Chapter 5

Translational regulation in response to environmental stress

Translational regulation in response to environmental stress

This chapter focuses on the translational response of fission yeast cells to environmental stress, and builds on data gained from translational profiling in vegetatively growing cells (Chapter 3). Cells were exposed to oxidative stress, heat stress and DNA damage. Translational profiling was performed to identify mRNAs with an altered translational status, and changes in total mRNA levels were measured using microarrays. Integration of these data revealed mRNAs that are regulated at the translational level only, mRNAs that only showed a change in total mRNA abundance, and mRNAs that showed regulation at both levels.

Introduction

Upon exposure to stress or in response to changing environmental conditions, cells need to reprogramme their pattern of gene expression. This is especially important for single-celled organisms, as they need to adapt swiftly to unexpected fluctuations in nutrient-availability, pH, temperature, external osmolarity as well as exposure to UV radiation and potentially toxic chemical compounds.

Depending on the nature and dose of the stress signal, yeast cells show a variety of cellular responses, such as adaptation or resistance to the stress, delay of cell division, growth arrest or cell death (for reviews see Gasch and Werner-Washburne 2002; Mager and Siderius 2002; Temple et al. 2005; Gasch 2007). Common to all these cellular responses is an underlying change in global gene expression patterns, and it is important to identify these changes on a global scale to better understand the mechanisms of the cellular response to changing environmental conditions. Furthermore, it needs to be pointed out that cells are usually studied under optimal growth conditions in the laboratory environment, but these conditions do not necessarily reflect the natural environment of these cells, and it was probably sub-optimal conditions that helped to shape cellular gene expression patterns in evolutionary terms (Gasch and Werner-Washburne 2002).

Using microarray technology and genome-wide approaches, many mRNAs could be identified to be regulated at the level of mRNA abundance in budding yeast (Gasch et

al. 2000; Causton et al. 2001; Gasch et al. 2001) and fission yeast (Chen et al. 2003; Gatti et al. 2004; Watson et al. 2004; Rustici et al. 2007) cells exposed to stress.

Despite the fact that up to 35% of mRNAs were found to be significantly regulated in terms of mRNA abundance in these conditions, it is also important to study changes at other levels of gene expression regulation. Studies using genome-wide translational profiling in budding yeast cells exposed to stresses, such as a shift to a non-fermentable carbon source (Kuhn et al. 2001), treatment with rapamycin or heat shock (Preiss et al. 2003), or oxidative stress (Shenton et al. 2006; Swaminathan et al. 2006), could show global changes in translation and identify mRNAs, that were specifically regulated at the translational level.

Given that such data on translational regulation do not yet exist for fission yeast on a genome-wide scale, I decided to use translational profiling in fission yeast cells exposed to oxidative stress, heat shock and DNA damage.

In these stress conditions, fission yeast cells launch two transcriptional responses: the core environmental stress response (CESR) is a set of genes that is commonly regulated at the level of mRNA abundance in response to all, or most, stresses, whereas the specific environmental stress response (SESR) is a set of genes that is likely to play a specific role in stress adaptation (Chen at al. 2003). Induced CESR genes encode proteins that are involved in a variety of functions such as carbohydrate metabolism, signaling and transcriptional regulation, lipid or fatty acid metabolism, antioxidants, DNA repair, or proteins involved in protein folding and protein degradation. The genes repressed in the CESR are mainly associated with protein synthesis, transport, transcription, cellular signaling, and cytoskeletal organization.

As an example of the SESR, additional genes that function in antioxidant pathways or genes encoding mebrane transporters are induced specifically in the response to oxidative stress; genes that participate in the ubiquitin pathway and additional genes involved in protein folding or degradation are induced specifically in the response to heat stress. The regulation of many genes of the CESR is dependent on the Sty1p MAP-kinase and also on the transcription factor Atf1p, whereas these factors are less important for the SESR (Chen et al. 2003).

Translation profiling done in these stress conditions should complement the existing data on changes in mRNA expression levels and identify translationally regulated mRNAs.

Medium resolution translational profiling

To study translational changes in stress conditions, I wanted to screen for mRNAs that change their association with ribosomes, reflecting changes in the efficiency with which they are translated. To this end, polysome profiles were prepared from cells exposed to stress and unstressed control cells. mRNA was extracted from 4 equally spaced fractions throughout the polysome profile, labelled and hybridized on DNA microarrays against labelled genomic DNA as reference (Figure 5.1). Using this medium resolution approach with 4 fractions should give more detailed data on translational changes than a comparison of only monosomal and polysomal fractions, especially as there were also strong changes in total mRNA abundance in the conditions tested. To test if translational profiles obtained from this approach reflected translational data from the high-resolution translational profiling (see Chapter 3), translational profiles from the mRNAs with the highest and lowest ribosome occupancy were compared between these 2 approaches (Figure 5.2). There was good agreement between the profiles from medium- and high-resolution translational profiling: mRNAs with high ribosome occupancy peaked mostly in the last fractions (fraction 3-4 in the medium-resolution translational profiling; fractions 7-12 in the high-translational profiling) corresponding to efficiently translated mRNAs, whereas mRNAs with low ribosome occupancy peaked mostly in the first fractions (fraction 1-2 in the medium-resolution translational profiling; fractions 1-6 in the high-translational profiling). Note that for the medium resolution profiling, cells were grown in full medium (YE) and normalization of the microarray data was performed using the standard normalization script without the use of spiked-in bacterial mRNAs (Lyne et al. 2003), as only relative changes in profiles between conditions were measured and not absolute mRNA levels in each fraction as for the high-resolution translational profiling (Chapter 3).

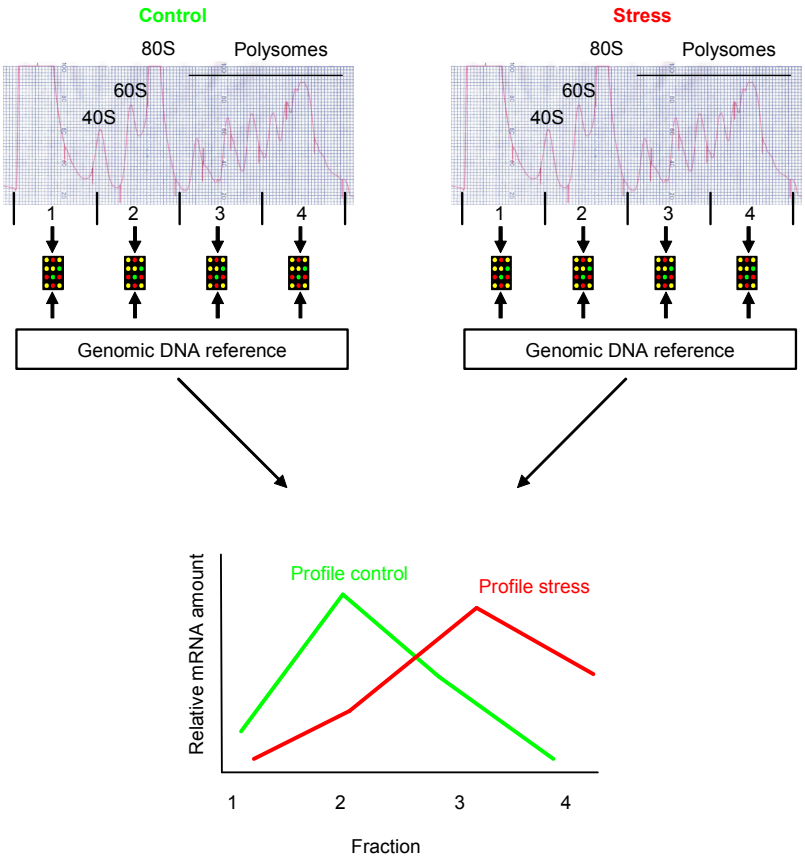


Figure 5.1 Experimental layout for medium resolution translational profiling under stress conditions
Polysome profiles were prepared from unstressed control cells and cells exposed to stress. mRNA was extracted from 4 fractions equally spaced throughout the profile, labelled and competitively hybridized on DNA microarrays against labelled genomic DNA as reference. Profiles were then compared to identify translationally regulated mRNAs. Shown is the translational profile for a hypothetical mRNA that is translationally up-regulated in the stress condition.

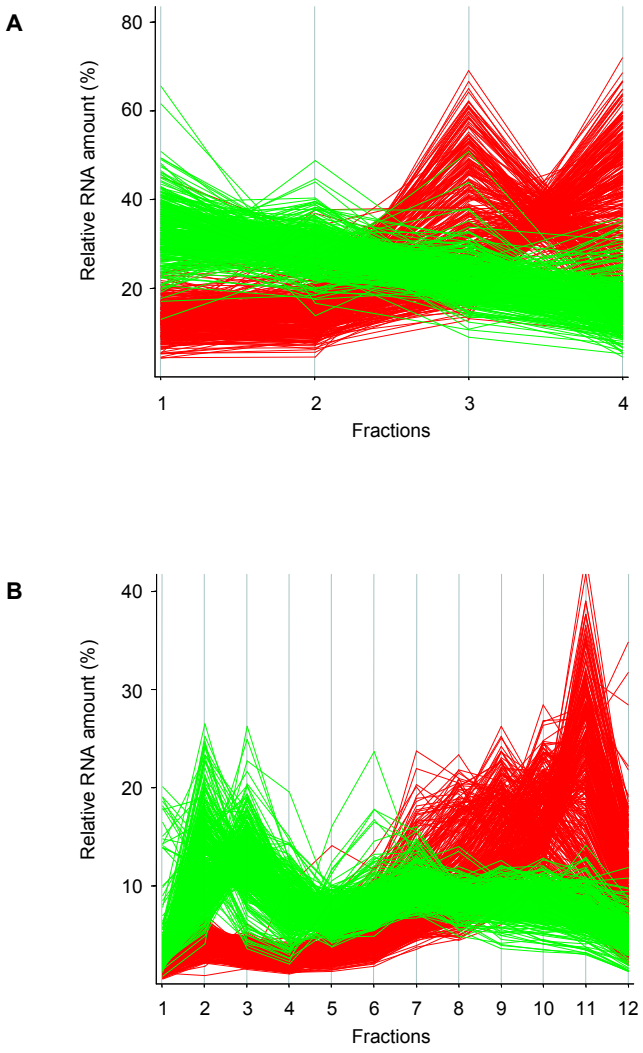


Figure 5.2 Comparison of the distribution of mRNAs with high and low ribosome occupancy between medium- and high-resolution translational profiling
Shown is the distribution of 10% of mRNAs with the highest ribosome occupancy (red lines) and 10% of mRNAs with the lowest ribosome occupancy (green lines) as determined by high-resolution translational profiling (Chapter 3). The profiles of these mRNAs are depicted as determined in medium-resolution **(A)** and high-resolution **(B)** translational profiling. For both cases, the profiles represent the average profile from 3 independent biological repeats.

