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

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This chapter focuses on how changes in translation efficiency could be identified in fission yeast cells deleted for the gene encoding protein arginine methyltransferase 3 (Rmt3p) using translational profiling. These mutant cells exhibit an imbalance in the ratio between the small (40S) and the large ribosomal (60S) subunit, but no other obvious phenotype (Bachand and Silver 2004), which made this mutant an interesting candidate to study the global biological response in terms of changes in total mRNA level and translational efficiency. Whereas total mRNA levels remained largely unchanged in *rmt3*-null compared to wild type (wt) cells, several mRNAs showed increased translational efficiency in the mutant cells, many of them mRNAs encoding ribosomal proteins of the 40S subunit.

Introduction

The post-translational modification of proteins is important to regulate a variety of protein features such as stability, localization, activity or interaction patterns. Protein arginine methyltransferases (PRMTs) catalyze the methylation of arginine residues in proteins. Several different PRMTs have been identified in eukaryotic genomes, but seem to be lacking from prokaryotes (Bachand 2007). Together with PRMT1 and PRMT5, mammalian PRMT3 shows the strongest conservation throughout evolution and has homologues in fission yeast and flies, but not in budding yeast or worm (Bachand 2007).

Rmt3p, the fission yeast PRMT3, has been identified to bind to the ribosomal protein Rps2p using tandem affinity purification followed by mass spectrometry (Bachand and Silver 2004). Other binding partners identified in this study – albeit not in a 1:1 stoicheometry as Rps2p – were the ribsomal protein Rps2401p and translation elongation factor Ef1a-cp. Furthermore, Rps2p could be identified as a substrate for methylation by PRMT3 both in fission yeast (Bachand and Silver 2004) and

mammalian cells (Swiercz et al. 2005). The activity of Rmt3p in fission yeast is not essential, as cells deleted for the *rmt3* gene are viable and do not exhibit any obvious growth or temperature-sensitive phenotype (Bachand and Silver 2004). However, *rmt3Δ* cells show an imbalance in their free 40S:60S subunit ratio as determined by polysome profiling (Figure 4.1; Bachand and Silver 2004): there is a strong increase in the peak for the 60S subunit in the mutant.

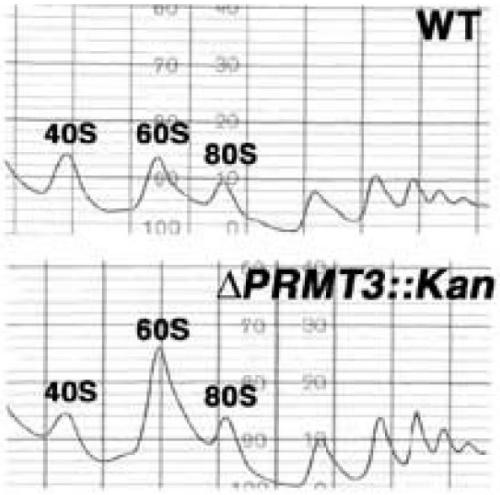

Figure 4.1 Imbalance in free 40S:60S ratio in *rmt3Δ* cells
Polysome profiles from wt (WT, top panel) and *rmt3Δ* (ΔPRMT3::Kan, bottom panel) cells. The position of free small (40S) and large (60S) ribosomal subunits and monosomes (80S) are indicated. This figure is taken from Bachand et al. (2004).

Given the connection of Rmt3p to the translational apparatus and the 40S:60S imbalance in *rmt3Δ* cells, we wanted to study the biological response to deletion of *rmt3* in fission yeast on the global scale, both in terms of mRNA levels and especially translation. We analyzed mRNA levels using standard expression profiling with DNA microarrays and performed translational profiling to look for changes in translational efficiency of specific mRNAs. Data from these experiments revealed an up-regulation of ribosome density for many mRNAs encoding 40S ribosomal proteins without changes in the overall levels of these mRNAs, suggesting enhanced translational efficiency for these mRNAs in *rmt3Δ* cells. This work was done in collaboration with Francois Bachand and Pamela Silver (Department of Systems Biology, Harvard Medical School, Boston, USA).

No changes in mRNA levels were detected in rmt3Δ cells using DNA microarrays

To investigate, whether *rmt3Δ* cells respond to the imbalance between large and small ribosomal subunits by altering steady-state levels of specific mRNAs, we compared mRNA levels between *rmt3Δ* and wt cells using DNA microarrays. Total RNA was isolated from *rmt3Δ* and an isogenic wt strain, labelled, and competitively hybridized to DNA microarrays. Three independent biological repeats were performed, including one dye-swap. Normalization of the microarray data was performed using the standard local normalization script (see Chapter 2). Only probes for RNAs for which data could be obtained for all 3 repeats were included for further analysis (4258 out of 5253).

