs after different times of exposure to oxidative stress	133
Figure 5.13	Translation profiles of an up-regulated mRNA after different times of exposure to oxidative stress	134
Figure 5.14	Translational regulation of mRNAs encoding ribosomal proteins	

	under oxidative stress	135
Figure 6.1	Summary of relationships between all aspects of gene expression	139
Figure 6.2	Comparison of poly(A) tail lengths between fission and budding yeast	140

List of tables

Table 3.1	Summary of all correlations between the different genome-wide data sets on key aspects of gene expression	92
Table 4.1	mRNAs with altered levels in <i>rmt3Δ</i> cells identified by SAM	99
Table 4.2	mRNAs translationally regulated in <i>rmt3Δ</i> cells identified by SAM	102
Table 4.3	GO terms enriched for mRNAs with an increased polysomal-to-monosomal ratio in <i>rmt3Δ</i> cells	105
Table 5.1	Curated list of translationally up-regulated mRNAs under heat and oxidative stress	119
Table 5.2	Curated list of translationally down-regulated mRNAs under heat and oxidative stress	122
Table 5.3	List of mRNAs that show translational regulation under oxidative stress, but are not regulated at the level of total mRNA abundance	127
Table 5.4	List of mRNAs that show translational regulation under heat stress, but are not regulated at the level of total mRNA abundance	128