Translational profiling in cells exposed to environmental stress

Cells were grown in YE medium at 32°C and exposed to oxidative stress (0.5 mM H_2O_2), heat stress (shift to 39°C) and DNA damage (MMS 0.02% final concentration) for 15 minutes (min). This short exposure period and the relatively mild doses of stress were chosen to prevent a genome-wide translational shut-down in the cell or cell death, and to identify specific and direct translational responses. Cells were harvested, and one aliquot of the sample was used for the preparation of total RNA for the comparison of mRNA levels; a second aliquot was directly used for polysome profiling as outlined in Figure 5.1. No difference could be detected between the overall polysome profile from cells exposed to any of the stresses and unstressed control cells (data not shown), indicating that translation was not altered on a global scale in these cells.

mRNAs extracted from the 4 polysomal fractions were labelled and hybridized against labelled genomic DNA as reference to obtain translational profiles. Total RNA samples were directly compared between control cells end cells exposed to stress on microarrays. At least 2 biological repeats were performed.

Identifying mRNAs with an altered translational status

It was not straightforward to identify mRNAs with an altered translational profile in any of the stress conditions: no single ratio could be used to identify changes as is usually done in expression profiling, but significant changes in the shape of profiles needed to be identified. Several approaches were tried, and ultimately best results were obtained by a combination of several methods: First, translation profiles were calculated as the percentage of a given mRNA for each fraction such that the total over all 4 fractions was 100%. Second, a measure of the difference of the profile of every mRNA between the stress sample and the corresponding control was calculated. This was done by summing up the total difference between the profiles for each fraction (Figure 5.3A). Third, I calculated a translational score for each mRNA in each condition by multiplying the percentage in each fraction with an arbitrary weight of 1, 2, 3 and 4 for fractions 1-4. The results for each fraction were then summed to obtain the translational score for each mRNA (Figure 5.3B). A higher translational

score indicates that a higher proportion of a given mRNA is associated with the later fractions. By dividing the translational score of a given mRNA in a stress condition by the translational score of the same mRNA in the control condition, a translational ratio is obtained. A ratio above 1 indicates a translational up-regulation, while a ratio under 1 indicates a translational down-regulation.

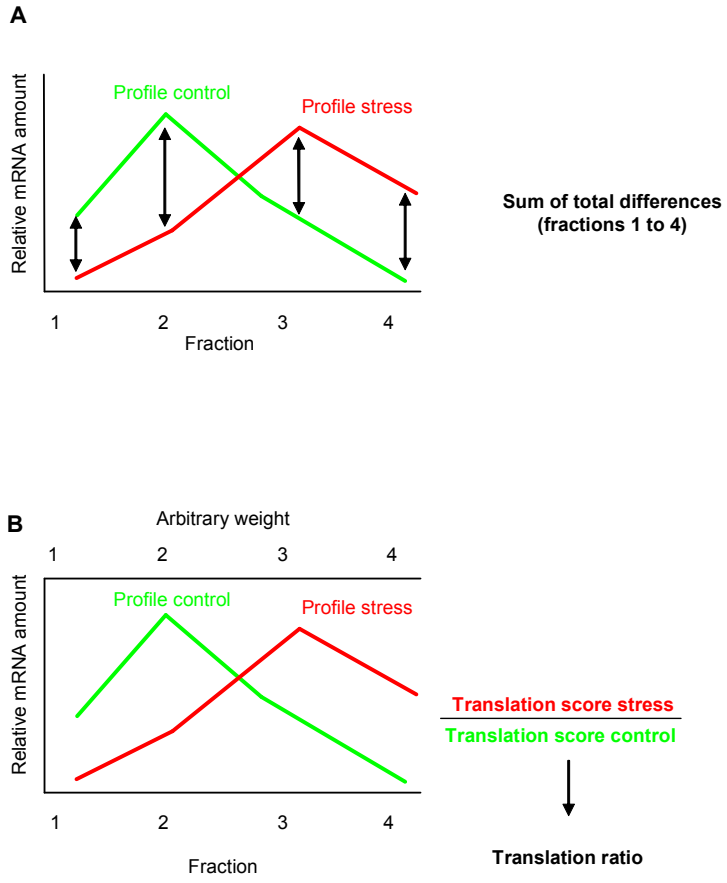


Figure 5.3 Outline of data analysis to define translationally regulated mRNAs
(A) The sum of total difference for a given mRNA between the translational profile under stress conditions and the translational profile in the control was calculated by summing the total difference in each fraction (indicated by an arrow).
(B) A translational score for each mRNA in each condition was calculated by multiplying the percentage in each fraction with an arbitrary weight of 1, 2, 3 and 4 for fractions 1-4. The results for each fraction were then summed to obtain the translational score for each mRNA. By dividing the translational score of a given mRNA in a stress condition by the translational score of the same mRNA in the control condition, a translational ratio was obtained.

Next, mRNAs were selected that showed a constant change in both repeats (heat shock, DNA damage) or in 2 out of 3 repeats (oxidative stress) according to arbitrary cut-offs: the total difference between the profiles had to be greater than 30; the cut-off for the translational ratio was set to a 1.15 fold change. These cut-offs were chosen based on trial and error using different cut-offs and visual inspection of the translation

profiles of the corresponding mRNAs compared to control cells. Data from these two approaches were combined and the translational ratio was used to determine the direction of the change.

Finally, a visual inspection of the data obtained by filtering on cut-offs was done to exclude profiles, which only showed changes due to noise in the data-set. Between 10% - 50% of mRNAs identified by filtering on cut-offs were discarded after visual inspection to obtain a high-confidence, conservative data set of translational changes. mRNAs in this data set will now be referred to as translationally regulated mRNAs.

Unfortunately, no mRNAs have been previously reported to be translationally regulated under these conditions in fission yeast, and as a consequence there are no positive or negative controls. Furthermore, statistical methods for the analyis of microarray data are usually developed to deal with expression data and as such not suitable for the anlysis of translational profiles. Moreover, the availability of only 2 repeats for most conditions (heat stress, DNA damage) is usually not enough for a statistical analysis.

However, assuming that the data from the 4 fractions of the translational profiles corresponds to time points in a time course experiment, I also compared the data from the control to data obtained under oxidative stress using a two class time course comparison in SAM (for details on SAM see chapter 2). Using a Δ value of 0.08 with a false discovery rate (FDR) of ~11%, SAM identified 55 up- and 115 down-regulated mRNAs in terms of changes in the translational profiles. Whereas there is a certain agreement with the curated data set of translationally up- (overlap 23) and down-regulated (overlap 34) mRNAs, SAM failed to identify several translationally regulated mRNAs, which don't show a simple change in their profile based on the change in their slope, which is used as a basis for the calculation of significant changes in this type of comparion in SAM. The examples of translationally regulated mRNAs presented in Figure 5.10, Figure 5.12 and Figure 5.13 were identified in both approaches using the automated method as described above followed by visual inspection and by SAM.

Translationally regulated mRNAs in oxidative and heat stress

The extent of translational regulation is reflected by the number of mRNAs showing high differences between the translation profile in stress conditions and the

translational profile in the control (Figure 5.4). Most changes were observed under heat stress, fewer changes were observed under oxidative stress, and only very little change was observed in cells exposed to the DNA damaging agent MMS. In the curated data-set of translationally regulated mRNAs, 157 mRNAs were up-regulated under heat stress and 25 mRNAs were up-regulated under oxidative stress; 13 mRNAs were shared between both conditions (Figure 5.5A; Table 5.1).

Whereas the number of translationally up-regulated mRNAs was much higher under heat stress compared to oxidative stress, the number of down-regulated mRNAs was roughly similar: 56 mRNAs were found to be translationally down-regulated under heat stress and 43 mRNAs were found to be translationally down-regulated under oxidative stress; 11 of these mRNAs were down-regulated in both conditions (Figure 5.6A, Table 5.2).

Further analysis will be focused on translational regulation under heat and oxidative stress, as only 1 mRNA was found to be consistently translationally regulated in cells exposed to MMS (SPAC23H3.15c); this mRNA encoding a protein of unknown function is translationally up-regulated in all 3 conditions tested.

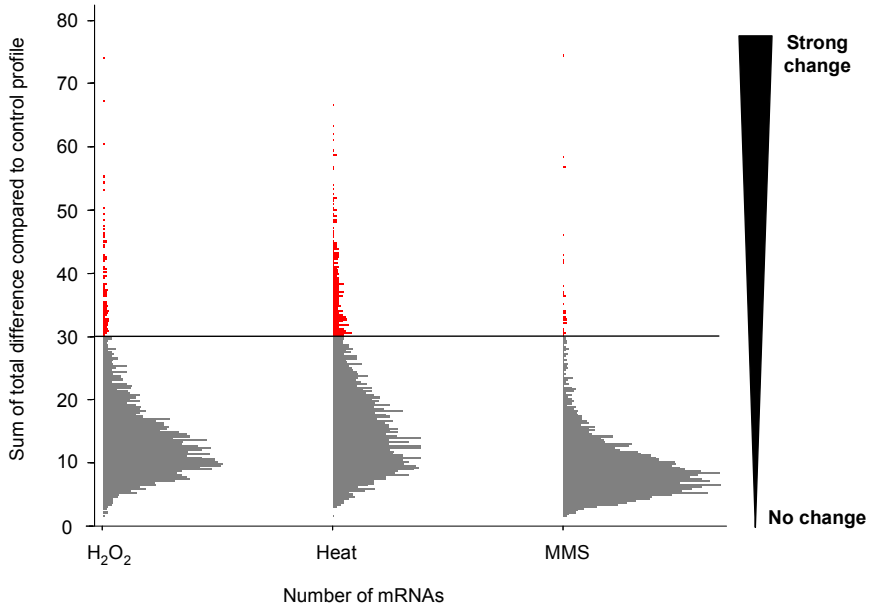


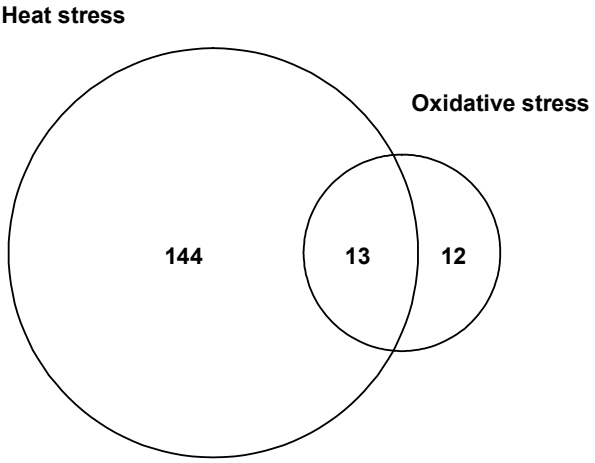
Figure 5.4 Sum of total difference between the translational profile in the stress conditions and in the control

Shown is a histogram of the sum of total difference between the translational profiles after 15 min exposure to oxidative stress (H_2O_2), heat stress (Heat) or DNA damage (MMS) and the corresponding control profile calculated as outlined in Figure 5.3A. mRNAs with a total difference higher than 30 are depicted in red.

