GENOME-WIDE TRANSLATIONAL CONTROL IN FISSION YEAST

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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

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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 a basis, I then used translational profiling to assess the extent of translational regulation in cells in respose 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.

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Chapter 1

Introduction – Post-transcriptional gene expression regulation

Post-transcriptional gene expression regulation

This chapter gives an overview of post-transcriptional gene expression regulation, with a strong focus on regulation at the level of mRNA translation. Translation initiation mechanisms are described and examples of translational regulation introduced. Furthermore, novel microarray-based techniques are discussed, which allow the study of post-transcriptional gene expression regulation on a genome-wide scale.

An overview

The phenotype of an organism is largely determined by the sum of functional proteins in the cell, the sequence of which are encoded as genes in its DNA. The control of gene expression is a fundamental process to bring the genome to life and mis-regulation at any level is usually associated with disease. Today, it is well established that gene expression is regulated at various levels and there is increasing evidence that the diverse processes involved in this regulation are integrated with each other (Maniatis and Reed 2002; Orphanides and Reinberg 2002; Proudfoot et al. 2002; Hieronymus and Silver 2004; Mata et al. 2005; Moore 2005; McKee and Silver 2007). Gene expression regulation can be divided into 2 main categories of (1) transcriptional control and (2) post-transcriptional control (Figure 1.1). Furthermore, downstream of these 2 processes, expressed proteins can still be regulated by post-translational modifications and protein degradation (post-translational control).

Transcriptional control has received much attention, through both traditional single gene studies (Kadonaga 2004) as well as through genome-wide approaches such as expression profiling (Lockhart and Winzeler 2000; Bertone et al. 2005), transcription factor binding studies and identification of regulatory sequence elements (Hanlon and Lieb 2004; Sandelin et al. 2007), and chromatin remodelling and epigenetics (Bernstein et al. 2007; Kouzarides 2007; Li et al. 2007). Post-transcriptional control has been less extensively studied. This discrepancy is apparent, when searching for these 2 terms within the scientific literature: roughly 55,000 articles are found on PubMed (www.ncbi.nlm.nih.gov/sites/entrez?db=PubMed) for the search query "transcriptional regulation", whereas "post-transcriptional regulation" only returns roughly 5700 hits. This bias is partially based on historical as well as technical reasons: it is clear that transcription is one of the fundamental and intuitively

important steps within the cascade of gene expression regulation and techniques to study transcription and transcriptional control are well established in the scientific community.

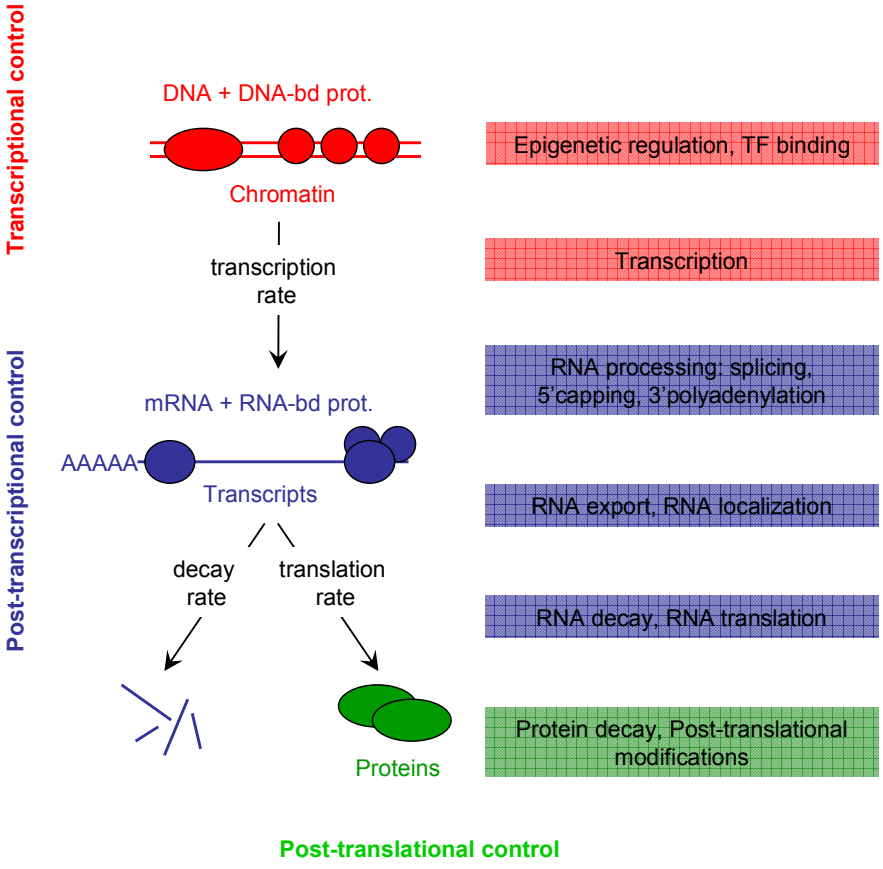


Figure 1.1 Layers of gene expression regulation
Shown is a schema of the various layers of gene expression regulation. The various regulatory processes are colour-coded according to their involvement in transcriptional control (red), post-transcriptional control (blue) or post-translational control (green). This figure is adapted from Mata et al. (2005).

However, recently there has been increasing appreciation of the necessity and importance of post-transcriptional gene expression regulation. Post-transcriptional regulation mechanisms comprise various processes such as mRNA processing (polyadenylation, capping and splicing), mRNA export and localization, mRNA decay, and mRNA translation (Figure 1.1). Despite this variety of regulatory mechanisms, there is one thing in common for all of them: they ultimately control if and where a given mRNA is translated into a protein. Consequently, translation and translational control are central to post-transcriptional gene expression regulation. Therefore, first translational initiation mechanisms and translational regulation will be

discussed in detail and then an overview of recent efforts to study post-transcriptional regulation on a genome-wide scale will be given.

Translational regulation

After transcription, before translation: RNA processing and export

Before an mRNA can be transported out of the nucleus into the cytoplasm in order to be available for the translational machinery, it has to undergo a series of processing steps: the mRNA acquires a cap structure at the 5' terminus, introns are spliced out from the pre-mRNA, and a specialized 3' end of the mRNA is generated, usually by polyadenylation. All these steps happen co-transcriptionally and can influence each other (Proudfoot et al. 2002). Only a brief overview of these processes will be given, especially as far as they are relevant to translational regulation, and some of the many reviews, which give a more detailed view of these specific RNA processing steps, will be pointed out.

The first processing step is the addition of the m^7G cap structure to the 5' end of the nascent mRNA and happens after 20-30 nucleotides have been synthesized (for reviews see Shatkin and Manley 2000; Gu and Lima 2005): In a three-step reaction, the GMP moiety from GTP is added to the first nucleotide of the pre-mRNA, and GMP is methylated at position N7. The m^7G cap is important for mRNA stability and translation (see below). In the nucleus, the m^7G cap gets bound by the cap binding complex (CBC), which consists of 2 subunits and after shuttling to the cytoplasm, it gets bound by translation initiation factor 4E, which is an essential step in translation initiation.

As the coding sequences (exons) of most mRNAs in higher eukaryotes are interrupted by introns, these introns must be spliced out of the pre-mRNA in order to generate a functional mRNA. Splicing requires consensus sequences on the mRNA, which mark the exon-intron boundaries, and the spliceosome, the catalytic complex which carries out the enzymatic reactions to remove the introns and ligate the flanking exons (for reviews see Kramer 1996; Collins and Guthrie 2000; Jurica and Moore 2003; Patel and Steitz 2003). The spliceosome consists of 5 small ribonucleoprotein particles (snRNPs: U1, U2, U4, U5 and U6), each of them made out of a small nuclear RNA (snRNA) and associated proteins, and many accesory proteins. In fact, well over a

hundred proteins are thought to be splicing factors (Jurica and Moore 2003). The catalysis of the splicing reaction itself is dependent on RNA-protein, RNA-RNA, and protein-protein interactions. Furthermore, the alternative use of exons (alternative splicing) can contribute to the creation of protein variety by allowing one gene to produce multiple isoforms (Matlin et al. 2005).

Most mRNAs also bear a specific structure in the form of a poly(A) tail at their 3' end. The only known protein-coding genes lacking poly(A) tails are histone mRNAs in most higher eukaryotes, but not in yeast (Fahrner et al. 1980). Polyadenylation is achieved in two steps: the nascent mRNA is cleaved at the site where polyadenylation is meant to begin, which is followed by poly(A) synthesis (for reviews see Zhao et al. 1999; Shatkin and Manley 2000; Proudfoot and O'Sullivan 2002). In analogy to splicing, formation of the poly(A) tail requires a multi-subunit polyadenylation complex and specific sequence-elements on the pre-mRNA. In mammalian cells, the site of cleavage lies mostly between an AAUAAA hexamer motif and a GU-rich downstream element (DSE) (McLauchlan et al. 1985). The AAUAAA hexamer is bound by the cleavage and polyadenylation specificity factor (CPSF), and the DSE interacts with the cleavage stimulatory factor (CstF). Cleavage factor I and II (CF I; CF II) are also required. Whereas both poly(A) polymerase (PAP) and CPSF are required for cleavage of the pre-mRNA and poly(A) addition, CstF is necessary for the endonucleolytic cleavage and – together with CPSF – for the recruitment of CF I and CF II (Takagaki et al. 1989; MacDonald et al. 1994; Murthy and Manley 1995). The principles of poly(A) tail formation are the same in yeast and mammalian cells and the protein complexes involved have orthologous components, but also specific accessory factors that are only found in one of the species (Shatkin and Manley 2000; Proudfoot and O'Sullivan 2002; Stevenson and Norbury 2006). Furthermore, in yeast, a variable A-rich element substitutes for the AAUAAA hexamer motif and there are 3 polyadenylation complexes: cleavage polyadenylation factor (CPF), which contains several factors homologous to CPSF and also the poly(A) polymerase, cleavage factor IA (CF IA) and cleavage factor IB (CF IB).