These experiments revealed no significant differences in steady-state mRNA levels in *rmt3Δ* cells compared to wt cells (Figure 4.2): no mRNA showed a consistent change beyond the 1.5 fold cut-off. These data were further corroborated by using the Significance Analysis of Microarrays (SAM) software (Tusher et al. 2001): only when using a low Delta value cut-off and thus a high false discovery rate (median FDR ~14%) could differentially expressed mRNAs be detected (9 up-regulated, 2 down-regulated; Table 4.1). Furthermore, the actual fold-changes of these mRNAs between *rmt3Δ* and wt cells were small, and the biological significance of these changes, if any, remains elusive (Table 4.1). The only mRNA showing a consistent down-regulation in all 3 experiments (~6 fold) was *rmt3* itself (Figure 4.2; Table 4.1). Despite being absent in the deletion strain, *rmt3* mRNA passes the quality criteria of the microarray normalization script (Lyne et al. 2003), as there is consistent strong signal from the wt labelling. Nevertheless, signal in the *rmt3Δ* cells is reduced to background levels.

In conclusion, *rmt3Δ* cells show little or no response to the 40S:60S ribosomal imbalance in terms of changes in mRNA abundance.

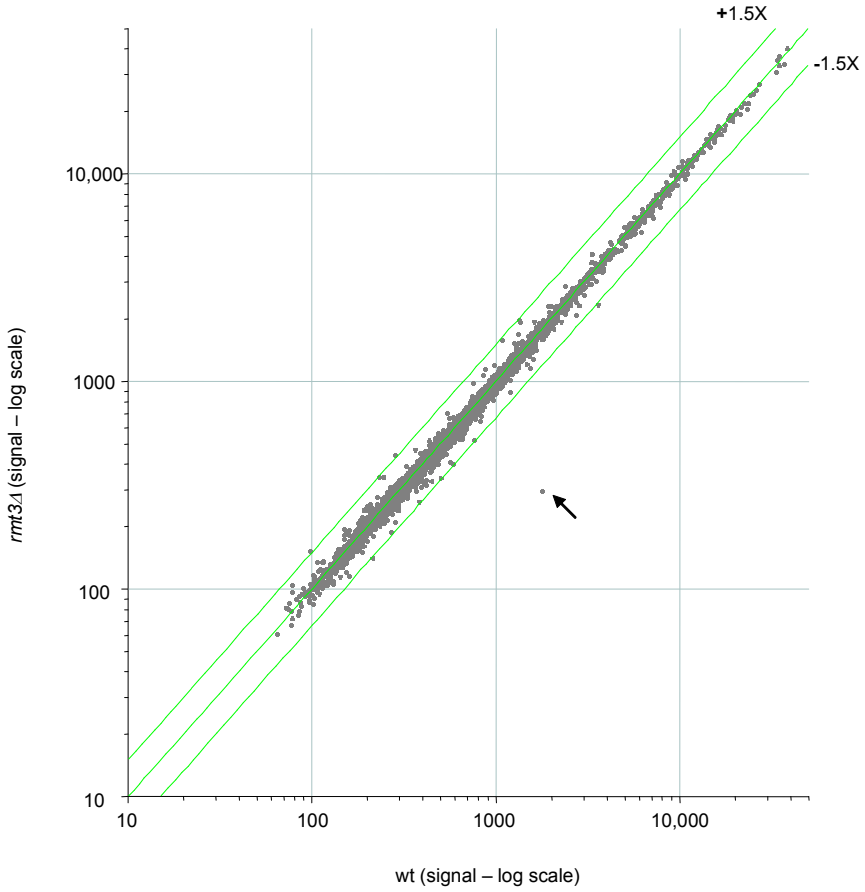

Figure 4.2 Genome-wide mRNA profiling comparing *rmt3Δ* and wt cells
Scatter blot of normalized mRNA levels from *rmt3Δ* and wt cells measured using DNA microarrays. Shown are the averaged data for 3 biological repeats. The green lines represent the 1.5 fold cut-off. The single mRNA indicated with an arrow corresponds to *rmt3*.