I next looked for enrichment of specific functional categories among the groups of translationally regulated mRNAs (Figure 5.5B; Figure 5.6B). Nearly all of the mRNAs that were translationally up- or down-regulated in both stress conditions were part of the core environmental stress response (CESR) genes. These genes are regulated at the level of mRNA abundance in response to most stresses (Chen et al. 2003). Among the mRNAs translationally up-regulated under heat stress, many mRNAs encoding ribosomal proteins were found (Figure 5.5B; Table 5.1). In contrast, many of these mRNAs encoding ribosomal proteins were found to be translationally down-regulated under oxidative stress (Figure 5.6B; Table 5.2). Furthermore the functional groups of highly abundant mRNAs ("10% most abundant mRNAs") and efficiently translated mRNAs ("20% of mRNAs with highest ribosome occupancy"; (Lackner et al. 2007)) were also enriched in the group of mRNAs translationally down-regulated under oxidative stress (Figure 5.6B). Note that despite the fact that many mRNAs encoding ribosomal proteins also belong to these groups of highly abundant and efficiently translated mRNAs, there were additional non-ribosomal mRNAs from these 2 groups among the mRNAs translationally down-regulated under oxidative stress. Some examples of these mRNAs are *eno1*, encoding enolase; *pgk1*, encoding phosphoglycerate kinase; or *eft2-1*, encoding translation elongation factor 2-1.

The average translation profiles for mRNAs translationally regulated under oxidative stress and heat stress are shown in Figure 5.7 and Figure 5.8, respectively.

A

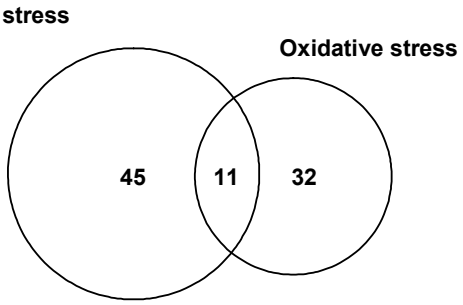


B

P	No. of overlapping genes	Functional group
Translationally up-regulated in both stresses (13 genes)		
1.7E-09	9	*sty1* or *atf1* dependent
1.3E-08	13	Up-regulated in any oxidative stress
2.7E-08	9	Up-regulated in CESR
Translationally up-regulated in heat-stress only (144 genes)		
3.6E-48	83	10% most abundant mRNAs
1.4E-47	53	Ribosomal Genes
1.5E-39	56	GO Structural constitutent of the ribosome
2.8E-27	59	GO Ribonucleoprotein complex
8.3E-25	60	GO Protein biosynthesis
5.7E-23	57	Down-regulated in CESR
1.3E-11	37	Up-regulated in CESR
Translationally up-regulated in oxidative stress only (12 genes)		
5.2E-05	7	Up-regulated in CESR

Figure 5.5 Translationally up-regulated mRNAs under heat and oxidative stress
(A) Venn diagram showing the overlap between mRNAs translationally up-regulated after 15 min exposure to heat and oxidative stress.
(B) Enrichment for functional groups among translationally up-regulated mRNAs depicted in **(A)**. Functional groups were either defined by Gene Ontology (GO) terms or correspond to curated gene lists obtained through other experiments. *sty1 or atf1 dependent*: mRNA expression levels dependent on the MAP kinase Sty1p or the transcription factor Atf1p (Chen et al. 2003). *Up-regulated in any oxidative stress*: mRNAs with increased abundance under oxidative stress induced through various oxidants (Chen et al., submitted). *Down-regulated in CESR*: mRNAs with down-regulated levels during the core environmental stress response (Chen et al. 2003). *Up-regulated in CESR*: mRNAs with up-regulated levels during the core environmental stress response (Chen et al. 2003). *10% most abundant mRNAs*: 10% mRNAs with the highest abundance measured using Affymetrix chips (see Chapter 3). *Ribosomal genes*: mRNAs encoding ribosomal proteins.

A



B

P	No. of overlapping genes	Functional group
Translationally down-regulated in both stresses (11 genes)		
7.0E-07	10	Down-regulated in CESR
Translationally down-regulated in heat-stress only (45 genes)		
2.5E-10	23	Down-regulated in CESR
Translationally down-regulated in oxidative stress only (32 genes)		
2.5E-11	21	10% most abundant mRNAs
4.6E-09	22	20% of mRNAs with highest ribosome occupancy
5.8E-08	12	Ribosomal Genes
1.8E-07	14	GO Ribosome
3.2E-07	22	GO Biosynthesis
7.7E-06	16	GO Protein Biosynthesis
1.2E-05	15	GO Ribonucleoprotein complex

Figure 5.6 Translationally down-regulated mRNAs under heat and oxidative stress
(A) Venn diagram showing the overlap between mRNAs translationally down-regulated after 15 min exposure to heat and oxidative stress.
(B) Enrichment for functional groups among translationally down-regulated mRNAs depicted in **(A)**. Functional groups were either defined by Gene Ontology (GO) terms or correspond to curated gene lists obtained through other experiments: *Down-regulated in CESR*: mRNAs with down-regulated levels during the core environmental stress response (Chen et al. 2003). *10% most abundant mRNAs*: 10% mRNAs with the highest abundance measured using Affymetrix chips (see Chapter 3). *20% of mRNAs with highest ribosome occupancy*: 20% of mRNAs with highest ribosome occupancy as determined using high-resolution translational profiling (see Chapter 3). *Ribosomal genes*: mRNAs encoding ribosomal proteins.

Table 5.1 Curated list of translationally up-regulated mRNAs under heat and oxidative stress

Systematic name	Common name	Function - GeneDB
Up-regulated mRNAs in both heat and oxidative stress		
SPAPB1A10.05		sequence orphan
SPAPB1A10.08		sequence orphan
SPCC1223.03c	*gut2*	glycerol-3-phosphate dehydrogenase
SPAC513.02		phosphoglycerate mutase family
SPAC19D5.01	*pyp2*	tyrosine phosphatase
SPAC2F3.05c		aldo/keto reductase
SPAC23H3.15c		sequence orphan
SPAC2E1P3.01		zinc binding dehydrogenase
SPAC31G5.21		conserved eukaryotic protein
SPBC660.05		hypothetical protein
SPBC106.02c	*srx1*	sulphiredoxin
SPAC4H3.03c		glucan 1,4-alpha-glucosidase
SPAC8C9.16c		mitochondrial protein
Up-regulated mRNAs oxidative stress only		
SPAC23C11.06c		conserved yeast protein
SPAC2H10.01		transcription factor
SPAC19B12.08		peptidase family C54
SPAC1751.01c	*gti1*	gluconate transporter inducer
SPCC16A11.15c		sequence orphan
SPAC513.07		cinnamoyl-CoA reductase
SPCC663.08c		short chain dehydrogenase
SPBC1773.06c		alcohol dehydrogenase
SPAC20G4.04c	*hus1*	checkpoint clamp complex protein
SPBC1773.05c	*tms1*	hexitol dehydrogenase
SPBC12D12.06	*srb11*	cyclin
SPAC139.05		succinate-semialdehyde dehydrogenase
Up-regulated mRNAs heat stress only		
SPAC27D7.11c		possibly S. pombe specific
SPBC1604.10	*srb7; med21*	mediator complex subunit
SPBC1604.07	*atp4*	F0-ATPase subunit
SPCC1259.01c	*rps18-2; rps18; rps1802*	40S ribosomal protein
SPAC4G8.10	*gos1*	SNARE
SPCC330.14c	*rpl24-2; rpl24; rpl2402*	60S ribosomal protein
SPBC18E5.06	*rps21*	40S ribosomal protein
SPBC1685.09	*rps29*	40S ribosomal protein
SPAPB17E12.13	*rpl18-2; rpl18; rpl1802*	60S ribosomal protein
SPAC6F6.07c	*rps13*	40S ribosomal protein
SPAC1F3.08c		dubious
SPBC13E7.04	*atp16*	F1-ATPase delta subunit
SPAC24H6.07	*rps9a; rps901; rps9; rps9-1*	40S ribosomal protein
SPAC144.11	*rps1102; rps11-2; rps11*	40S ribosomal protein
SPAC31G5.03	*rps1101; rps11; rps11-1*	40S ribosomal protein S11
SPAC922.04		sequence orphan
SPBC2F12.04	*rpl17-1; rpl1701; rpl17*	60S ribosomal protein
SPBC839.13c	*rpl1601; rpl13; rpl13-1; rpl16a; rpl16-1*	60S ribosomal protein
SPAC26A3.07c	*rpl1101; rpl11-1; rpl11*	60S ribosomal protein
SPBC17G9.10	*rpl1102; rpl11-2; rpl11*	60S ribosomal protein
SPAP8A3.04c	*hsp9; scf1*	heat shock protein
SPBC1289.14		adducin N-terminal domain protein