The emerging poly(A) tail is bound by the poly(A)-binding protein (PAPB). PABP is thought to influence the final length of the poly(A) tail on the one hand positively by stimulating the processivity of PAP, on the other hand negatively, by interacting with the poly(A) nuclease PAN (Mangus et al. 2003). Furthermore, PABPs are involved in nuclear export and are also important for the initiation of translation (see below). The

poly(A) tail is also crucial for several other post-transcriptional regulatory mechanisms in the cytoplasm and cytoplasmic polyadenylases can regulate the translational state and stability of various target mRNAs via modifying the length of the respective poly(A) tails (Read and Norbury 2002; Stevenson and Norbury 2006). The best studied example is probably that of translational regulation of maternal mRNAs in *Xenopus* oocytes, which are stock-piled in a translationally-repressed state with very short poly(A) tails, which become polyadenylated upon activation and as a consequence translated (see below and Mendez and Richter 2001; Richter 2007). mRNA decay by exonucleolytic mechanisms is also usually preceded by a shortening of the poly(A) tail (Wilusz et al. 2001; Parker and Song 2004), and recently deadenylation of poly(A) tails has also been shown to happen in microRNA (miRNA)-mediated expression regulation (Giraldez et al. 2006; Wu et al. 2006).

The last part in the journey from the site of transcription into the cytoplasm is the nuclear export of the mature mRNA. Export through the nuclear pore complex (NPC) happens in the context of messenger ribonuleoprotein particles (mRNPs) (for reviews see Daneholt 1997; Cole and Scarcelli 2006; Stewart 2007). mRNPs comprise the mRNA and associated RNA-binding proteins, which bind to the mRNA during the processing steps (Aguilera 2005; Moore 2005). Apart from the aforementioned CBC or PABP, such RNA-binding proteins include SR (serine/arginine rich) and hnRNP (heterogeneous nuclear RNP) proteins, or the exon junction complex (EJC), which is a set of proteins loaded onto the mRNA upstream of exon-exon junctions as a consequence of pre-mRNA splicing. These factors are important for the association of the mRNP with the NPC and the shuttling into the cytoplasm, and some of them stay associated with the mRNA as it is exported, whereas others are restricted to the nucleus. Furthermore, nuclear export is an important step in quality control, as faulty or un-processed mRNAs are not only useless, but potentially harmful, if translated in the cytoplasm. Only functional mRNAs are exported into the cytoplasm and this quality control step is closely coupled to RNA processing and the mRNP composition.

Again, it needs to be emphasized, that despite the introduction of mRNA transcription, capping, splicing, polyadenylation and nuclear export as sequential events, these events occur in the cell integrated with each other and not at all independently in spatial and temporal context (Proudfoot et al. 2002; Aguilera 2005; Moore 2005).

Molecular mechanism of translation initiation in eukaryotes

Translation can be divided in 3 major steps: initiation, elongation and termination. Translation initiation comprises the summary of events that lead to the positioning of an elongation-competent 80S ribosome over the AUG start codon of the mRNA. Polypeptide synthesis takes place during the elongation phase. The completed polypeptide is released after the ribosome encounters a stop codon during translation termination.

Much evidence points toward translation initiation being the rate limiting step in the process of translating an mRNA into a protein. When cells are treated with low doses of elongation inhibitors such as cycloheximide in a way that total protein synthesis is only minimally affected, most mRNAs are found to be resistant to low levels of elongation inhibitors and their translational efficiency is basically not altered (Lodish and Jacobsen 1972; Walden et al. 1981; Mathews et al. 2007). Furthermore, the average density of ribosomes along the mRNA is significantly lower than the maximum packing capacity of one ribosome per 30-40 nucleotides (Wolin and Walter 1988; Arava et al. 2003; Mathews et al. 2007). This maximum capacity can be obtained by treating mRNAs with drugs that slow down elongation. The complexity and importance of translation initiation compared to elongation and termination is further underscored by the fact that only few dedicated factors are needed for the latter two processes, whereas more than 25 proteins are needed to ensure proper translational initiation (Preiss and Hentze 2003; Pestova et al. 2007). Therefore, it is not surprising that most translational regulation is executed at the level of initiation (Preiss and Hentze 2003; Gebauer and Hentze 2004; Holcik and Sonenberg 2005; Mathews et al. 2007). An overview of the molecular mechanisms of translation initiation will be introduced here, as far as they are directly relevant to the regulation of translation and the examples presented below. For a more detailed view of the molecular events regulating translation initiation in mammalian cells and yeast, see references (Hinnebusch et al. 2007; Pestova et al. 2007).

43S pre-initiation complex formation. Translation initiation starts with the formation of the 43S pre-initiation complex (Figure 1.2). As physiological conditions favour the association of 40S ribosomal subunits and 60S ribosomal subunits to form 80S subunits (i.e. full ribosomes) but only free ribosomal subunits can initiate

translation, it is important that post-termination ribosomes dissociate (Pestova et al. 2001; Preiss and Hentze 2003). In prokaryotes this dissociation is achieved through a ribosome-recycling factor, but there is no known eukaryotic equivalent (Kisselev and Buckingham 2000). The eukaryotic initiation factors (eIFs) eIF3, eIF1 and eIF1A are thought to promote this dissociation in eukaryotes, but the mechanism for it is unknown and recent data suggest that the activity of these factors is not sufficient to prevent formation of 80S subunits (Preiss and Hentze 2003; Pestova et al. 2007), and it is thought that dissociation of empty 80S subunits is directly linked to 43S pre-initiation complex formation (Pestova et al. 2007).

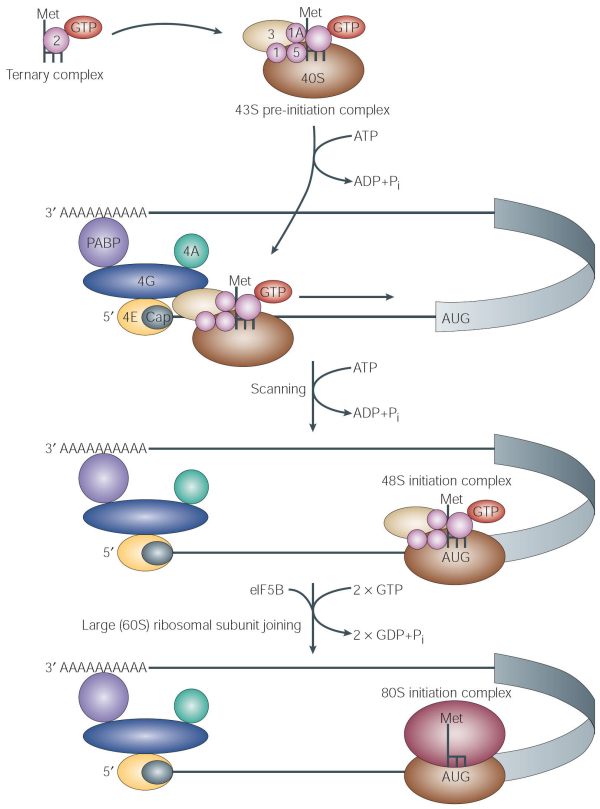


Figure 1.2 Molecular mechanisms of translation inititation
Shown are the major molecular events that lead to cap-dependent translation inititation. For a detailed description see main text. This figure is taken from Gebauer et al. (2004).

The first step in 43S pre-initiation complex formation is the formation of the ternary complex (Figure 1.3, Figure 1.2). The ternary complex consists of eIF2, a hetero-trimer of α, β and γ subunit, methionyl-initiator tRNA (Met-$tRNA_i^{Met}$) and GTP, and its assembly is regulated by the guanine nucleotide exchange factor (GEF) eIF2B (Figure 1.3): GTP is hydrolyzed after recognition of the AUG start codon producing

eIF2 bound to GDP, which has a 10-fold reduced affinity for Met-tRNA$_i^{Met}$ (Hinnebusch et al. 2007). eIF2B promotes the GDP-GTP exchange to re-generate active eIF2 (Figure 1.3) (Preiss and Hentze 2003; Hinnebusch et al. 2007; Pestova et al. 2007). Binding of the active ternary complex to the 40S ribosomal subunit is aided independently by eIF1, eIF1A and eIF3 in mammalian cells (Preiss and Hentze 2003; Pestova et al. 2007). In budding yeast eIF1, eIF3, eIF5 and the ternary complex can be isolated as a multifactor complex (MFC), which raises the possibility that this MFC is recruited to the 40S subunit as pre-formed unit (Hinnebusch et al. 2007). The 43S pre-initiation complex is then ready to bind to the 5' end of the mRNA.