Table 4.1 mRNAs with altered levels in *rmt3Δ* cells identified by SAM

Systematic name	Common name	Function - GeneDB	Total mRNA ratio (*rmt3Δ*/wt)
Up-regulated mRNAs in *rmt3Δ*			
SPCC18B5.01c	*hba2; bfr1*	ABC transporter family	1.24
SPCC965.07c	*gst2*	glutathione S-transferase	1.26
SPBPB2B2.06c		calcineurin-like phosphoesterase	1.32
SPBC215.11c		aldo/keto reductase	1.26
SPAC27D7.10c		possibly S. pombe specific	1.49
SPAC27D7.09c		predicted N-terminal signal sequence	1.45
SPBC8D2.11	*pi054*	sequence orphan	1.38
SPCC965.14c		cytosine deaminase	1.63
SPAC1039.02		calcineurin-like phosphoesterase	1.41
Down-regulated mRNAs in *rmt3Δ*			
SPBC8D2.10c	***pi055; rmt3***	**type I ribosomal protein arginine methytransferase 3**	**0.18**
SPAC1B3.16c	*vht1*	vitamin H transporter	0.77

List of mRNAs, which were identified to show different levels in *rmt3Δ* cells using SAM. Total mRNA ratios (*rmt3Δ*/wt) displayed are the average of 3 biological repeats. *rmt3* mRNA – indicated in bold letters – is the only mRNA with a consistent differential expression.

Genome-wide translational profiling in rmt3Δ cells

The absence of changes in the steady-state mRNA levels in the *rmt3Δ* cells does not rule out changes at other levels of gene expression regulation in these cells, especially given the evidence for a role of Rmt3p in the regulation of translation through methylation of Rps2p (Bachand and Silver 2004). To test whether there are changes in the translational efficiency of certain mRNAs in *rmt3Δ* cells, we wanted to study the distribution of mRNAs in monosomal (weakly translated; only associated with a maxiumum of one ribosome) and polysomal fractions (heavily translated; associated with 2 or more ribosomes). To this end, cellular extracts were prepared from *rmt3Δ* and wt cells and separated in 5-45% sucrose gradients. The gradients were then fractionated into 15 fractions. Fractions corresponding to free ribosomal subunits and monosomes (fractions 4 to 9; Figure 4.3) and to polysomes (fractions 11 to 15; Figures 4.3) were pooled and RNA was extracted from these pools. RNA from the monosomal pool of *rmt3Δ* and wt cells were labelled and competitively hybridized to DNA microarrays; RNAs from the polysomal pools were processed and analyzed in the same way (Figure 4.3). Normalization of the microarray data was performed using the standard local normalization script (see Chapter 2).

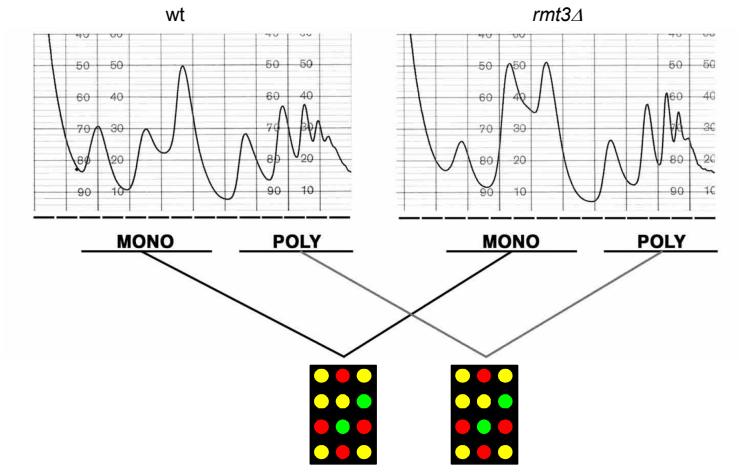

Figure 4.3 Experimental layout for translational profiling comparing monosomal and polysomal fractions between *rmt3Δ* and wt cells
Fractions from polysome profiles of *rmt3Δ* and wt cells were pooled as indicated into monosomal (MONO) and polysomal (POLY) fractions. mRNAs were extracted from these pools, labelled and competitively hybridized onto DNA microarrays. This figure is adapted from Bachand et al. (2006).

For further analysis, we focused on probes from RNAs for which we could obtain data in at least two of the three biological repeats in both monosomal and polysomal fractions from *rmt3Δ* and wt cells (3389 out of 5253). There was enrichment for low-abundance mRNAs in the excluded genes (data not shown; Lackner et al. 2007). For mRNAs with a change in their translational status in *rmt3Δ* cells, we expected one of the following changes in distribution between mono- and polysomal fractions: an up-regulation in translation for a specific mRNA should manifest itself by an increased mRNA ratio in the polysomal pool and a decreased mRNA ratio in the monosomal pool comparing *rmt3Δ* and wt cells, whereas a down-regulation in translation for a specific mRNA should be seen by a lower mRNA ratio in the polysomal pool and higher ratio in the monosomal pool comparing *rmt3Δ* and wt cells.