SPBC365.03c	*rpl2101;rpl21;rpl21-1*	60S ribosomal protein
SPAC6G9.09c	*rpl24;rpl24-1*	60S ribosomal protein
SPCC1739.08c		short chain dehydrogenase
SPBC18E5.04	*yL9; rpl10-1; rpl1001; rpl10*	60S ribosomal protein
SPAP7G5.05	*rpl10-2; rpl1002; rpl10*	60S ribosomal protein
SPAC21E11.03c	*pcr1; mts2*	bZIP (basic leucine zipper) transcription factor family
SPCC576.08c	*rps2*	40S ribosomal protein
SPCC24B10.09	*rps1702; rps17; rps17-2*	40S ribosomal protein
SPAC18G6.14c	*rps7*	40S ribosomal protein
SPCC1739.04c		sequence orphan
SPAC589.10c		ribosomal-ubiquitin fusion protein
SPAC343.06c		scramblase
SPBC18H10.12c	*rpl7-2; rpl702; rpl7c; rpl701*	60S ribosomal protein
SPAC11D3.01c		conserved fungal protein
SPBP4H10.13	*rps23-2; rps2302*	40S ribosomal protein
SPCC338.05c	*mms2; spm2*	ubiquitin conjugating enzyme
SPCC364.03	*rpl1702; rpl17; rpl17-2*	60S ribosomal protein
SPCC622.15c		double-strand break repair protein
SPBC29B5.03c	*rpl26*	60S ribosomal protein
SPAC4F8.10c	*stg1*	SM22/transgelin-like actin modulating protein
SPCC1919.09	*tif6*	translation initiation factor
SPBC1198.05		guanylate kinase
SPBC83.17		multiprotein bridging factor
SPAC1805.11c	*rps26-2; rps2602*	40S ribosomal protein
SPAC7D4.07c	*trx2; trx; trx1*	thioredoxin
SPBC11C11.06c		sequence orphan
SPAC3H5.10	*rpl32; rpl32-2; rpl3202*	60S ribosomal protein
SPBC16C6.11	*rpl32; rpl3201; rpl32-1*	60S ribosomal protein
SPBC3E7.16c	*leu3*	2-isopropylmalate synthase
SPCC364.02c	*bis1*	stress response protein
SPAC27F1.02c	*cdc8; fus4*	tropomyosin
SPAC4A8.15c	*cdc3*	profilin
SPBC83.02c	*rpl43;rpl4302;rpl37a-2;rpl43-2*	60S ribosomal protein
SPAC31A2.05c	*mis4*	adherin
SPBC3D6.15	*rps25; rps25-1; rps2501; rps25a*	40S ribosomal protein
SPAC31A2.04c		20S proteasome component beta 4
SPAC694.05c	*rps25; rps2502; rps25b; rps25-2; rps31*	40S ribosomal protein
SPBC56F2.02	*rpl1901; rpl19-1*	60S ribosomal protein
SPAC26H5.10c	*tif51; tif511*	translation initiation factor
SPBC32F12.03c	*gpx1*	glutathione peroxidase
SPBC26H8.13c		sequence orphan
SPCC191.01		sequence orphan
SPBP4H10.10		rhomboid family protease
SPAPB1E7.07		glutamate synthase
SPBC12D12.07c	*trx2*	thioredoxin
SPBC3B9.13c	*rpp1-2; rpp102; rpa3; rpap1-3*	60S acidic ribosomal protein
SPAC4G9.16c	*rpl9; rpl9-1; rpl901*	60S ribosomal protein
SPCC613.06	*rpl9; rpl9-2; rpl902*	60S ribosomal protein
SPAC5H10.03		phosphoglycerate mutase family
SPAC18B11.07c	*sng1; rhp6; ubc2*	ubiquitin conjugating enzyme
SPAC821.10c	*sod1*	superoxide dismutase
SPAC2C4.17c		MS ion channel
SPCC962.04	*rps12-1; rps12; rps1201*	40S ribosomal protein
SPCC191.07	*cyc1*	cytochrome c
SPBC1685.02c	*rps1202; rps12; rps12-2*	40S ribosomal protein
SPBC21C3.13	*rps16; rps1901; rps19-1*	40S ribosomal protein
SPBC649.02	*rps19; rps1902; rps19-2*	40S ribosomal protein
SPCC338.18		sequence orphan

SPCC1442.14c		adenosine 5'-monophosphoramidase
SPCC338.08		sequence orphan
SPCC31H12.04c	*rpl1202; rpl12-2; rpl12*	60S ribosomal protein
SPBC29A3.12	*rps9-2; rps9b; rps902*	40S ribosomal protein
SPCC1672.08c	*tfa2*	transcription factor TFIIE beta subunit
SPAC4H3.07c		rhodanese-like domain
SPBC16A3.02c		mitochondrial peptidase
SPAC24C9.14		OTU-like ubiquitin-specific protease
SPAC6G10.11c	*rps31; ubi3*	ribosomal-ubiquitin fusion protein
SPAC1687.06c	*rpl44; rpl28*	60S ribosomal protein
SPAC22A12.17c		short chain dehydrogenase
SPBC337.08c	*ubi4*	ubiquitin
SPBC839.17c	*fkh1; fkbp12*	peptidyl-prolyl cis-trans isomerase
SPCC757.12		alpha-amylase
SPBC119.02	*ubc4*	ubiquitin conjugating enzyme
SPAC10F6.05c	*ubc6*	ubiquitin conjugating enzyme
SPAC22G7.08	*ppk8*	serine/threonine protein kinase
SPCC285.15c	*rps28-2; rps28; rps2802*	40S ribosomal protein
SPBC3B9.07c	*rpa43; rpa21*	DNA-directed RNA polymerase I complex subunit
SPBC336.10c	*tif512*	translation initiation factor
SPAC22H12.01c		sequence orphan
SPBC776.11	*rpl29; rpl2801; rpl27a; rpl28-1*	60S ribosomal protein
SPCC16C4.13c	*rpl12-1; rpl12.1; rpl1201*	60S ribosomal protein
SPCC757.07c	*cta1; ctt1*	catalase
SPCC1494.03		sequence orphan
SPBC16A3.08c		nuclear telomere cap complex subunit
SPBC725.03		conserved yeast protein
SPBC3E7.07c		conserved eukaryotic protein
SPAC144.04c	*spe1*	ornithine decarboxylase
SPCC576.04		bax inhibitor-like protein
SPCC757.03c		conserved fungal protein
SPBC1289.06c		sequence orphan
SPBC1289.03c	*fyt1; mal25-1; spi1*	Ran GTPase
SPAC13G6.07c	*rps601; rps6; rps6-1*	40S ribosomal protein
SPAC3A12.10	*rpl20-1; rpl20; rpl18a-2; rpl2001*	60S ribosomal protein
SPAC26A3.04	*rpl2002; rpl20; rpl18a; rpl20-2; rpl18a-1*	60S ribosomal protein
SPCC330.06c	*pmp20*	thioredoxin peroxidase
SPAC19G12.09		NADPH-dependent alpha-keto amide reductase
SPAC12B10.13		CTLH domain
SPBC21H7.06c		inositol metabolism protein
SPBC8D2.11	*pi054*	sequence orphan
SPACUNK4.17		dehydrogenase
SPBC18H10.14	*rps1601; rps16; rps16-1*	40S ribosomal protein
SPAC664.04c	*rps1602; rps16-2; rps16*	40S ribosomal protein
SPAC323.02c		20S proteasome component alpha 5
SPAC29B12.11c		human WW domain binding protein-2 ortholog
SPBC685.07c	*rpl27-1; rpl2701*	60S ribosomal protein
SPAC26F1.04c	*mrf1; etr1*	enoyl-[acyl-carrier protein] reductase
SPAPB1E7.12	*rps6; rps6-2; rps602*	40S ribosomal protein
SPAC20G4.06c	*adf1; cof1*	cofilin
SPBP8B7.24c	*atg8*	autophagy associated protein
SPBC25H2.05	*ucp15; egd2*	nascent polypeptide-associated complex
SPBC405.07	*rpl3602; rpl36; rpl36-2*	60S ribosomal protein
SPBC56F2.11	*met6*	homoserine O-acetyltransferase
SPAC1F7.04	*rho1*	Rho family GTPase
SPBC28F2.03	*ppi1; cyp1; cyp2*	cyclophilin
SPAC11E3.14		conserved fungal protein
SPAC1071.07c	*rps15-2; rps15; rps1502; rps15-3*	40S ribosomal protein

SPBC16E9.16c		sequence orphan
SPAC688.13	*scn1*	TatD DNase family
SPAC1F12.02c	*p23fy*	translationally controlled tumor protein homolog
SPAC13G6.02c	*rps3a-1; rps1-1; rps101*	40S ribosomal protein
SPBC24C6.09c		phosphoketolase
SPAC57A10.12c	*ura3*	dihydroorotate dehydrogenase

List of mRNAs that were identified to be translationally up-regulated after 15 min exposure to heat stress, oxidative stress or in both conditions. Shown is the systematic name, the common name and the functional category according to GeneDB.

Table 5.2 Curated list of translationally down-regulated mRNAs under heat and oxidative stress

Systematic name	**Common name**	**Function - GeneDB**
Down-regulated mRNAs in both heat- and oxidative stress		
SPBC56F2.12	*ilv5*	acetohydroxyacid reductoisomerase
SPCC622.12c		NADP-specific glutamate dehydrogenase
SPCC417.08	*ef-3; ef3*	translation elongation factor 3
SPBC17D1.06		DEAD/DEAH box helicase
SPAC959.07	*rps4-3; rps403; rps4*	40S ribosomal protein
SPCPB16A4.03c	*ade10*	IMP cyclohydrolase
SPAC56F8.09	*rrp8*	methyltransferase
SPBC1709.05	*sks2; hsc1*	heat shock protein 70 family
SPBC25B2.05	*mis3*	KH domain
SPAC30C2.02	*mmd1*	deoxyhypusine hydroxylase
SPAC140.02	*gar2*	GAR family
Down-regulated mRNAs oxidative stress only		
SPBC3H7.08c		conserved fungal protein
SPAC57A7.12		heat shock protein 70 family
SPBC428.05c		argininosuccinate synthase
SPAC29A4.15		serine-tRNA ligase
SPCP31B10.07	*eft1; eft202*	translation elongation factor 2
SPAC513.01c	*eft2-1; eft201; etf2; eft2; etf201*	translation elongation factor 2
SPBC14F5.04c	*pgk1*	phosphoglycerate kinase
SPAC22E12.12		dubious
SPAC24H6.10c		phospho-2-dehydro-3-deoxyheptonate aldolase
SPAC3H5.12c	*rpl5; rpl501; rpl5-1*	60S ribosomal protein
SPBC1815.01	*eno101; eno1*	enolase
SPCC622.18	*rpl6*	60S ribosomal protein
SPAC3H5.07	*rpl7-2; rpl702; rpl7-A; rpl7; rpl7-1; rpl701*	60S ribosomal protein
SPBC19F8.08	*rps4-1; rps401; rps4*	40S ribosomal protein
SPBPB2B2.08		sequence orphan
SPAC56E4.04c	*cut6*	acetyl-CoA carboxylase
SPCC576.08c	*rps2*	40S ribosomal protein
SPAC17H9.05	*ebp2*	P40
SPBC725.11c	*php2*	CCAAT-binding factor complex subunit
SPCC18.14c	*rpp0*	60S acidic ribosomal protein
SPAC22H10.06c		dubious
SPAPB8E5.06c	*rpl3-b; rpl3-2; rpl302; rpgL3-2*	60S ribosomal protein
SPAC1F7.13c	*rpk5a; rpl8-1; rpl2-1; rpk5; rpl18; rpl801*	60S ribosomal protein
SPAC13G6.07c	*rps601; rps6; rps6-1*	40S ribosomal protein
SPBC839.13c	*rpl13a-1; rpl1601; rpl13; rpl13-1; rpl16a; rpl16-1*	60S ribosomal protein
SPCC576.11	*rpl15*	60S ribosomal protein