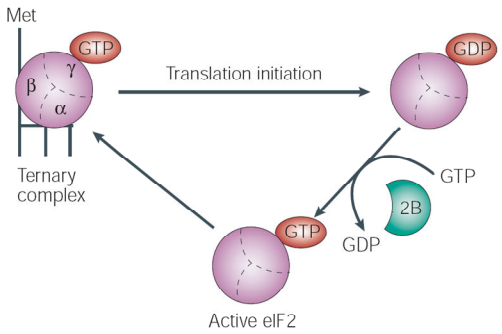


Figure 1.3 Formation of avtive ternary complex
The ternary complex consists of eIF2, a hetero-trimer of α, β and γ subunit, methionyl-initiator tRNA (Met-tRNA$_i^{Met}$) and GTP, and its assembly is regulated by the guanine nucleotide exchange factor (GEF) eIF2B: GTP is hydrolyzed after recognition of the AUG start codon producing eIF2 bound to GDP, which has a 10-fold reduced affinity for Met-tRNA$_i^{Met}$. eIF2B promotes the GDP-GTP exchange to re-generate active eIF2. This figure is taken from Gebauer et al (2004).

Recruitment of the pre-initiation complex to the mRNA. Recognition of the m^{7}G cap structure at the 5' end of the mRNA is mediated by the cap-binding complex eIF4F, which comprises the 3 subunits eIF4E, eIF4G and eIF4A (Figure 1.2). eIF4E binds directly to the m^{7}G cap structure; eIF4A is a dead-box RNA helicase that is thought to unwind secondary structures in the 5' UTR (Un-Translated Region) so that the 43S complex can scan along the mRNA; and eIF4G is thought to act as scaffold protein (Preiss and Hentze 2003; Hinnebusch et al. 2007; Pestova et al. 2007). In mammalian cells, eIF3 from the pre-initiation complex interacts with the central domain of eIF4G (Lamphear et al. 1995). This interaction has not yet been found in budding yeast, where eIF4A is also not stably associated with eIF4E and eIF4G (Goyer et al. 1989; Hinnebusch et al. 2007). Altogether, the binding of the pre-

initiation complex to the mRNA involves the cooperative activities of eIF4F, eIF3, eIF4B and possibly the poly(A)-binding protein (PABP). PABP was initially identified as a protein that associates with the poly(A) tail at the 3' UTR of the mRNA. The concerted binding of PABP and eIF4E to eIF4G is thought to pseudo-circularize the mRNA (Figure 1.2) (Wells et al. 1998). Furthermore, in budding yeast poly(A)-binding protein PAB1 is essential for translation initiation (Sachs 2000). This circularization provides a possible framework by which 3' UTR-binding proteins can regulate translation initiation, as most known regulatory sequences are found in the 3' UTR, despite the fact that translation starts at 5' end of the mRNA (Gebauer and Hentze 2004).

Scanning of the mRNA and AUG recognition. After proper assembly at the 5' end of the mRNA, the pre-initiation complex needs to scan along the mRNA until the recognition of the AUG start codon (Kozak 1989; Kozak 2002). The model of scanning had originally been proposed by Kozak, and despite the fact that most biochemical and genetic data are consistent with the model (Kozak 1999), direct physical intermediates of the scanning process have not been identified to date. The 43S pre-initiation complex can bind to an mRNA having an unstructured 5' UTR independent of eIF4F, eIF4A and ATP, but needs eIF1 or eIF4G to scan to the start codon. However, an mRNA with a structured 5' UTR additionally requires eIF4F, eIF4B, ATP and eIF1A (Pestova et al. 1998; Pestova and Kolupaeva 2002). eIF4A helicase and eIF4F are thought to promote unwinding of the secondary structure of the mRNA, while eIF1 and eIF1A are thought to promote a structural conformation of the 43S pre-initiation complex, which allows scanning in 5'-3' direction.

Ready to go: formation of the translation competent 80S subunit. The 43S pre-initiation complex recognizes the start codon through formation of base-pairs between the anticodon loop of the initiator tRNA and the AUG start codon (Figure 1.2). This stable complex is referred to as 48S initiation complex. Selection of the correct start codon is dependent on eIF1 (Pestova et al. 1998; Pestova and Kolupaeva 2002). Then, several events take place in order for the 60S subunit to join the 48S complex and form the 80S subunit. eIF5 catalyzes the hydrolysis of eIF2-GTP, and as a consequence most of the initiation factors including eIF2-GDP disassociate from the small ribosomal subunit, leaving the initiator tRNA bound to the start codon

(Hinnebusch et al. 2007). Recently, it has been found that a second step of GTP hydrolysis is necessary for 60S joining and to render the resulting 80S subunit competent for polypeptide synthesis: GTPase activity of eIF5B is stimulated by 60S subunit and even stronger by 80S subunits. GTP-bound eIF5B stimulates 60S subunit joining and GTP hydrolysis occurs after 80S subunit formation has happened and is essential for the release of eIF5B (Pestova et al. 2000; Lee et al. 2002; Shin et al. 2002). Taken toghether, 2 steps of GTP-hydrolysis are required for 80S complex formation and also provide a checkpoint for proper start codon recognition.

Cap-independent translation initiation. The events of translational initiation described above are an account of cap-dependent translational initiation, which usually occurs for most cellular mRNAs. However, an alternative way of initiating translation can happen in a cap-independent way through internal ribosomal entry sites (IRES). IRES are heavily structured sequence elements in the 5' UTR of the mRNA with no obvious conserved consensus sequence (Baird et al. 2006). The structured IRES segment in the 5' UTR of the mRNA has an active role in the recruitment of the 40S subunit. IRES elements are found in viral mRNAs and also certain cellular mRNAs, which are involved in growth control, differentiation, apoptosis or oncogenesis (Doudna and Sarnow 2007; Elroy-Stein and Merrick 2007). These mRNAs are usually only weakly translated under normal conditions, but can be more efficiently translated upon down-regulation of cap-dependent translation. For further in-depth reviews on the topic of IRES see references (Hellen and Sarnow 2001; Stoneley and Willis 2004; Jackson 2005; Spriggs et al. 2005; Fraser and Doudna 2007).
For a detailed view of translation elongation and translation termination see references (Ehrenberg et al. 2007; Taylor et al. 2007).

Why translational regulation?

Why do cells need translational regulation and how do they benefit from it? There are several possible answers to this question, which are also addressed in reference (Mathews et al. 2007): first, regulation at the translational level can happen as a quick response without the necessity of going through all the upstream processes of gene expression such as transcription, mRNA processing or mRNA export. Furthermore,

translational regulation is usually reversible, as it is often mediated through reversible protein modifications such as the phosphorylation of initiation factors. The need for translational control is also apparent for systems, where transcriptional control is not possible. Examples for such systems are reticulocytes, which lack a nucleus, oocytes or RNA viruses. Another reason for the regulation of translation is spatial control of gene expression within the cell (St Johnston 2005; Schuman et al. 2006). The requirement for localized protein production in neurons or during development can only be met by translational regulation, as regulation of transcription is restricted to the nucleus of the cell. Another reason, which makes translational regulation a good option for the cell to regulate gene expression, is its flexibility. As can be seen by the complex mechanisms of translation initiation outlined above, there are many molecular targets for translational regulation, which consequently can change translational efficiencies for many or only a few mRNAs. A last but important point, why cells regulate translation, is fine tuning of gene expression, as there are numerous examples of genes that are regulated at both the transcriptional and translational level (e.g. GADD45α or TNF-α; Saklatvala et al. 2003; Lal et al. 2006).

Targets for translational regulation: initiation factors, mRNA and the ribosome

Translational control can in principle be divided into global regulation of translation and mRNA-specific translational regulation (Gebauer and Hentze 2004). Global regulation affects the translational efficiency of most mRNAs through a general switch-on or switch-off of translation. mRNA-specific regulation only affects the translation of a subset of mRNA. However, in some cases, this simple distinction cannot be made; for example, the general down-regulation of cap-dependent translation enhances translation of a subset of IRES-bearing mRNAs (see above).

What are the targets for translational control at the initiation step and what are the basic priciples? A simple answer to this question would be that most translational regulation prohibits or allows the association of the mRNA with the translational apparatus. Given the plethora of translation initiation factors, it is not surprising that many of them are targets for translational regulation and many have been shown to be modified post-translationally, which affects translational efficiency (Dever 2002; Raught and Gingras 2007). A key target in many regulatory mechanisms is the cap-

binding protein eIF4E that can be bound by inhibitory proteins, which subsequently hinders binding of the mRNA (see below for more details). Global regulation of translation is generally mediated through such modifications of translation initiation factors.

Another target for translational regulation is the mRNA itself, through cis-regulatory elements, which can be bound by trans-acting factors. The cis-regulatory elements on the mRNA could be found anywhere along the mRNA, but for most well characterized examples of translational regulation these elements are found in the 3' UTR or 5' UTR (Figure 1.4). mRNA-specific translational regulation happens mostly via regulatory proteins, that bind to the cis-regulatory elements of a given mRNA.