To identify the mRNAs with significantly changed polysomal-to-monosomal ratios, a two-class paired comparison in SAM was used. A high Delta value cut-off of 2 was chosen in order to minimize the FDR. At this threshold the FDR was estimated to be below 0.001% (Tusher et al. 2001). SAM identified 59 up-regulated and 12 down-regulated mRNAs in the *rmt3Δ* deletion mutant (Figure 4.4A,B; Table 4.2).

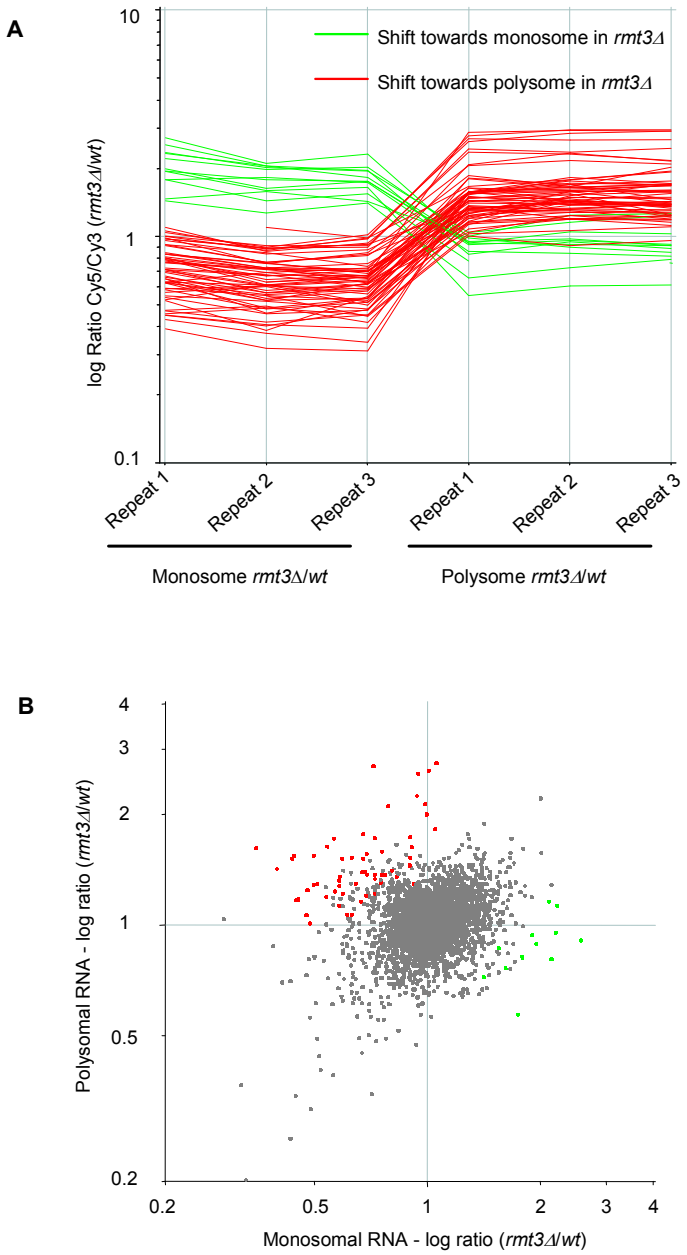

Figure 4.4 Translational changes in *rmt3Δ* cells
(A) Displayed are translational changes in *rmt3Δ* cells identified using translational profiling as outlined in Fig. 4.3 and identified using SAM (see main text). Shown are 3 independent biological repeats each comparing monosomal and polysomal fractions between *rmt3Δ* and wt cells (*x* axis). Intensity ratios (*rmt3Δ*/wt) are displayed in a log scale (*y* axis). Genes displayed in red (n = 59) show a shift towards polysomal fractions in *rmt3Δ* cells compared to wt, wheres genes displayed in green (n = 12) show a shift towards monosmal fractions in *rmt3Δ* cells compared to wt.
(B) Averaged monosomal and polysomal RNA ratios determined as in **(A)**, but displayed using a scatter plot. Displayed are averaged ratios for mRNAs that could be detected in at least 2 of the 3 biological repeats. Colouring of mRNAs has been done as in **(A)**.