SPBC660.16	*gnd*	phosphogluconate dehydrogenase
SPAC19A8.07c		U3 snoRNP-associated protein
SPAC17A5.03	*rpl3; rpl3-1; rpl301; rpgL3-1*	60S ribosomal protein
SPCC576.01c		sulfonate dioxygenase
SPBC839.16		C-1-tetrahydrofolate synthase
SPBC776.03		homoserine dehydrogenase
Down-regulated mRNAs heat-stress only		
SPBC1703.05		protein kinase (RIO family)
SPBC1703.07		ATP citrate synthase
SPBC119.16c		conserved fungal protein
SPAPB2B4.03	*cyc17; cig2*	cyclin
SPBC1773.04		cinnamoyl-CoA reductase
SPBC13G1.04c		alkB homolog
SPBC2G2.13c		deoxycytidylate deaminase
SPCC320.11c		RNA-binding protein
SPBC26H8.08c		GTPase
SPCC364.01		sequence orphan
SPBC28F2.11		chromatin remodeling complex subunit
SPBC16D10.01c		TPR repeat protein
SPBC1105.07c		THO complex
SPAC8E11.10	*sou1*	short chain dehydrogenase
SPAC144.03	*min3; ade2; min10*	adenylosuccinate synthetase
SPAP7G5.02c	*gua2*	GMP synthase [glutamine-hydrolyzing]
SPBC1604.09c		exonuclease
SPAC694.04c		conserved eukaryotic protein
SPAC637.09		ribonuclease H70
SPAC19D5.10c		sequence orphan
SPAC2C4.12c		tRNA 2'-phosphotransferase
SPBC713.05		WD repeat protein
SPAC16E8.06c	*nop12*	RNA-binding protein
SPBP16F5.05c		ankyrin repeat protein
SPAC3H8.07c		prefoldin subunit 3
SPAC890.05		G-patch domain
SPBC365.11		GRIP domain
SPAC1002.07c	*ats1*	N-acetyltransferase
SPBC1709.02c		valine-tRNA ligase
SPAC23H4.06	*gln1*	glutamate-ammonia ligase
SPBC887.14c	*rph1; pif1; pfh1*	pif1 helicase homolog
SPBC4B4.07c	*U1-A*	small nuclear ribonucleoprotein (snRNP)
SPBC428.15		GTP binding protein
SPAC13D6.02c	*byr3*	zinc finger protein
SPCC126.03	*lps1; pus1*	tRNA pseudouridylate synthase
SPAC589.05c		conserved protein
SPBC17D1.02		diphthamide biosynthesis protein
SPBC9B6.07		nucleolar protein Nop52 family
SPAC4C5.01		haloacid dehalogenase
SPBP8B7.16c	*dbp2*	DEAD/DEAH box helicase
SPBC16C6.12c		Las1-like protein
SPCC1393.09c		RWD domain
SPAC18B11.06		small nucleolar ribonucleoprotein
SPAC9G1.12	*cpd1*	tRNA (m1A) methyltransferase complex subunit
SPCC550.05	*nse1*	Smc5-6 complex non-SMC subunit 1

List of mRNAs that were identified to be translationally down-regulated after 15 min exposure to heat stress, oxidative stress or in both conditions. Shown is the systematic name, the common name and the functional category according to GeneDB.

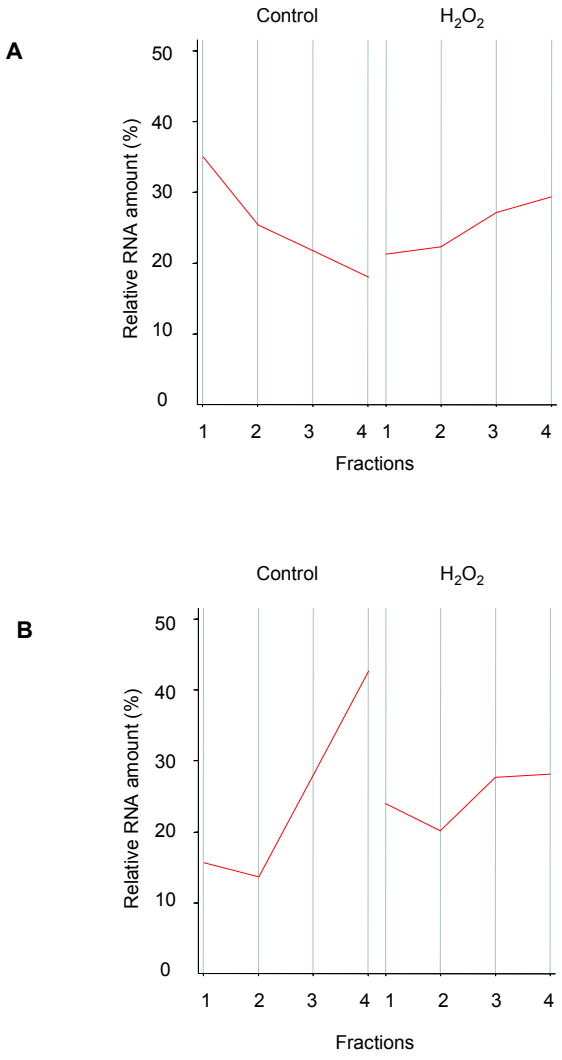


Figure 5.7 Average translation profiles for mRNAs translationally regulated under oxidative stress
(A) Average translation profile shown for 25 mRNAs identified to be translationally up-regulated after 15 min exposure to oxidative stress.
(B) Average translation profile shown for 43 mRNAs identified to be translationally down-regulated after 15 min exposure to oxidative stress.

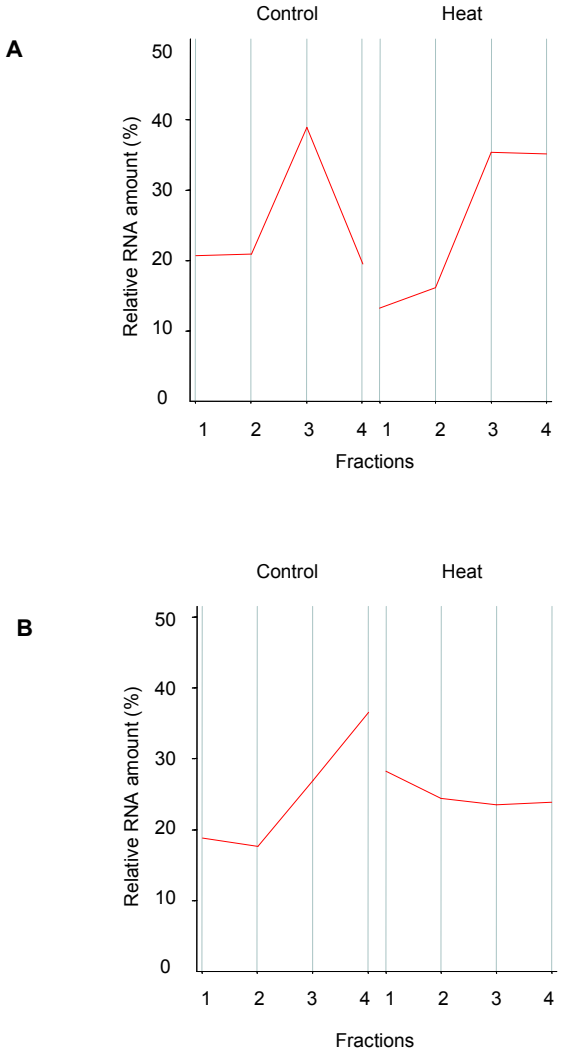


Figure 5.8 Average translation profiles for mRNAs translationally regulated under heat stress
(A) Average translation profile shown for 157 mRNAs identified to be translationally up-regulated after 15 min exposure to heat stress.
(B) Average translation profile shown for 56 mRNAs identified to be translationally down-regulated after 15 min exposure to heat stress.

Coordination between changes in mRNA abundance and translation

Many mRNAs that have been found to be translationally regulated in heat and oxidative stress were members of the CESR genes, which are regulated at the level of mRNA abundance in the response to stress (Chen et al. 2003). To see if there was a general coordination between changes in mRNA abundance and translation, the changes in total mRNA levels for these mRNAs were compared (Figure 5.9). This comparison revealed a clear trend: many, but not all, mRNAs that were regulated at the translational level were also regulated at the level of mRNA abundance. This connection was especially strong for up-regulated mRNAs.

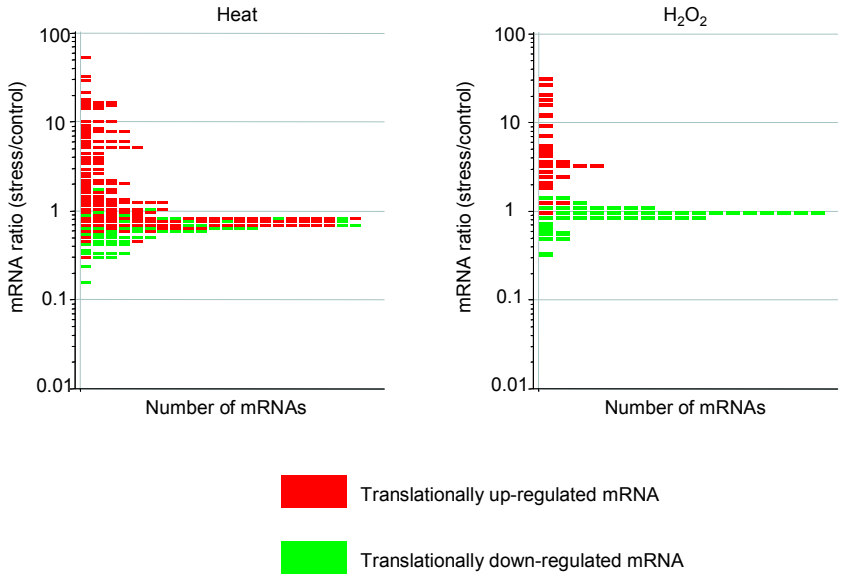


Figure 5.9 Changes in total mRNA levels for translationally regulated mRNAs in stress conditions
Changes in total mRNA abundance for mRNAs identified to be translationally regulated under heat or oxidative stress. mRNA levels are shown as ratios relative to control cells. The ratios represent the normalized averages of 2 (Heat) or 3 (H_2O_2) independent biological repeats after 15 min exposure to stress. mRNAs are colour-coded according to translational up-regulation (red) or translational down-regulation (green) in the corresponding conditions.