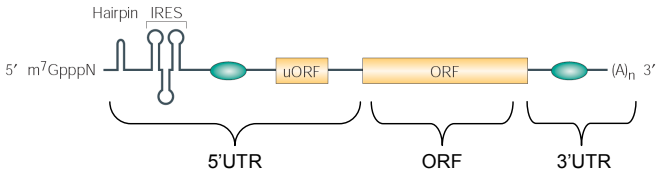


Figure 1.4 Cis-acting sequence elements that influence translation initiation of specific mRNAs
The m^7G cap structure at the 5' end and the poly(A) tail at the 3' end of the mRNA are both essential elements for cap-dependent translation initiation. Additionally, specific sequence elements in the 5' or 3' UTR (green ovals) can influence translation initiation in combination with bound trans-acting factors. Structured elements such as hairpins can inhibit translation initiation and structured internal ribosomal entry sites (IRES) can mediate cap-independent translation initiation. Upstream open reading frames (uORFs) usually inhibit translation initiation for the downstream start codon. This figure is taken from Gebauer et al. (2004).

The ribosome itself can also be targeted to exert translational regulation and several of its protein constituents can undergo post-translational modifications; a well studied example is the phosphorylation of ribosomal protein S6 (RPS6) by ribosomal S6 kinase (S6K), which was first shown more than 30 years ago (Gressner and Wool 1974). A correlation of RPS6 phosphorylation with an increase in translation initiation, especially of mRNAs posessing a 5'-terminal oligopyrimidine sequence (TOP mRNAs), prompted the hypothesis that translation of TOP mRNAs is actually regulated through this phosphorylation (Jefferies et al. 1994). However, recent data contradict this model and a simple causal relationship between S6 phosphorylation and translational efficiency: a double knock-out of both S6K homologues in mouse cells (Pende et al. 2004) or a knock-in of un-phosphorylatable RPS6 (Ruvinsky et al. 2005) does not affect translational regulation of TOP mRNAs. The elucidation of the

exact mechanism of RPS6 phosphorylation on translation is further aggravated by the discovery of various alternative substrates of S6K, which also include factors involved in translational initiation (Ruvinsky and Meyuhas 2006). Ribosomal proteins can also be modified through ubiquitination (Spence et al. 2000) or methylation (Bachand and Silver 2004; Swiercz et al. 2005).

In budding yeast, due to an ancient duplication event (Kellis et al. 2004), most genes encoding ribosomal proteins are duplicated. The open reading frame (ORF) and the protein sequence of the paralogues are very similar, but the UTRs and intron sequences can differ. Ribsomal gene pairs were generally considered to be functionally equivalent. However, recent genome-wide screens for genes required for various cellular processes such as telomere length homeostasis (Askree et al. 2004), centromeric cohesion (Marston et al. 2004), or for genes that exhibit deleterious haploinsufficient interactions with actin (Haarer et al. 2007), identified in several cases a specific effect for only one of the paralogues of the ribosomal protein, whereas deletion of the other paralogue would not affect the studied biological process. To date, the biological reason for this specialization is not clear. One possibility could be that specific ribosomal proteins are involved in cellular processes other than translation. Another intriguing hypothesis is heterogeneity of ribosomes: the cell could construct various kinds of ribosomes, which differ in terms of paralogue composition and post-translational modifications, and "specialized" ribosomes could play a role in the regulation of translation of specific subsets of mRNAs.

Classic examples of translational regulation

Translational regulation is crucial for diverse physiological processes. It is involved in the response to cellular stress (Holcik and Sonenberg 2005), in the mis-regulation of gene expression during cancer (Schneider and Sonenberg 2007), in apoptosis (Morley and Coldwell 2007), during development (Thompson et al. 2007), or in the establishment of synaptic plasticity and consequently in learning and memory (Klann and Richter 2007). Many examples of translational regulation have been reported within and also outside these areas. Instead of giving a broad overview of these regulatory mechanisms, I will focus below on several well-studied examples, for which the underlying molecular mechanisms have been reasonably well identified. Most of the regulatory mechanisms presented here - such as the regulation of ternary

complex formation, the regulation of translation via eIF4E-binding proteins, or the post-transcriptional regulation via ARE-elements - are probably conserved for most eukaryotes, despite the fact that these processes have mostly been studied in budding yeast and mammlian cells. Other regulatory mechanisms – such as the translational regulation of gene expression in *Drosophila* or *Xenopus* development – are probably specialized mechanisms to meet the specific requirements of gene expression regulation in the corresponding organism. However, the underlying principles for these regulatory mechanisms can be found in diverse variations in many eukaryotic cells.

Regulation of ternary complex formation. Exposure of cells to stress conditions (e.g. oxidative stress, nutrient limitation, hypoxia, temperature stress) results often, if not always, in a global down-regulation of translation (Holcik and Sonenberg 2005). One of the best studied examples of how this down-regulation is achieved, is regulation of the availability of active ternary complexes (Figure 1.5). Binding of the Met-tRNA$_i^{Met}$ to the 40S subunit through the ternary complex is an essential step in translation inititation, as described above (Figure 1.2; Figure 1.3). After the exposure to stress, the α-subunit of eIF2 (eIF2α) is phosphorylated and thereby inhibits the exchange of GDP for GTP by eIF2B and as a consequence formation of active ternary complexes is strongly reduced, and translation is down-regulated globally (Dever et al. 1992; Gebauer and Hentze 2004; Holcik and Sonenberg 2005; Ron and Harding 2007). The molecular mechanism for this inhibition is based on the fact that phosphorylated eIF2α-GDP turns into a competitive inhibitor of eIF2B, as eIF2B has a much higher affinity towards phosphorylated eIF2α-GDP than towards un-phosphorylated eIF2α-GDP (Rowlands et al. 1988). There are at least 4 kinases that have been identified to phosphorylate eIF2α at Ser51 in the response to various stresses (Figure 1.4; Dever et al. 2007): the haem-regulated inhibitor (HRI) is induced by haem depletion; general control non-depressible 2 (GCN2) is mainly activated by amino acid starvation; protein kinase activated by double-stranded RNA (PKR) is stimulated in response to viral infection; PKR-like endoplasmic reticulum kinase (PERK) is activated during endoplasmatic reticulum (ER) stress and the unfolded protein response (UPR).

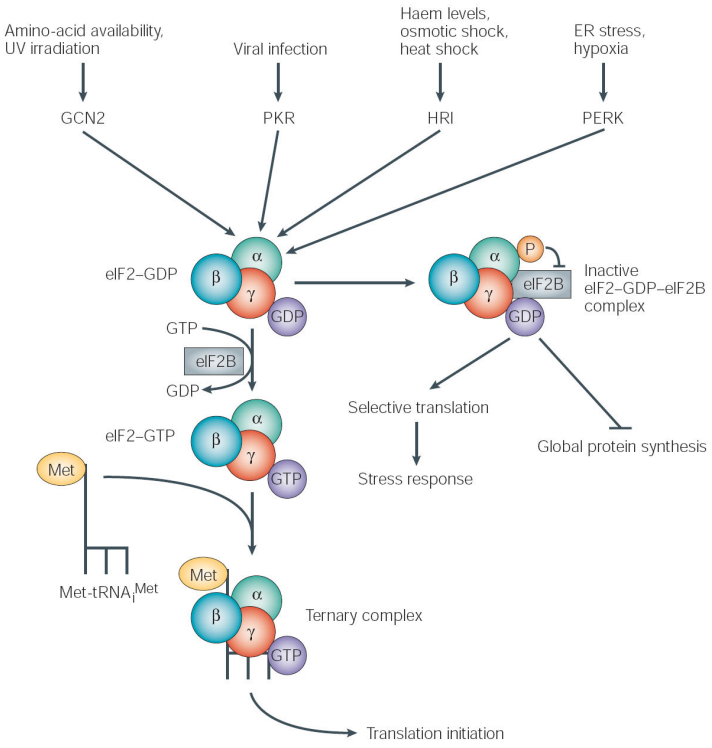


Figure 1.5 Inhibition of global protein synthesis in response to various stress stimuli through phosphorylation of eukaryotic initiation factor-2α
Several protein kinases (GCN2, PKR, HRI, or PERK) can phosphorylate the α-subunit of eIF2 in response to a variety of stress conditions. This phosphorylation inhibits the necessary GTP-GDP exchange on eIF2 by reducing the dissociation rate of the guanine nucleotide exchange factor eIF2B and active ternary complex formation is inhibited. As a consequence, translation initiation and global translation is down-regulated. This figure is taken from Holcik et al. (2005).