Table 4.2 mRNAs translationally regulated in *rmt3Δ* cells identified by SAM

				Ratio (*rmt3Δ*/wt)			
Systematic name	**Common name**	**Function - GeneDB**	**SAM score**	**Mono**	**Poly**	**P/M**	**T**
Up-regulated mRNAs							
SPBC14C8.04		acetolactate synthase regulatory unit	3.2	0.5	1.4	2.7	1.0
SPBC1539.06		acyl-coenzyme A binding protein	7.1	0.4	1.5	3.5	1.0
SPAC15F9.03c	*nft2; ntf2; nxt2*	NTF2 domain	7.6	0.3	1.7	4.9	1.0
SPBC1604.11	*atp17*	F-type ATPase subunit F	5.5	0.4	1.4	3.7	1.0
SPBC1709.10c		metallochaperone	3.2	0.8	1.4	1.8	1.0
SPAC17H9.07		signal recognition particle subunit	4.8	0.6	1.7	3.0	1.0
SPAC1F12.02c	*p23fy*	translationally controlled tumor protein homolog	4.5	0.8	1.6	2.0	1.0
SPAC24C9.16c	*cox8*	cytochrome c oxidase subunit VIII	3.9	0.6	1.3	2.3	1.0
SPAC25B8.01		MAPR family steroid-binding protein	3.0	0.5	1.0	1.9	0.9
SPBC29A3.07c		RNA-binding protein	9.1	0.9	2.7	2.8	1.1
SPBC2D10.19c		sequence orphan	3.3	0.5	1.5	2.8	1.1
SPBC31F10.15c	*atp15*	F0-ATPase epsilon subunit	3.1	0.6	1.1	1.9	1.0
SPAC3A12.16c	*tim17*	TIM23 translocase complex subunit	4.1	0.8	1.4	1.8	1.0
SPBC3H7.08c		conserved fungal protein	3.4	0.8	1.4	1.8	1.0
SPAC4G8.02c	*sss1*	translocon gamma subunit	4.4	0.6	1.3	2.3	1.0
SPAC823.02		sequence orphan	4.0	0.8	1.6	1.9	1.0
SPBC83.18c		C2 domain	4.1	0.7	1.6	2.3	1.0
SPCC965.14c		cytosine deaminase	3.1	1.0	1.8	1.7	1.6
SPAC1851.03	*ckb1*	CK2 family	3.3	0.9	1.5	1.7	1.0
SPAC3G6.02	*dss1*	mRNA export protein	3.3	0.7	1.3	1.8	1.0
SPBC428.02c	*eca39B2.11c; eca39*	branched chain amino acid aminotransferase	3.2	0.6	1.1	1.8	1.1
SPBC20F10.09	*lsm5*	U6 snRNP-associated protein	5.1	0.7	1.7	2.4	1.0
SPAP27G11.04c		tRNA-specific adenosine deaminase subunit	3.3	0.6	1.7	2.7	1.0
SPAPB1E7.12	*rps6; rps6-2;rps602*	40S ribosomal protein	3.5	0.5	1.4	2.8	1.0
SPBC16C6.06	*vps10; pep1*	sorting receptor for CPY	4.6	0.5	1.3	2.7	1.0
SPAC1687.01	*rpa17; rpc19*	DNA-directed RNA polymerase I and III subunit	3.1	0.8	1.4	1.7	1.1
SPBC17G9.10	*rpl1102; rpl11-2; rpl11*	60S ribosomal protein	3.7	0.6	1.4	2.2	1.0
SPBC776.01	*rpl29*	60S ribosomal protein	8.1	0.8	2.2	2.9	1.1
SPCC663.04	*rpl39*	60S ribosomal protein	7.9	0.9	2.4	2.6	1.1
SPAC1687.06c	*rpl44; rpl28*	60S ribosomal protein	6.8	0.4	1.6	3.9	1.0
SPBP22H7.08	*rps10B; rps10; rps1002; rps10-2;*	40S ribosomal protein	6.3	0.6	1.6	2.6	1.1
SPAC31G5.03	*rps1101; rps11; rps11-1*	40S ribosomal protein	3.2	0.7	1.5	2.1	1.0
SPCC962.04	*rps12-1; rps12; rps1201*	40S ribosomal protein	3.6	0.6	1.4	2.2	1.1
SPAC1071.07c	*rps15-2; rps15; rps1502; rps15-3*	40S ribosomal protein	3.4	0.9	1.8	2.1	1.0
SPBC18H10.14	*rps1601; rps16; rps16-1*	40S ribosomal protein	3.1	0.9	1.4	1.5	1.0
SPCC24B10.09	*rps1702; rps17; rps17-2*	40S ribosomal protein	11.6	1.0	2.9	3.0	1.1
SPBC21C3.13	*rps16; rps1901; rps19-1*	40S ribosomal protein	5.8	0.6	1.6	2.7	1.1
SPBC18E5.06	*rps21*	40S ribosomal protein	9.6	1.0	2.8	2.8	1.0
SPBP4H10.13	*rps23-2; rps2302*	40S ribosomal protein	5.3	0.5	1.8	3.2	1.0
SPAC806.03c	*rps26; rps2601; rps26-1*	40S ribosomal protein	4.2	0.6	1.3	2.3	1.0
SPAC1805.11c	*rps26-2; rps2602*	40S ribosomal protein	12.5	0.7	2.9	4.2	1.1
SPCC285.15c	*rps28-2; rps28; rps2802*	40S ribosomal protein	6.7	0.9	2.3	2.4	1.1
SPBC1685.09	*rps29*	40S ribosomal protein	6.3	1.0	2.1	2.2	1.1