To find mRNAs that were strongly regulated at the translational level, but did not show strong changes in total mRNA abundance, I further focused on mRNAs whose total mRNA level did not change more than 1.5 fold in the stress conditions. Under

oxidative stress, 3 of the 25 translationally up-regulated and 33 of the 43 translationally down-regulated mRNAs fell into this category (Table 5.3). Under heat stress, 42 of the 157 translationally up-regulated and 9 of the 56 translationally down-regulated mRNAs were not strongly regulated in terms of mRNA abundance (Table 5.4). I will refer to these mRNA as "regulated at the translational level only". Among these mRNAs were still many mRNAs encoding ribosomal proteins. These were found to be translationally up-regulated under heat stress (12 ribosomal mRNAs), but translationally down-regulated (13 ribosomal mRNAs) under oxidative stress.

Table 5.3 List of mRNAs that show translational regulation under oxidative stress, but are not regulated at the level of total mRNA abundance

Systematic name	Common name	Function - GeneDB	Total mRNA ratio
Up-regulated mRNAs oxidative stress			
SPBC12D12.06	*srb11*	cyclin	1.00
SPAC31G5.21		conserved eukaryotic protein	1.29
SPAC20G4.04c	*hus1*	checkpoint clamp complex protein	1.23
Down-regulated mRNAs oxidative stress			
SPAC30C2.02	*mmd1*	deoxyhypusine hydroxylase	0.82
SPBC839.13c	*rpl13a-1; rpl1601; rpl13; rpl13-1; rpl16a; rpl16-1*	60S ribosomal protein	1.06
SPAC22H10.06c		dubious	0.90
SPAC29A4.15		serine-tRNA ligase	0.87
SPCC576.11	*rpl15*	60S ribosomal protein	0.94
SPCC18.14c	*rpp0*	60S acidic ribosomal protein	0.94
SPCPB16A4.03c	*ade10*	IMP cyclohydrolase	0.81
SPCC576.08c	*rps2*	40S ribosomal protein	0.87
SPAC3H5.07	*rpl7-2; rpl702; rpl7-A; rpl7; rpl7-1; rpl701; rpl7b*	60S ribosomal protein	0.92
SPAC3H5.12c	*rpl5; rpl501; rpl5-1*	60S ribosomal protein	0.94
SPCC622.18	*rpl6*	60S ribosomal protein	0.92
SPAC57A7.12		heat shock protein 70 family	0.85
SPCC622.12c		NADP-specific glutamate dehydrogenase	1.16
SPAC22E12.12		dubious	0.90
SPAC17A5.03	*rpl3; rpl3-1; rpl301; rpgL3-1*	60S ribosomal protein	0.97
SPCC576.01c		sulfonate dioxygenase	0.80
SPBC1709.05	*sks2; hsc1*	heat shock protein 70 family	0.83
SPAPB8E5.06c	*rpl3-b; rpl3-2; rpl302; rpgL3-2*	60S ribosomal protein	0.95
SPAC959.07	*rps4-3; rps403; rps4*	40S ribosomal protein	0.89
SPBC19F8.08	*rps4-1; rps401; rps4-1B.01c; rps4*	40S ribosomal protein	0.96
SPBC3H7.08c		conserved fungal protein	0.96
SPCP31B10.07	*eft1; eft202*	translation elongation factor 2	0.81
SPAC513.01c	*eft2-1; eft201; etf2; eft2; etf201*	translation elongation factor 2	0.83
SPBC56F2.12	*ilv5*	acetohydroxyacid reductoisomerase	0.92
SPBC839.16		C-1-tetrahydrofolate synthase	1.03
SPBC776.03		homoserine dehydrogenase	1.05
SPAC13G6.07c	*rps601; rps6; rps6-1*	40S ribosomal protein	0.92
SPAC1F7.13c	*rpl8-1; rpl2-1; rpk5; rpl18; rpl801*	60S ribosomal protein	0.95
SPBC725.11c	*php2*	CCAAT-binding factor complex subunit	1.05

SPAC56E4.04c	*cut6*	acetyl-CoA carboxylase	0.86
SPBC1815.01	*eno101; eno1*	enolase	1.02
SPAC24H6.10c		phospho-2-dehydro-3-deoxyheptonate aldolase	0.99
SPBC14F5.04c	*pgk1*	phosphoglycerate kinase	1.03

List of mRNAs that were identified to be translationally regulated after 15 min exposure to oxidative stress, but did not show a more than 1.5 fold change in total mRNA levels in any of the 3 biological repeats. Shown is the systematic name, the common name, the functional category according to GeneDB and the average of the normalized total mRNA level ratio relative to control cells.

Table 5.4 List of mRNAs that show translational regulation under heat stress, but are not regulated at the level of total mRNA abundance

Systematic name	Common name	Function - GeneDB	Total mRNA ratio
Up-regulated mRNAs heat stress			
SPBC18E5.06	*rps21*	40S ribosomal protein	0.69
SPAC1F3.08c		dubious	1.22
SPBC1604.07	*atp4*	F0-ATPase subunit	1.10
SPBC13E7.04	*atp16*	F1-ATPase delta subunit	1.16
SPAPB17E12.13	*rpl18-2; rpl18; rpl1802*	60S ribosomal protein	0.81
SPBC1604.10	*srb7; med21*	mediator complex subunit	0.72
SPAC6F6.07c	*rps13*	40S ribosomal protein	0.83
SPAC922.04		sequence orphan	0.79
SPCC338.05c	*mms2; spm2*	ubiquitin conjugating enzyme	1.09
SPCC24B10.09	*rps1702; rps17; rps17-2*	40S ribosomal protein	1.03
SPAC20G4.06c	*adf1; cof1*	cofilin	0.88
SPBC1198.05		guanylate kinase	0.97
SPAC4F8.10c	*stg1*	SM22/transgelin-like actin modulating protein	1.37
SPBC18E5.04	*rpl10-1; RL10; rpl1001; rpl10*	60S ribosomal protein	0.84
SPAP7G5.05	*rpl10-2; rpl1002; rpl10*	60S ribosomal protein	0.80
SPCC1919.09	*tif6*	translation initiation factor	0.99
SPAC1805.11c	*rps26-2; rps2602*	40S ribosomal protein	0.94
SPAC27F1.02c	*cdc8; fus4*	tropomyosin	1.12
SPAC4A8.15c	*cdc3*	profilin	0.85
SPAC31A2.05c	*mis4*	adherin	0.80
SPBC83.02c	*rpl43; rpl4302; rpl37a-2; rpl43-2*	60S ribosomal protein	0.82
SPAC694.05c	*rps25; rps2502; rps25b; rps25-2; rps31*	40S ribosomal protein	0.79
SPAC31A2.04c		20S proteasome component beta 4	1.27
SPCC1442.14c		adenosine 5'-monophosphoramidase	1.32
SPBC26H8.13c		sequence orphan	1.26
SPBC3B9.13c	*rpp1-2; rpp102; rpa3; rpap1-3*	60S acidic ribosomal protein	0.78
SPBC1685.02c	*rps1202; rps12; rps12-2*	40S ribosomal protein	0.94
SPCC191.07	*cyc1*	cytochrome	1.24
SPAC18B11.07c	*sng1; rhp6; ubc2*	ubiquitin conjugating enzyme	1.37
SPBC119.02	*ubc4*	ubiquitin conjugating enzyme	1.28
SPCC1672.08c	*tfa2*	transcription factor TFIIE beta subunit	1.32
SPAC4H3.07c		rhodanese-like domain	0.85
SPBC839.17c	*fkh1; fkbp12*	peptidyl-prolyl cis-trans isomerase	1.15
SPBC336.10c	*tif512*	translation initiation factor	0.88
SPAC6G10.11c	*rps31; ubi3*	ribosomal-ubiquitin fusion protein	0.80
SPBC3E7.07c		conserved eukaryotic protein	0.84
SPBC1289.03c	*fyt1; mal25-1; spi1*	Ran GTPase	1.03

SPBC8D2.11	*pi054*	sequence orphan	1.26
SPAC323.02c		20S proteasome component alpha 5	1.24
SPAC1F7.04	*rho1*	Rho family GTPase	1.00
SPBC28F2.03	*ppi1; cyp1; cyp2*	cyclophilin	1.04
SPAC1071.07c	*rps15-2; rps15; rps1502; rps15-3*	40S ribosomal protein	0.90
Down-regulated mRNAs heat stress			
SPBC1773.04		cinnamoyl-CoA reductase	0.80
SPCC622.12c		NADP-specific glutamate dehydrogenase	0.97
SPBC28F2.11		chromatin remodeling complex subunit	0.84
SPAC144.03	*min3; ade2; min10*	adenylosuccinate synthetase	0.85
SPAC19D5.10c		sequence orphan	1.08
SPAC2C4.12c		tRNA 2'-phosphotransferase	0.73
SPAC16E8.06c	*nop12*	RNA-binding protein	0.87
SPBC365.11		GRIP domain	0.91
SPAC589.05c		conserved protein	0.78

List of mRNAs that were identified to be translationally regulated after 15 min exposure to heat stress, but did not show a more than 1.5 fold change in total mRNA levels in any of the 2 biological repeats. Shown is the systematic name, the common name, the functional category according to GeneDB and the average of the normalized total mRNA level ratio relative to control cells.