Regulation through uORFs. Interestingly, whereas translation of most mRNAs is down-regulated by eIF2α phosphorylation, translation of several specific mRNAs can be up-regulated in response to reduced availability of ternary complex. In response to various starvation conditions and amino acid deprivation in budding yeast, Gcn2p kinase is up-regulated through a mechanism that recognizes lack of amino acids; this is mediated through binding of un-charged tRNAs to the kinase (Dong et al. 2000). Ternary complex formation and global translation are down-regulated as a consequence. However, Gcn4p, a master transcriptional regulator, which activates transcription of amino acid-biosynthesis genes, is translationally up-regulated under these conditions (Hinnebusch and Natarajan 2002). This up-regulation is achieved by regulatory upstream open reading frames (uORFs). Four of these uORFs can be found in the 5' UTR of the *GCN4* mRNA (Hinnebusch and Natarajan 2002; Hinnebusch

2005): In optimal growth conditions and availability of ternary complex, translation usually starts at uORF1 and ribosomes can resume scanning afterwards to resume translation at uORF2, uORF3 and uORF4 (Figure 1.6). However, ribosomes cannot re-initiate translation after termination at these latter uORFs and as a consequence, *GCN4* mRNA is not translated. After eIF2α phosphorylation, when ternary complexes become limited, ribosomes are more likely to resume scanning without re-initiating at the downstream uORFs and translation is initiated at the actual start codon of *GCN4* (Figure 1.6). The response to amino acid starvation via the GCN2 kinase seems to be an evolutionarily conserved mechanism, as was shown by two recent reports that GCN2 activity in the mouse brain is essential for the restriction of intake of diets lacking essential amino acids (Hao et al. 2005; Maurin et al. 2005): in these studies it was show that the GCN2 pathway is used to recognize depressions in serum amino acid levels that occur during consumption of food with an imbalanced composition of amino acids, which results in a behavioral response that limits the consumption of imbalanced foods and favours the intake of a balanced diet

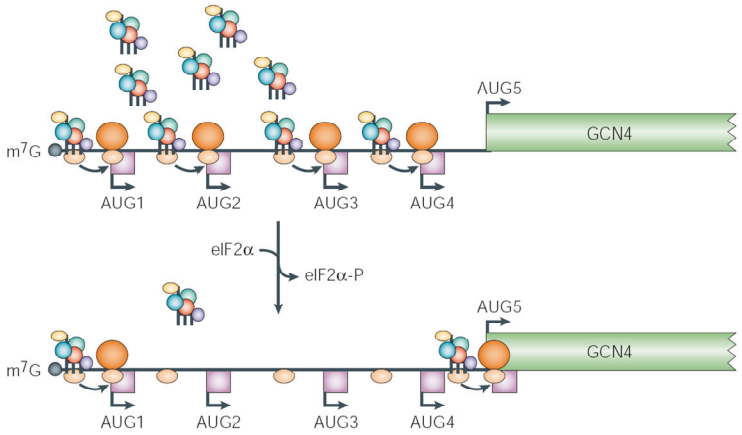


Figure 1.6 Translational regulation of *GCN4* by upstream open reading frames (uORFs) With low levels of eIF2α-phosphorylation and abundant active ternary complex, ribosomes initiate translation at uORF1, resume scanning, and re-initiate translation at uORF2, uORF3 or uORF4. However, they do not resume scanning to re-initiate translation at the start codon of *GCN4*. When cells are starved for amino acids, eIF2α is phosphorylated and as a consequence the number of active ternary complexes is down-regulated. In these conditions, re-initiation at uORF2-uORF4 happens less frequently and scanning can resume to the actual start codon of *GCN4*, which is then translated. This figure is taken from Holcik et al. (2005).

In fission yeast eIF2α phosphorylation has also been reported to be mediated by the kinases Gcn2p, Hri1p and Hri1p (Zhan et al. 2002; Dunand-Sauthier et al. 2005). However, no homologue of Gcn4p exists in fission yeast.

The mammalian transcription factor ATF4 is regulated in a similar way in response to ER stress or amino acid starvation by uORFs (Harding et al. 2000; Scheuner et al. 2001), and there is evidence that GCN2 also regulates synaptic plasticity through modulation of ATF4 translation (Costa-Mattioli et al. 2005 and references therein).

There are numerous other examples of mRNAs whose translation is regulated by uORFs (Dever 2002). Recent genome-wide bioinformatics approaches in yeast and mammals suggest that the occurrence of functional uORFs is widespread and might be a common regulatory mechanism of translation (Iacono et al. 2005; Cvijovic et al. 2007).

Regulation by eIF4E inhibitory proteins. An important step during translation initiation is the binding of the m^7G cap by eIF4F (Figure 1.2). The backbone of this complex is eIF4G, which interacts with the cap-binding complex eIF4E and the helicase eIF4A. Translational initiation can be regulated by the disruption of eIF4E-eIF4G binding through inhibitory proteins, which were originally called 4E-BP (for 4E binding proteins) (Richter and Sonenberg 2005). These inhibitory proteins have been reported to control a variety of biological processes such as development, cell growth, and may repress tumour formation (Richter and Sonenberg 2005). 4E-BPs compete with eIF4G for the binding to eIF4E, and the binding affinity is regulated through phosphorylation of 4E-BPs (Gingras et al. 1999): in the hypo-phosphorylated state, 4E-BPs bind to eIF4E and prevent translation initiation; in the hyper-phosphorylated state, 4E-BPs binding to eIF4E is blocked.

In addition to 4E-BPs, several other proteins can bind eIF4E in an mRNA-specific manner and inhibit translation initiation. The mRNA specificity for these proteins comes through interactions with sequence-specific elements within the mRNA or through the interaction with RNA-binding proteins.

In *Xenopus* oocytes, many mRNAs remain dormant with short 3' poly(A) tails. When the oocytes are stimulated, these mRNAs become polyadenylated and translationally active. A cytoplasmic polyadenylation element (CPE) in the 3' UTR of the mRNA is important for both masking and translational activation of the mRNA and is bound by

the cytoplasmic polyadenylation element binding protein (CPEB) (Mendez and Richter 2001; Richter 2007). When dormant, CPEB is bound by Maskin, which inhibts the binding between eIF4E and eIF4G (Figure 1.7), acting as a mRNA specific 4E-BP (Cao and Richter 2002). After stimulation of the oocyte to complete meiosis, CPEB stimulates polyadenylation of the mRNA; the poly(A) tail is then bound by PABP, which then can bind eIF4G and displace Maskin (Figure 1.7; Cao and Richter 2002). During translational repression, the CPEB-containing complex also inculdes PARN, a poly(A)-specific ribonuclease, which overrides the polyadenylating activity of the poly(A) polymerase GLD2, which contributes to the short poly(A) tail of target mRNAs during translational repression (Kim and Richter 2006).

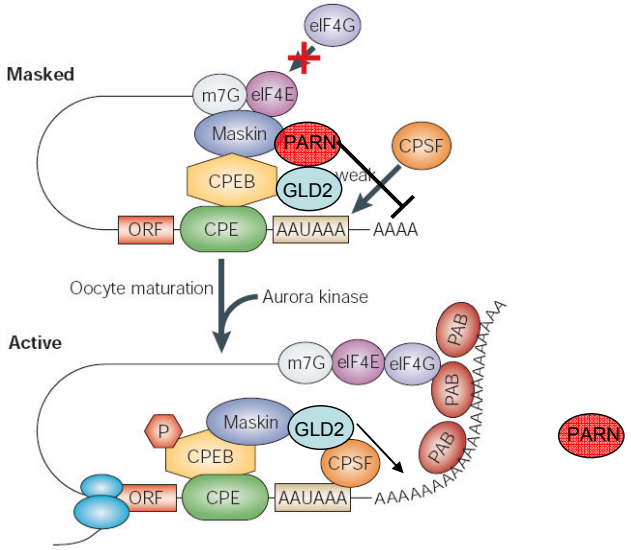


Figure 1.7 Regulation of translation by the cytoplasmic polyadenylation element (CPE) mRNAs that bear a CPE in their 3' UTR are translationally repressed in developing oocytes by the binding of the cytoplasmic polyadenylation element binding protein (CPEB) and Maskin. Maskin interacts directly with the cap-binding protein eIF4E and prevents its association with eIF4G, which is crucial for translation initiation. The short poly(A) tail is maintained by blocking access of cleavage and polyadenylation specificity factor (CPSF) for the AAUAAA sequence and by the poly(A)-specific ribonuclease PARN, which counteracts the polyadenylating activity of GLD2. Induction of oocyte maturation results in phosphorylation of CPEB. Consequently, Maskin and PARN dissociate from the complex and CPSF binds to the AAUAAA sequence. Binding of CPSF activates the poly(A) polymerase GLD2 that extends the poly(A) tail. These events lead to successful translation initiation and translation of the previously translationally repressed mRNA. This figure is adapted from Kuersten et al. (2003).

Another example of an mRNA-specific 4E-BPs is the homeodomain transcription factor Bicoid, which apart from its activity as transcription factor inhibits translation of Caudal mRNA in *Drosophila* (Dubnau and Struhl 1996; Rivera-Pomar et al. 1996). Similar to Maskin, Bicoid has an eIF4E-binding motif, and it was initially thought that Bicoid directly binds to eIF4E (Niessing et al. 2002). However, recent work

showed that Bicoid interacts with d4EHP (Drosophila 4E-homologous protein), an eIF4E-like protein that can interact with the m^7G cap but not with eIF4G (Cho et al. 2005).

Recent studies have also identified Cup as a translational regulator in *Drosophila*, which interacts with eIF4E and prevents eIF4F complex formation and translational initiation (Wilhelm et al. 2003; Nakamura et al. 2004; Nelson et al. 2004). Nanos and Oskar are examples of mRNAs regulated by Cup.