SPBC16G5.14c	*rps3*	40S ribosomal protein	4.5	0.4	1.3	2.8	1.0
SPAC19B12.04	*rps3001; rps30-2; rps30; rps30-1*	40S ribosomal protein	5.1	0.7	1.7	2.3	1.0
SPAC13G6.02c	*rps3a-1;rps1-1;rps101*	40S ribosomal protein	3.4	0.7	1.3	1.9	1.0
SPAC22H12.04c	*rps1-2;rps102;rps3a-2*	40S ribosomal protein	3.6	0.7	1.3	1.9	1.0
SPAC8C9.08	*rps5*	40S ribosomal protein	4.5	0.7	1.6	2.3	1.0
SPAC328.10c	*rps5; rps5-2; rps502*	40S ribosomal protein	4.0	0.7	1.4	2.2	1.1
SPAC13G6.07c	*rps601; rps6; rps6-1*	40S ribosomal protein	4.3	0.7	1.4	2.1	1.0
SPAC18G6.14c	*rps7*	40S ribosomal protein	4.3	0.5	1.2	2.3	1.0
SPAC24H6.07	*rps9a; rps901; rps9; rps9-1*	40S ribosomal protein	4.0	0.6	1.4	2.5	1.1
SPBC29A3.12	*rps9-2; rps9b; rps902*	40S ribosomal protein	3.9	0.7	1.4	1.8	1.1
SPAPJ698.02c	*rps002; rps0; rpsa-2;rps0-2*	40S ribosomal protein	3.5	0.6	1.2	2.0	1.0
SPAC19A8.01c	*sec7C; sec7c; sec73*	guanyl-nucleotide exchange factor	4.2	0.5	1.2	2.6	1.0
SPAC6G9.11	*syb1; snc1*	synaptobrevin	3.1	0.6	1.2	1.8	1.0
SPBC23G7.05	*psu1; sui1; tif1; tif38*	translation initiation factor	3.4	0.6	1.4	2.3	1.0
SPAC16E8.15	*tif451; tif45; eIF4E1; tif1*	translation initiation factor	3.5	0.5	1.1	2.3	1.0
SPCC1919.09	*tif6*	translation initiation factor	3.2	0.7	1.4	2.0	1.0
Down-regulated mRNAs							
SPBC11G11.03		60S acidic ribosomal protein	-4.3	2.1	1.2	0.6	1.1
SPAC144.12	*ppi*	ribose 5-phosphate isomerase	-4.5	2.4	0.9	0.4	1.0
SPAC1F3.08c		dubious	-3.5	2.1	0.9	0.4	1.0
SPBC2G5.02c		CK2 family	-3.8	1.6	0.8	0.5	1.0
SPBC4C3.07	*eIF3f*	translation initiation factor	-3.6	1.8	1.0	0.6	1.0
SPBC8E4.01c		inorganic phosphate transporter	-3.9	1.5	0.9	0.6	1.1
SPBC2D10.10c	*fib; fib1*	fibrillarin	-3.4	2.1	1.1	0.5	1.2
SPAC26F1.06	*gpm1*	monomeric 2,3-bisphosphoglycerate (BPG)-dependent phosphoglycerate mutase (PGAM)	-4.8	1.8	0.9	0.5	1.0
SPBC119.11c	*snm1; pac1; hcs*	double-strand-specific ribonuclease	-3.8	1.4	0.7	0.5	1.0
SPAC31G5.11	*pac2*	cAMP-independent regulatory protein	-6.2	1.8	0.8	0.5	1.0
SPAC1783.08c	*rpl15-2; rpl1502*	60S ribosomal protein	-5.4	2.1	0.9	0.4	1.0
SPAC57A10.12c	*ura3*	dihydroorotate dehydrogenase	-4.8	1.7	0.6	0.3	1.0

List of mRNAs that were identified to show significant changes in the distribution between monosomal and polysomal fractions in *rmt3Δ* cells compared to wt using SAM. Monosomal (Mono), polysomal (Poly), polysomal-to-monosomal (P/M), and total RNA (T) ratios between *rmt3Δ* and wt cells are shown as average of 3 independent biological repeats.