Several examples of mRNAs that are regulated at the translational level only are shown in Figure 5.10 and Figure 5.11. SPAC31G5.21 is an mRNA encoding an uncharacterized, but conserved eukaryotic protein. It was translationally up-regulated in response to both stresses (Figure 5.10B), but was only weakly up-regulated in terms of total mRNA level under oxidative stress. Under heat stress, it slightly missed the arbitrary 1.5 fold cut-off on changes in total mRNA levels, as it is 1.55 fold up-regulated in one of the two repeats. Another mRNA that is translationally up-regulated under oxidative stress is *srb11* (Figure 5.10B), which encodes a putative G1-to-S phase-specific cyclin and is a component of the mediator sub-complex that functions in the negative regulation of transcription (Spahr et al. 2001; Samuelsen et al. 2003). *Sks2* mRNA is strongly translationally down-regulated under oxidative stress (Figure 5.10A). It encodes a heat shock protein, which is moderately down-regulated in response to heat shock at the level of mRNA abundance (Oishi et al. 1996; Chen et al. 2003).

Two mRNAs that showed a strong translational down-regulation under heat stress are shown in Figure 5.11A: SPAC589.05c encodes a conserved eukaryotic protein of unknown function; SPCC622.12c encodes a predicted NADP-specific glutamate dehydrogenase (Yoshioka et al. 1997), whose expression is dependent on the MAP kinase Sty1p and the transcription factor Atf1p (Chen et al. 2003). SPBC8D2.11 is an

mRNA encoding a protein of unknown function, which is translationally up-regulated under heat stress.

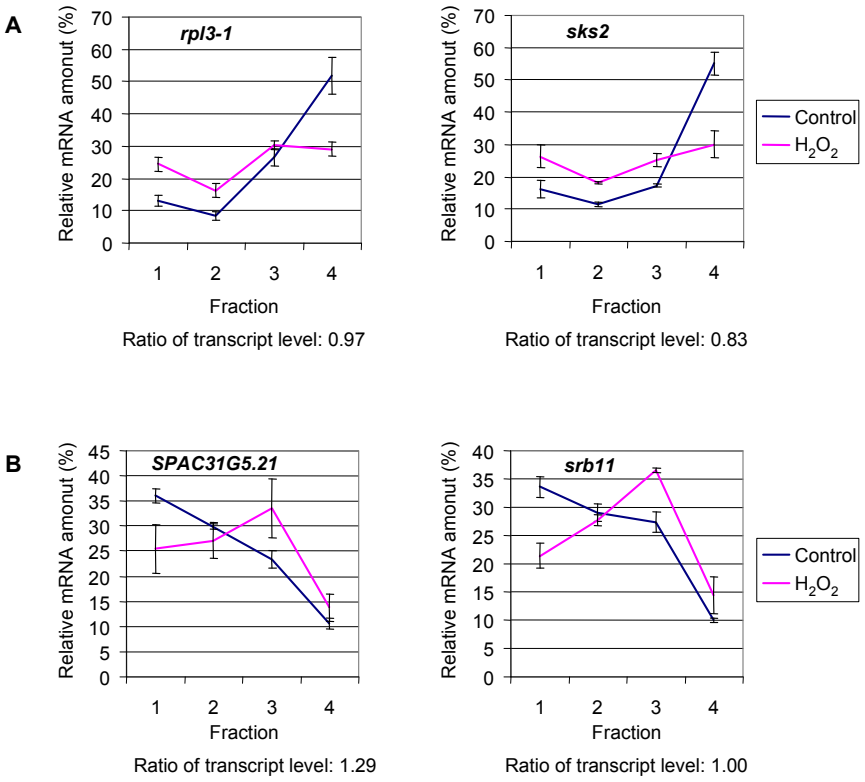


Figure 5.10 Example profiles of mRNAs that show translational regulation under oxidative stress, but are not regulated at the level of total mRNA abundance
(A) Translation profiles for mRNAs translationally down-regulated after 15 min exposure to oxidative stress. Shown are the averaged profiles from 3 independent biological repeats for the mRNA encoding the 60S ribosomal protein L3 (*rpl3-1*) and the mRNA encoding heat shock protein Sks2p (*sks2*). Error bars represent the standard deviation. The normalized and averaged ratio of transcript level relative to the control is also shown.
(B) Translation profiles for mRNAs translationally up-regulated after 15 min exposure to oxidative stress. Shown are the averaged profiles from 3 independent biological repeats for the mRNA encoding an uncharacterized, conserved protein (SPAC31G5.21) and the mRNA encoding the cyclin Srb11p. Error bars represent the standard deviation. The normalized and averaged ratio of transcript level relative to the control is also shown.

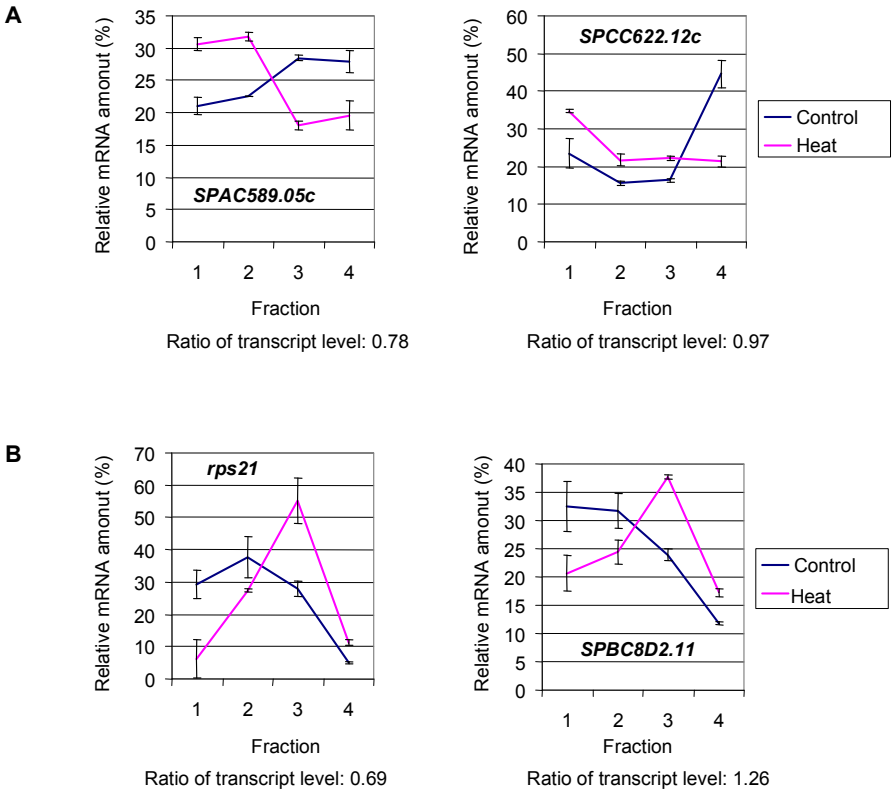


Figure 5.11 Example profiles of mRNAs that show translational regulation under heat stress, but are not regulated at the level of total mRNA abundance

(A) Translation profiles for mRNAs translationally down-regulated after 15 min exposure to heat stress. Shown are the averaged profiles from 2 independent biological repeats for the mRNA encoding a conserved eukaryotic protein (*SPAC589.05c*) and the mRNA encoding a predicted NADP-specific glutamate dehydrogenase (*SPCC622.12c*). Error bars represent the standard deviation. The normalized and averaged ratio of transcript level relative to the control is also shown.

(B) Translation profiles for mRNAs translationally up-regulated after 15 min exposure to heat stress. Shown are the averaged profiles from 2 independent biological repeats for the mRNA encoding the 40S ribosomal protein S21 (*rps21*) and the mRNA encoding an uncharacterized protein (*SPBC8D2.11*). Error bars represent the standard deviation. The normalized and averaged ratio of transcript level relative to the control is also shown.

Regulation of translation under oxidative stress in a time course experiment

Despite the fact that an arbitrary 1.5 fold cut-off for changes in total mRNA levels was used to identify mRNAs that were only regulated at the translational level, many of them showed slight changes in total mRNA abundance in the same direction as the change in translation (Table 5.3; Table 5.4), and several of them have been reported to show delayed changes in total mRNA levels in response to stress (Chen et al. 2003). To also obtain information on the temporal regulation of translation under stress, additional translational profiling was performed for cells exposed to oxidative stress not only for 15 min, but also for 5 min and 60 min. By looking at the total difference between the translational profiles under stress and control conditions as outlined in Figure 5.3A and using the arbitrary cut-off of 30 for the total difference calculated between profiles (Figure 5.4), roughly the same number of mRNAs was translationally regulated after 5 min (166) and 15 min (142) exposure to oxidative stress. The number of 166 translationally regulated mRNAs after 5 min exposure to stress needs to be taken with caution, as only one repeat of the experiment was performed, and this number would be lower if only changes were taken into account that happen consistently in several biological repeats. However, the number of mRNAs with changed translational profiles increased to 878 mRNAs after 60 min exposure to oxidative stress, most of them being down-regulated (575 mRNAs). Taken together, these data indicate that few mRNAs rapidly respond on the translational level to the exposure to stress, whereas many more mRNAs are translationally regulated after longer exposure to stress. Note that no complete analysis on translationally regulated mRNAs after 5 and 60 min exposure to stress will be presented here due to the lack of biological repeats. Instead, I will focus on several clear-cut examples and mRNAs that have already been defined as translationally regulated after 15 min exposure to oxidative stress.

20 of the 33 mRNAs that showed strong regulation only at the translational level after 15 min were already down-regulated after 5 min exposure to oxidative stress. Two examples are shown in Figure 5.12. Both *sks2* and *eft2-2* (encoding translation elongation factor 2-2) already show strong changes in their translation profile after 5 min exposure to stress. After 60 min, an even stronger down-regulation in translation could be seen. At this time-point, also total mRNA levels of these mRNAs are

decreased. An example of an mRNA that is translationally up-regulated already after 5 min exposure to oxidative stress is *srb11* (Figure 5.13). In summary, data from the time course experiment suggest that translational regulation can happen at different rates. Several mRNAs show strong regulation already after 5 min exposure to stress, whereas others only respond after longer exposure.