Other mechanisms of mRNA-specific translation regulation. AU-rich elements (AREs) are present in the 3' UTR of many mRNAs and are potent sequence elements for post-transcriptional regulation of gene expression. AREs influence the stability or translation of a given mRNA usually through binding of ARE-specific RNA-binding proteins (Barreau et al. 2005). AUF1 was the first ARE-binding protein to be identified and was shown to exist in 4 isoforms (Wilson et al. 1999). Binding of ARE-binding proteins of the AUF1 family to AREs have been shown to promote degradation of mRNAs encoding cytokines (IL-3, GM-CSF) or cell cycle regulators ($p16I^{NK4a}$, $p21^{WAF1/CIP1}$, cyclin D1) (Lal et al. 2004; Raineri et al. 2004; Wang et al. 2005), and AUF1 has been shown to interact with the heat shock proteins hsc70-hsp70, eIF4G and PABP (Laroia et al. 2002). Despite its role in promoting mRNA decay, recent work showed that AUF1 can induce translation of MYC proto-oncogene mRNA (Liao et al. 2007): down-regulation of AUF1 abundance by RNA-interference (RNAi) *in vivo* did not result in altered MYC mRNA levels, as expected based on earlier *in vitro* studies (Brewer 1991), but significantly reduced MYC mRNA translation. In contrast, TIAR, another ARE-binding protein, was shown to suppress translation of MYC mRNA. Despite competitive binding of AUF1 and TIAR to the MYC ARE, translational up-regulation through AUF1 was not simply achieved by suppression of TIAR binding, as shown in double knockdown experiments (Liao et al. 2007).

Repression of translation through the ARE-binding protein TIAR has been shown for several mRNAs such as GADD45α (Lal et al. 2006), the translation initiation factors eIF4A and eIF4E, especially in response to UV radiation (Mazan-Mamczarz et al. 2006), and TNFα (Gueydan et al. 1999).

To date, many more ARE-binding proteins have been identified (e.g. HuR (Myer et al. 1997); TTP (Carballo et al. 1998); or KSRP (Gherzi et al. 2004)), and it is well recognized that AREs in conjunction with their ARE-binding proteins can influence gene expression through the modulation of mRNA turnover and translation. However, despite the identification of a large number of ARE bearing mRNAs and ARE-binding proteins, the full complexity of this regulatory mechanism is far from understood.

Multistep mechanisms of translational regulation. As is already evident from some of the examples given above, translational regulation can also be exerted as a multistep mechanism, which means that more than one mechanism is used to ensure tight translational regulation for critical proteins, whose mis-expression would be deleterious for the cell. One good example for this kind of control is the translational regulation of male-specific-lethal (*msl-2*) mRNA in *Drosophila*. Expression of MSL-2 in females causes inappropriate assembly of dosage compensation regulators on the X chromosomes and female lethality in *Drosophila* (Kelley et al. 1995). MSL-2 expression is inhibited by Sex-lethal (SXL), a female specific RNA binding protein, which also regulates sex determination via alternative splicing (Forch and Valcarcel 2003). First, SXL promotes retention of a facultative intron in the 5' UTR of *msl-2* and then represses its translation (Bashaw and Baker 1997; Kelley et al. 1997; Gebauer et al. 1998). SXL binds to sites in the 3' UTR and the intronic 5' UTR of *msl-2* (Figure 1.8) and represses translation in a dual way: SXL bound to the 3' UTR inhibits recruitment of the 43S pre-initiation complex, and SXL bound to the 5' UTR can inhibit scanning of the 43S pre-initiation complex, in case of escape from the first inhibitory mechanism (Beckmann et al. 2005). Furthermore, to exert its function via the 3' UTR, SXL requires the RNA-binding protein UNR (upstream of N-*ras*) as corepressor (Grskovic et al. 2003; Abaza et al. 2006; Duncan et al. 2006).

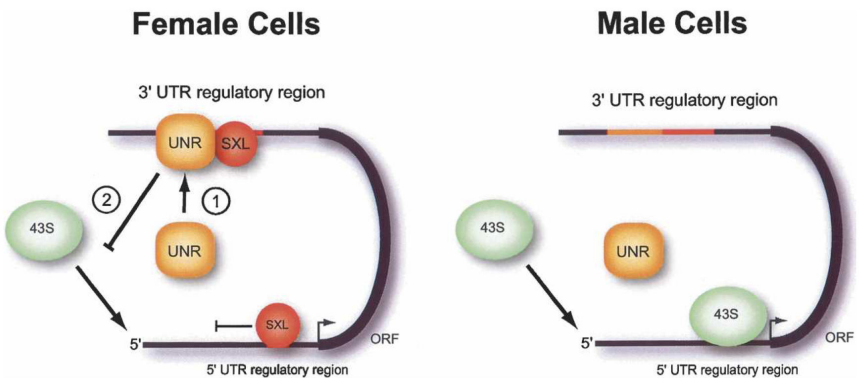


Figure 1.8 Translational regulation of male-specific-lethal (*msl-2*) mRNA in *Drosophila melanogaster* through a multi-step mechanism
msl-2 translation is inhibited by Sex-lethal (SXL), a female specific RNA binding protein. First, SXL promotes retention of a facultative intron in the 5' UTR of *msl-2* and then represses its translation. SXL binds to sites in the 3' UTR and the intronic 5' UTR of *msl-2* and represses translation in a dual way: SXL bound to the 3' UTR inhibits recruitment of the 43S pre-initiation complex, and SXL bound to the 5' UTR can inhibit scanning of the 43S pre-initiation complex, in the case that it escaped the first inhibitory mechanism. Furthermore, to exert its function via the 3' UTR, SXL requires the RNA-binding protein UNR (upstream of N-*ras*) as corepressor. In male cells, *msl-2* translation can be initiated, as SXL is not expressed. This figure is taken from Duncan et al. (2006).

Novel concepts in translational control: P-bodies and microRNAs

In the past few years, two new ways to modulate mRNA fate at the post-transcriptional level have attracted a great deal of attention. One is the discovery of cytoplasmic processing bodies (P-bodies), initially described as foci within the cell with a high concentration of mRNA decay enzymes (Bashkirov et al. 1997; Ingelfinger et al. 2002; Lykke-Andersen 2002; van Dijk et al. 2002; Sheth and Parker 2003; Cougot et al. 2004). The other discovery is that of small RNAs, which can regulate stability and translation of target mRNAs (Bartel 2004; Filipowicz 2005; Valencia-Sanchez et al. 2006). Interestingly, recent work suggests that there is also a connection between P-bodies and microRNA (miRNA)-mediated gene silencing (Liu et al. 2005; Liu et al. 2005; Sen and Blau 2005). These novel concepts will be introduced here, with a focus on their involvement in translational regulation.

P-bodies and polysomes. P-bodies were first visualized by various groups using microscopy of factors involved in mRNA decay and accessory factors such as DCP1, DCP2, XRN1 and LSM (Bashkirov et al. 1997; Ingelfinger et al. 2002; Lykke-Andersen 2002; van Dijk et al. 2002; Sheth and Parker 2003; Cougot et al. 2004). In

mammalian cells, GW182 protein is another marker of P-bodies and they are therefore sometimes also referred to as GW bodies (Eystathioy et al. 2002; Eystathioy et al. 2003).

mRNA decay in eukaryotes can be controlled in different ways via endonuleolytic or exonucleolytic pathways (for reviews see Wilusz et al. 2001; Parker and Song 2004). Exonucleolytic degradation is usually initiated by deadenylation of the poly(A) tail of the mRNA. Transcripts will then be degraded from their 5′ ends by the exonuclease XRN1, following removal of the 5' cap (decapping). Alternatively, the exosome complex can degrade transcripts from their 3′ ends before decapping.

P-bodies are probably a site of mRNA decay, as intermediates in the 5'-3' degradation pathway can be found localized to P-bodies (Sheth and Parker 2003). Furthermore, mutations in the decapping enzymes (DCP1, DCP2) or in the 5'-3' exonuclease XRN1 increase the size and number of P-bodies, which corresponds to a clogging of the system (Sheth and Parker 2003). Factors of the nonsense-mediated decay (NMD) pathway, which is responsible for the rapid degradation of mRNAs with a premature stop codon (Conti and Izaurralde 2005), can also be found in mammalian P-bodies (Unterholzner and Izaurralde 2004). However, it is not clear whether P-bodies are the only site of 5'-3' decay, as enzymes involved in this process can also be found elsewhere in the cytoplasm of yeast (Heyer et al. 1995) or mammalian cells (Bashkirov et al. 1997). It is also unclear whether mRNAs need to be deadenylated in order to enter P-bodies. In yeast, the deadenylase Ccr4p does not visibly localize to P-bodies (Sheth and Parker 2003), but the mammalian homolog does (Cougot et al. 2004). In mammalian and yeast cells, depletion of Ccr4p results in a reduction of P-bodies (Sheth and Parker 2003; Andrei et al. 2005), which is in favour of a model that mRNAs need to be deadenylated before entering P-bodies.

What are the connections between P-bodies and translation? Several lines of evidence indicate that mRNAs exist in 2 states: actively translated and associated with polysomes or in a translationally repressed state associated with P-bodies. When yeast cells are exposed to stress, such as glucose deprivation, translation is inhibited at the level of initiation, which is reflected by a strong decrease of polysomes, which corresponds to less mRNAs being associated with many ribosomes (Coller and Parker 2005). While translation gets down-regulated, P-bodies increase in size (Coller and Parker 2005). After removal of the stress, P-bodies decrease in size and polysomes reform, even in the absence of new transcription (Figure 1.9; Brengues et al. 2005).