Translational up-regulation of mRNAs encoding 40S ribosomal proteins

As there are many more mRNAs translationally up-regulated than down-regulated, we first focused on the analysis of the 59 mRNAs up-regulated in the *rmt3Δ* deletion mutant. According to a test in GeneSpring, these mRNAs were mostly enriched for GO terms related to eukaryotic pre-initiation complex, cytosolic ribosome, ribonucleoprotein complex, protein biosynthesis, and small ribosomal subunit ($P \sim 1e^{30}$-$6e^{-14}$). A similar enrichment for functional categories related to the protein synthesis machinery was obtained when these mRNAs were analyzed using the web-based tool FuncAssociate (Table 4.1; Berriz et al. 2003). Significantly, 25 mRNAs encoding ribosomal proteins of the 40S subunit were among the 59 translationally up-regulated mRNAs (Table 4.2). When analyzed globally, data from the translational profiling revealed that the majority of mRNAs encoding 40S ribosomal proteins exhibited a similar trend of up-regulation in the polysomal fractions and down-regulation in the monosomal fractions. This is evident from a scatter plot showing the monosomal ratios plotted against the polysomal ratios comparing *rmt3Δ* and wt cells (Figure 4.5), as well as in a heat map showing monosomal, polysomal and the polysomal-to-monosomal ratios (Figure 4.6). According to the expression profiling microarray data, this increase in association with ribosomes for mRNAs encoding 40S ribosomal proteins is not due to increased total mRNA levels in the *rmt3Δ* cells (Figure 4.2; Figure 4.6). Figure 4.5 and Figure 4.6 also show that mRNAs encoding 60S ribosomal proteins do not exhibit the overall increase in polysomal-to-monosomal ratios observed for mRNAs encoding 40S ribosomal proteins. Interestingly, several mRNAs encoding translation initiation factors (*tif45*, *sui1* and *tif6*) were also up-regulated translationally in the *rmt3Δ* deletion mutant.

The 12 translationally down-regulated mRNAs in the *rmt3Δ* deletion mutant did not show significant enrichment for any functional category.

Table 4.3 GO terms enriched for mRNAs with an increased polysomal-to-monosomal ratio in *rmt3Δ* cells

GO term	No. of genes	*P* value
Eukaryotic 43S preinitiation complex	24	<0.001
Eukaryotic 48S preinitiation complex	24	<0.001
Ribosome	28	<0.001
Protein translation	31	<0.001
Ribonucleoprotein complex (RNP)	29	<0.001
Protein biosynthesis	31	<0.001

GO terms enriched in the list of 59 mRNAs with increased polysomal/monosmal ratio in *rmt3Δ* cells determined using the web-based tool FuncAssociate. The number of mRNAs identified by the program to overlap with the functional list and the *P* values are displayed.

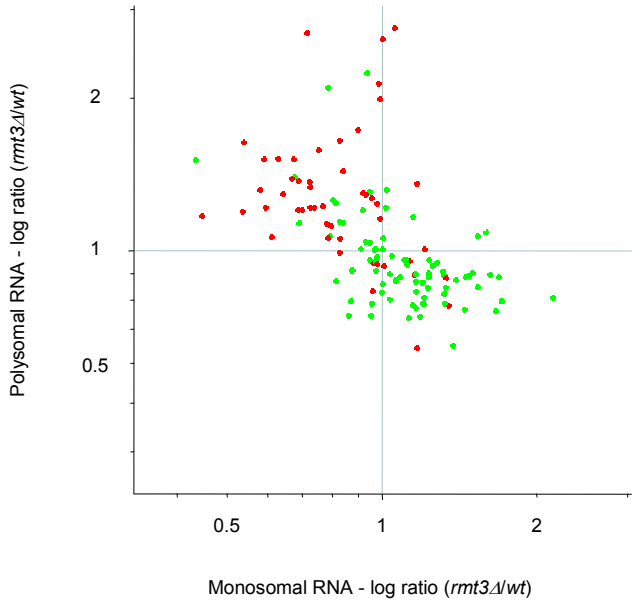

Figure 4.5 Polysomal and monosomal ratios for mRNAs encoding ribosomal proteins
Averaged monosomal and polysomal RNA ratios between *rmt3Δ* and wt cells displayed as in Fig. 4.4B for mRNAs encoding ribosomal proteins. Red dots: mRNAs encoding proteins of the 40S subunit. Green dots: mRNAs encoding proteins of the 60S subunit.