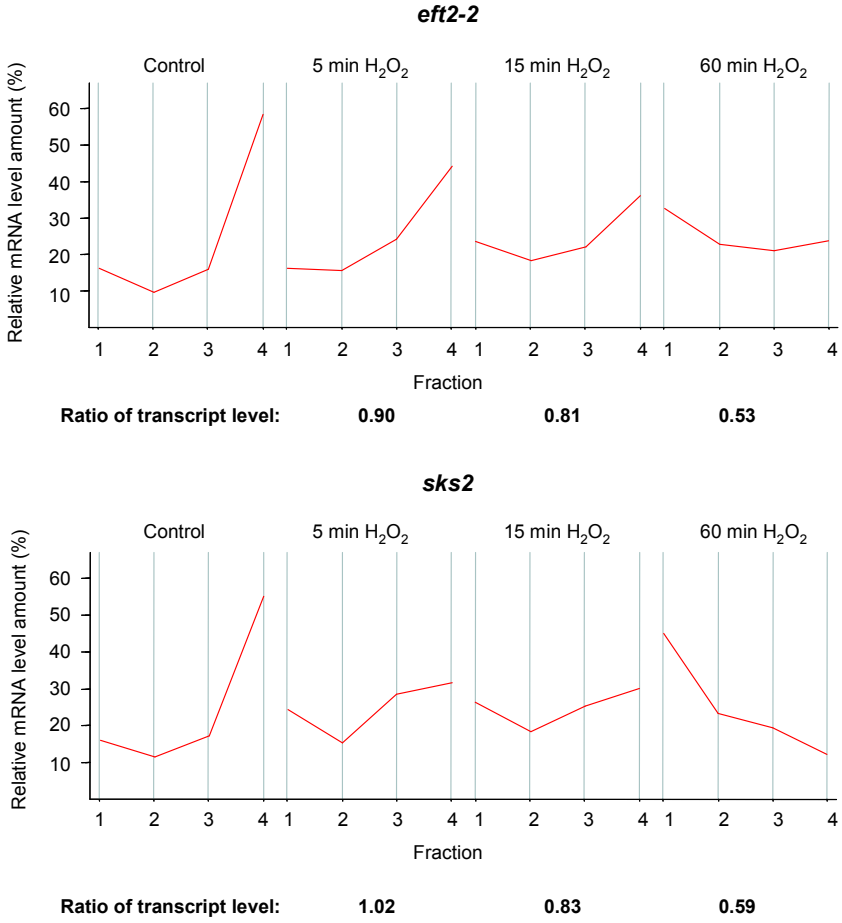


Figure 5.12 Translation profiles of down-regulated mRNAs after different times of exposure to oxidative stress
Shown are translational profiles for *eft2-2* (encoding translation elongation factor 2-2) and *sks2* (encoding a heat shock protein) after different times of exposure to oxidative stress. Translational profiles for the control and the 15 min time point represent the average of 3 independent biological repeats. Translational profiles for the 5 min and 60 min time point represent data from one experiment. The change of transcript level for each time point is also indicated as ratio relative to control cells.

From the single translational profiling experiment performed in cells exposed to oxidative stress for 60 min, 575 mRNAs were found to be translationally down-regulated. These mRNAs were again enriched for the most abundant mRNAs, CESR genes, and mRNAs encoding ribosomal proteins ($P \sim 5e^{-172}$-$7e^{-100}$). As several

mRNAs encoding ribosomal proteins are present in all these lists, this group of mRNAs was further analysed. Several ribosomal mRNAs were already translationally down-regulated after 15 min exposure to oxidative stress (Figure 5.6; Table 5.3), whereas nearly all of them were strongly down-regulated after 60 min. This is evident by comparing the average translation profiles of all mRNAs encoding ribosomal proteins for each time-point (Figure 5.14A). Despite this strong translational down-regulation, total mRNA levels showed only a moderate, albeit steady decrease after 60 min (Figure 5.14B).

In contrast, after the exposure to heat stress for 15 min, many ribosomal mRNAs exhibited higher translational efficiency (Figure 5.5; Table 5.4), despite the fact that most of them were actually slightly down-regulated in terms of total mRNA abundance with an average ratio for 130 ribosomal mRNAs relative to control of 0.74. These data indicate independent mechanisms regulating mRNA levels and translational efficiency for mRNAs encoding ribosomal proteins.

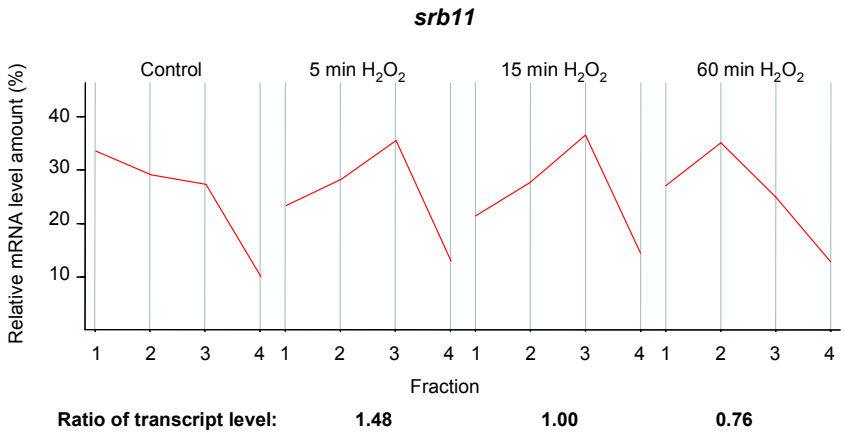


Figure 5.13 Translation profiles of an up-regulated mRNA after different times of exposure to oxidative stress
Translational profile for *srb11* (encoding a cyclin) after different times of exposure to oxidative stress. The translational profile for the control and the 15 min time point represent the average of 3 independent biological repeats. The translational profile for the 5 min and 60 min time-points represent data from one experiment. The change of transcript level for each time point is also indicated as ratio relative to control cells.

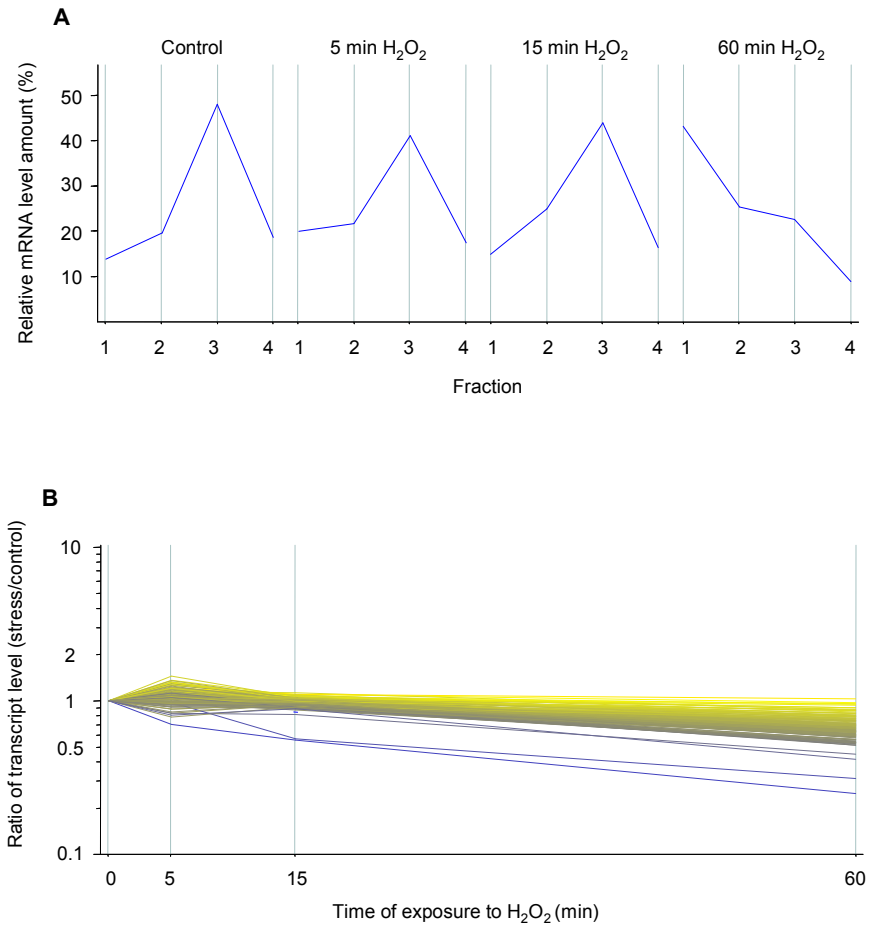


Figure 5.14 Translational regulation of mRNAs encoding ribosomal proteins under oxidative stress

(A) Average translational profile for 130 mRNAs encoding ribosomal proteins for different times of exposure to oxidative stress. The translational profiles for the control and the 15 min time point represent the average of 3 independent biological repeats. The translational profiles for the 5 min and 60 min time point represent data from one experiment.

(B) Shown is the change of total mRNA abundance for the same mRNAs encoding ribosomal proteins as in **(A)**. For each time-point, the normalized ratio of transcript level relative to control cells is shown. Each line represents one mRNA, which is colour-coded according to the amount of down-regulation: no strong regulation: yellow; strong down-regulation: blue. Data for the control and the 15 min time point represent the average of 3 independent biological repeats. Data for the 5 min and 60 min time points represent data from one experiment.

Conclusion

Polysome profiling combined with micorarray analysis of the mRNAs distributed over four fractions was used to study the translational response of fission yeast to environmental stress. The chosen stress conditions were oxidative stress, heat stress and DNA damage. Data analysis was performed using automated methods and visual inspection of the data to obtain a curated set of translationally regulated mRNAs. It has to be pointed out that this way of data analysis is rather crude and probably creates a conservative set of translationally regulated mRNAs. However, the verification of the translational regulation of several candidate mRNAs should help to improve analysis of translational profiling in fission yeast in the future and the availabilty of more repeats of the individual experiments will enable statistical tesing of the data sets.

Several mRNAs could be identified to be translationally regulated under oxidative or heat stress, whereas only one mRNA was found to be consistently translationally regulated after the exposure of cells to the DNA damage agent MMS. Whereas many translationally regulated mRNAs also showed changes in total mRNA levels under the stress conditions, several mRNAs showed a very rapid change in their translational profile without a concomitant change in total mRNA levels.

Furthermore, our data indicate that mRNAs encoding ribosomal proteins are concertedly regulated at the level of translation independent of their mRNA levels: a strong down-regulation at the level of translation could be seen after 60 min exposure to oxidative stress. In contrast to this translational down-regulation, many ribosomal mRNAs were translationally up-regulated after 15 min exposure to heat stress, despite a subtle but consistent down-regulation of this group of mRNAs in terms of total mRNA levels under this condition.

In summary, these data highlight that it is important to consider regulation of gene expression not only at the level of total mRNA abundance but to also include other layers of gene expression regulation to obtain a more comprehensive picture of altered gene expression patterns in response to changing environmental conditions.