Therefore, P-bodies in yeast seem to serve as sites of mRNA storage, which can then be released back into the translating pool without actually undergoing decay. The idea that the recruitment of mRNAs to P-bodies interferes with translation initiation and that only mRNAs not yet associated with ribosomes can be localized to P-bodies is strengthened by the finding that inhibition of translation elongation causes P-bodies to disappear, whereas inhibition of translation initiation increases P-bodies in size and number (Sheth and Parker 2003; Cougot et al. 2004; Andrei et al. 2005; Brengues et al. 2005; Teixeira et al. 2005). In budding yeast, the decapping activators Dhh1p and Pat1p are required for translational repression (Coller and Parker 2005). In mammalian cells, several proteins with established roles in translational repression localize to P-bodies: RCK/p54, CPEB and the eIF4E inhibitory protein eIF4E-T (Andrei et al. 2005; Ferraiuolo et al. 2005; Kedersha et al. 2005; Wilczynska et al. 2005; Chu and Rana 2006). However, the exact mechanism how mRNAs shuttle into P-bodies and become translationally repressed is not clear at the moment.

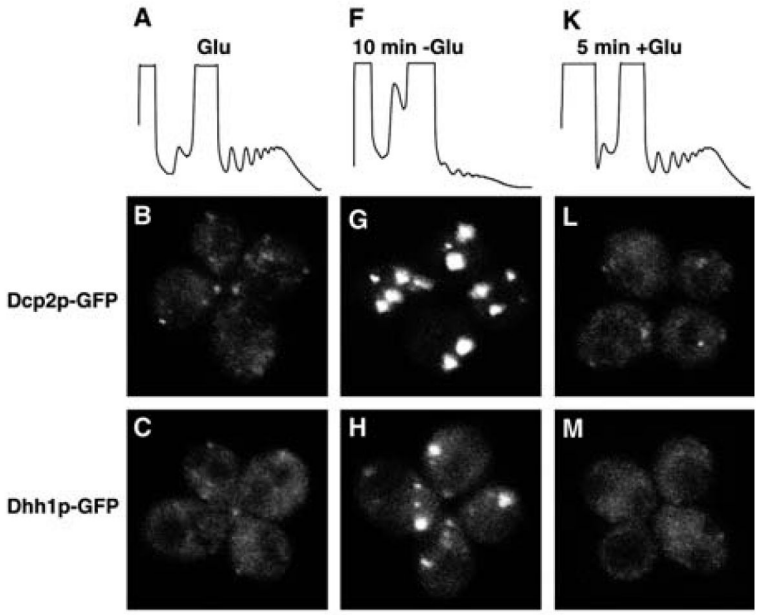


Figure 1.9 Movement of mRNAs between polysomes and P-bodies
Deprivation of glucose leads to repression of translation, which can be seen by diminished polysomes (**A**, **F**). This translation inhibition also results in increased number and size of P-bodies, which were visualized using the GFP-tagged reporters Dcp2p (**G**) and Dhh1p (**H**), whose presence in P-bodies is dependent on mRNA. After the re-addition of glucose, polysomes re-appear (**K**) and P-bodies basically disappear (**L**, **M**). These data are consistent with a move of mRNAs from polysomes to P-bodies after the inhibition of translation, and re-entering of mRNAs into the translation pool after translation is restored. This figure is taken from Brengues et al. (2005).

Another kind of cytoplasmic foci linked to translational repression can be observed in mammalian cells after the exposure to stress: stress granules (SGs) contain translationally silent mRNAs. These mRNAs are associated with pre-initiation complexes lacking the ternary complex and can also be shuttled back into polysomes after the removal of the stress (Kedersha and Anderson 2002). Despite the analogy to P-bodies and some shared components, SGs are distinct subcellular entities, as they also contain SG specific components such as 40S ribosomal subunits and translation initiation factors, which apart from eIF4E are not found in P-bodies, or ARE-binding proteins (Kedersha et al. 2005). However, fusion events and close association between SG and P-bodies could be observed in cells (Kedersha et al. 2005; Wilczynska et al. 2005).

Foci resembling stress granules have also been described in fission yeast (Dunand-Sauthier et al. 2002): Sum1p, a component of the translation initiation factor eIF3 complex, relocalizes to multiple cytoplasmic foci after the exposure to osmotic stress. In response to heat stress Sum1p is additionally localized to the inner nuclear periphery and furthermore colocalizes with eIF4E. All these data point to a spatial re-organization of the translational machinery to specific foci in these conditions. Furthermore, Sum1p interacts with components of the 26S proteasome and Sum1p relocalization in response to heat stress is dependent on an intact 26S proteasome.

Post-transcriptional gene expression regulation by small RNAs. Two types of small RNA molecules have emerged as regulators of mRNA stability and translation in the last decade: microRNAs (miRNAs) and short interfering RNAs (siRNAs). Current estimates from bioinformatic analysis suggest that the human genome encodes hundreds of different miRNAs and that they potentially regulate up to 30% of all genes (Lewis et al. 2005). However, only a few miRNAs and their targets have been validated to date.

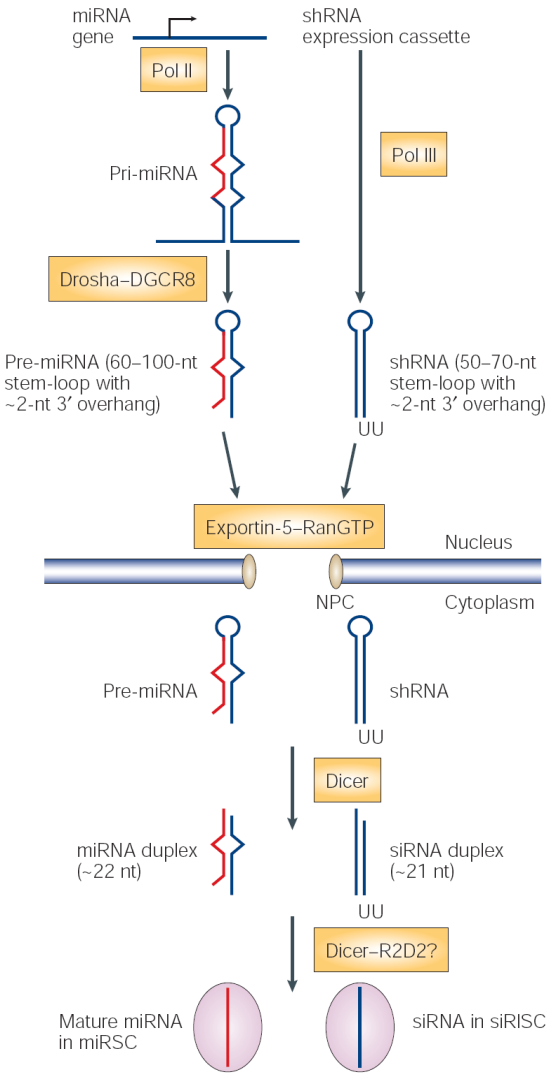


Figure 1.10 Biogenesis of miRNAs and siRNAs
Shown are the different ways of biogenesis for miRNAs and siRNAs. miRNAs are derived from longer precursors that include a ~70 nt imperfectly based hairpin segment and are usually transcribed by RNA polymerase II; siRNAs are of similar length but are derived from perfectly complementary RNA precursors, which are usually transcribed by RNA polymerase III. Despite the different mode of biogenesis, processing for both siRNAs and miRNAs is dependent on Dicer, and the regulatory function for both RNAs is exerted through proteins of the Argonaute (Ago) family. This figure is taken from Kim et al. (2005).

miRNAs and siRNAs are short RNAs of 21-26 nucleotides (nt) and are distinguished based on their biogenesis (Kim 2005; Jackson and Standart 2007): miRNAs are derived from longer precursors that include a ~70 nt imperfectly based hairpin segment; siRNAs are of similar length but are derived from perfectly complementary RNA precursors (Figure 1.10). Despite the different mode of biogenesis, processing for both siRNAs and miRNAs is dependent on Dicer, and the regulatory function for

both RNAs is exerted through proteins of the Argonaute (Ago) family: miRNAs and siRNAs associate with Ago proteins to form RNA-induced silencing complexes (RISCs), through which they modulate gene expression. During RNA-interference (RNAi), exogenously introduced siRNAs target mRNAs for endonucleolytic cleavage (Tomari and Zamore 2005). Such endonucleolytic cleavage has now also been described for plant (Llave et al. 2002; Allen et al. 2005) and mammalian (Yekta et al. 2004) miRNAs. Initially it was thought that perfect base-pairing between the miRNA/siRNA and the target mRNA favours endonucleolytic cleavage, whereas imperfect base-pairing results in the repression of the target by alternative mechanisms. However, it was shown that endonucleolytic cleavage still can occur when there are mismatches between the miRNA and the target mRNA (Mallory et al. 2004; Yekta et al. 2004).