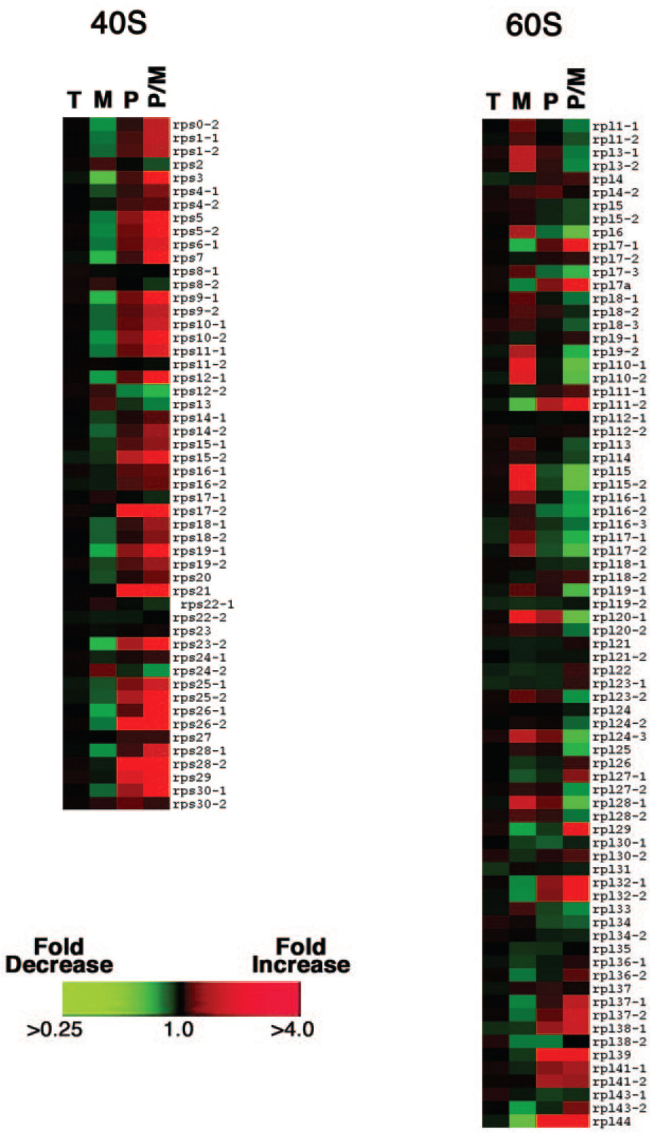

Figure 4.6 Gene expression changes of mRNAs encoding ribosomal proteins in *rmt3Δ* cells

Heat map representing the average ratio of total (T), monosomal (M), or polysomal (P) mRNAs isolated from *rmt3*-null cells relative to wild-type cells from three biological repeats. Polysomal-to-monosomal (P/M) RNA ratios are also represented and were calculated based on the average and normalized monosomal and polysomal ratios. Black squares denote no significant alteration in the amount of RNA isolated from *rmt3Δ* or wt cells; red and green squares denote ribosomal protein mRNAs that were more or less abundant, respectively. The intensity of the colour is proportional to the log2 increase or decrease, as indicated on the intensity scale. This figure is adapted from Bachand et al. (2006).

Conclusion

Fission yeast cells lacking the protein methyltransferase Rmt3p show a loss of methylation of the ribosomal protein Rps2p and exhibit an imbalance in the ratio of free ribosomal subunits (Figure 4.1; Bachand and Silver 2004). We explored the genome-wide response of these cells in terms of changes in total mRNA levels and translation by using DNA microarrays. Whereas no or only little changes in total mRNA levels between the *rmt3* deletion mutant and wt could be observed, several mRNAs exhibited an altered translational efficiency. In particular, many mRNAs encoding proteins of the small 40S ribosomal subunit and several translation initiation factors were translationally up-regulated. It is tempting to speculate that this post-transcriptional response is an autoregulatory mechanism ensuring homeostasis for proper functioning ribosomal subunits. Lack of Rps2p methylation might result in a 40S ribosomal subunit which is not totally functional, and a sub-sequent up-regulation of translation of 40S ribosomal proteins might help to compensate for this defect. Accordingly, overexpression of *rps2* in *rmt3Δ* cells could partially rescue the 40S:60S imbalance and restore the polysomal-to-monosomal ratios for two mRNAs encoding 40S ribosomal proteins as tested by real-time PCR (Bachand et al. 2006).

It is also important to mention at this point that genome-wide translational profiling was capable of detecting gene expression changes which would not have been discovered by looking only at steady-state mRNA levels or focusing only on a subset of genes.

Furthermore, several mRNAs, which were identified as being translationally up-regulated in the *rmt3Δ* deletion mutant using translational profiling with DNA microarrays, could be validated by high-resolution mRNA analysis using real-time PCR in 12 fractions spaced throughout the polysome profile by our collaborators (Bachand et al. 2006). A further validation of the translational profiling data came from immunoblotting for two of the translation initiation factors, which were identified as translationally up-regulated using microarrays: Sui1p and Tif45p both showed a 1.5 fold increase in actual protein levels (Bachand et al. 2006).