In animal cells, most miRNAs are only partially complementary to their target mRNAs and the down-regulation of protein levels of the target is usually greater than the down-regulation of its mRNA abundance, which suggests regulation at the level of translation in these cases (Jackson and Standart 2007). The classic example is that of *lin-4* miRNA regulating *lin-14* protein levels in *Caenorhabditis elegans* through interactions with the 3' UTR of the mRNA (Arasu et al. 1991; Wightman et al. 1991). Regulation of *lin-14* through *lin-4* does not involve changes in mRNA levels, but protein levels are dramatically altered. As *lin-14* mRNA could be found associated with polysomes in both the active and the repressed state, it was suggested that translation of the mRNA is repressed at a point after initiation (Olsen and Ambros 1999). A recent study using an artificial CXCR4 siRNA directed against a luciferase reporter with six bulged target sites in its 3' UTR reported a similar result as described for *C. elegans lin-14* repression (Petersen et al. 2006): luciferase expression is down-regulated by 95% without large changes in mRNA abundance and repressed mRNAs were still associated with polysomes. Furthermore, repression is also seen for IRES-initiated translation, which further suggests a repressive mechanism that acts after translation initiation (Petersen et al. 2006). The authors suggest a drop-off of ribosomes at various points along the ORF resulting from miRNA repression (Petersen et al. 2006). However, it is unclear how this mechanism works, and it is hard to imagine how the polysomal distribution under repressed conditions would be similar to the distribution in a un-repressed state if ribosomal drop-off would occur continuously (Jackson and Standart 2007).

In contrast to the idea that miRNAs regulate mRNAs at a step after translation initiation, two reports point towards initiation as the regulated step (Humphreys et al. 2005; Pillai et al. 2005). Using the same CXCR4 system, Humphreys et al. (2005) could show a similar strong down-regulation at the protein-level of a luciferase reporter mRNA bearing four partially complementary binding sites for the CXCR4 siRNA. However, this down-regulation is not seen with IRES-containing mRNAs. Furthermore, the down-regulation is dependent on the 5' cap and 3' poly(A) sequences. Pillai et al. (2005) also used luciferase reporters, which contained either one perfectly complementary or three imperfectly complementary target sites for *let-7* miRNA. Expression of the reporter is down-regulated and reporter mRNA containing imperfect let-7 target sites is found in lighter polysomal fractions upon expression of let-7 miRNA, but not in a control reaction, when let-7 is bound by an antisense 2'-O-Me oligonucleotide (Pillai et al. 2005). Furthermore, using in vitro synthesized mRNAs, it could be shown that the 5' cap is necessary for miRNA-mediated repression (Pillai et al. 2005). However, in contrast to the study by Humphreys et al. (2005), repression is not markedly relieved when the poly(A) tail is absent (Pillai et al. 2005). Taken together, the two latter studies strongly support miRNA-mediated respression at the level of translation initiation.

What could be the explanation for the discrepancies in miRNA-mediated translational repression reported by these various groups? First, in their study, Petersen at al. (2006) used a reporter mRNA that was transcribed in the nucleus by RNA polymerase II, whereas in the other two studies by Humphreys et al. (2005) and Pillai et al. (2005) the reporter mRNAs were co-transfected with the miRNA. Secondly, the number, origin, specificity and location of target sites on the reporter might influence the observed effect. Furthermore, in a recent paper, Thermann et al. (2007) describe the formation of heavy miRNPs after repression by the miRNA miR2 in *Drosophila*. These miRNA-mRNA assemblies, which the authors call "pseudo-polysomes" show the same sedimentation characteristics as polysomes, but even form under conditions of effectively blocked 60S subunit joining (Thermann and Hentze 2007). One could speculate that the association with polysomes described for miRNA-repressed mRNAs could actually be an association with such "pseudo-polysomes".

However, it is also plausible that miRNAs exert their repression on translation through various mechanisms, and as a consequence it may be necessary to validate the regulatory mechanism for each miRNA-target pair individually. Furthermore,

translation could also be influenced by miRNAs indirectly, as recent studies have shown that miRNAs can accelerate deadenylation of their target mRNAs (Giraldez et al. 2006; Wu et al. 2006).

Apart from Drosha, fission yeast has homologues of all important genes involved in the RNAi machinery such as Dicer (Dcr1p), Argonaute (Ago1p) and RNA-dependent RNA polymerase (Rdp1p). However, post-transcriptional gene expression silencing for protein coding genes has not been described in fission yeast yet, but the RNAi machinery is involved in heterochromatin silencing (Zofall and Grewal 2006). In this case, RNAi-mediated silencing involves the processing of repeat transcripts from *dg*/*dh*/*cenH* repeat elements into an RNAi-induced initiation of transcriptional silencing (RITS) complex, which then targets proteins such as the histone methyl-transferase Clr4p, or the heterochromatin binding protein Swi6p, to these repeat regions, which ultimately leads to heterochromatin formation and limited accessibility for Pol II.

The connection between miRNAs and P-bodies. Several recent reports have found connections between the gene silencing pathway via miRNAs/siRNAs and P-bodies. Pillai at al. (2005) show that mRNAs, which are translationally repressed by *let-7* miRNA, localize to P-bodies or to cytoplasmic foci adjacent to P-bodies. Apart from the localization of translationally repressed mRNAs to P-bodies, Ago proteins, the effector molecules of miRNA-mediated silencing, have also been found to localize to P-bodies (Liu et al. 2005; Sen and Blau 2005). Argonaute proteins also interact with GW182, a key P-body subunit in mammalian cells, and depletion of GW182 impairs the repression of miRNA-reporters (Jakymiw et al. 2005; Liu et al. 2005).

A recent report also shows the reversibility of miRNA-mediated repression and the involvement of P-bodies: Bhattacharyya et al. (2006) used the cationic amino acid transporter (CAT-1) mRNA or reporter mRNAs bearing its 3' UTR, which is negatively regulated by miRNA miR-122. In Huh7 cells, miR-122 is endogenously expressed and CAT-1 protein levels are significantly down-regulated and both CAT-1 and miR-122 can be found in P-bodies (Bhattacharyya et al. 2006). However, after exposure to stress, CAT-1 mRNA can escape the translational repression, and the de-repression and the exit from P-bodies is dependent on ARE elements in the 3' UTR. Bhattacharyya et al. (2006) could further show that the ARE-binding protein HuR is necessary for the release from translational repression and P-body entrapment.

All the above examples make it clear that P-body components are important for gene silencing via miRNA/siRNA-mediated repression. However, it is not clear if the spatial environment of the P-body itself or P-body components are important for this interaction. Recent work suggests that disruption of P-bodies does not necessarily affect siRNA-mediated silencing (Chu and Rana 2006). Therefore, concentration of miRNAs and miRNA-targets in P-bodies could be a consequence rather than a prerequisite of miRNA/siRNA-mediated gene silencing.

Taken together, regulation of gene expression via small RNAs and sequestration to P-bodies and its interplay between mRNA translation and decay adds further complexity to the control of post-transcriptional mRNA fate. As mentioned above, 30% of human genes are potential miRNA targets (Lewis et al. 2005), and it is entirely possible that miRNAs exert their function in a combinatorial mode: a given mRNA might be regulated by several miRNAs and a given miRNA might target several mRNAs. However, further research will be needed to elucidate the exact molecular events behind these regulatory mechanisms.

Functional genomics of post-transcriptional gene expression

Genome-wide approaches to identify targets of post-transcriptional gene expression regulation

The advent of microarray technology allowed the genome-wide study of gene expression at the level of steady-state mRNA abundance. Furthermore, microarray technology combined with chromatin immunopreciptitations is an invaluable tool to identify transcription factor binding sites and chromatin modifications on a global scale. Together, these studies revealed global networks of transcriptional control in a variety of organisms and physiological conditions (Babu et al. 2004; Luscombe et al. 2004; Barrera and Ren 2006; Walhout 2006).

However, as gene expression is often regulated at the post-transcriptional level, it is important to also gain an understanding of these regulatory processes and their targets on a genome-wide scale. In the same way as DNA and its interaction with transcription factors and chromatin modifiers is integral to transcriptional regulation, mRNA and its association with RNA-binding proteins is essential for the regulation of gene expression at the post-transcriptional level. Consequently, recent work of many groups has focused on the large-scale systems analysis of mRNA-protein interactions and mRNA dynamics. Many of these studies employ microarray-based approaches to study a variety of processes on a genome-wide scale such as (1) the association of mRNAs with specific RNA-binding proteins, (2) mRNA stability, or (3) the association of mRNAs with ribosomes and thus the efficiency with which these mRNAs are translated. These large-scale approaches are especially useful to identify potential targets for each of the myriads of possible post-transcriptional regulatory mechanisms, and building on this knowledge can in turn be useful to examine the underlying molecular mechanisms of the regulatory process at the molecular level. Here, some of these techniques and the interesting findings obtained from them will be introduced.

Translation

Translational efficiency can be measured on a genome-wide scale by assessing the number of ribosomes that are bound to a given mRNA. This can be achieved by

combining the traditional method of polysome profiling with microarray technology, which is referred to as translational profiling (Figure 1.11): Usually, cells are treated with the elongation-inhibitor cycloheximide, which "traps" ribosomes on the mRNA they are translating. Cellular lysates are then resolved according to their density on a sucrose gradient by ultracentifugation. As the ribosome is a huge macromolecular complex with a molecular mass above 3 megadalton (Taylor et al. 2007), the density of the mRNA-ribosome particles is determined by the amount of ribosomes bound to the mRNA. The sucrose gradient is then fractionated and a polysome profile is obtained by measuring the RNA abundance (Figure 1.11; right panel). Going along from the light to the heavy density fractions, free mRNAs are obtained, followed by the ribosomal 40S and 60S subunits, the monosome or 80S subunit, and the polysome fractions corresponding to mRNAs with increasing numbers of bound ribosomes. mRNAs from diverse fractions can then be extracted and quantified using microarrays.

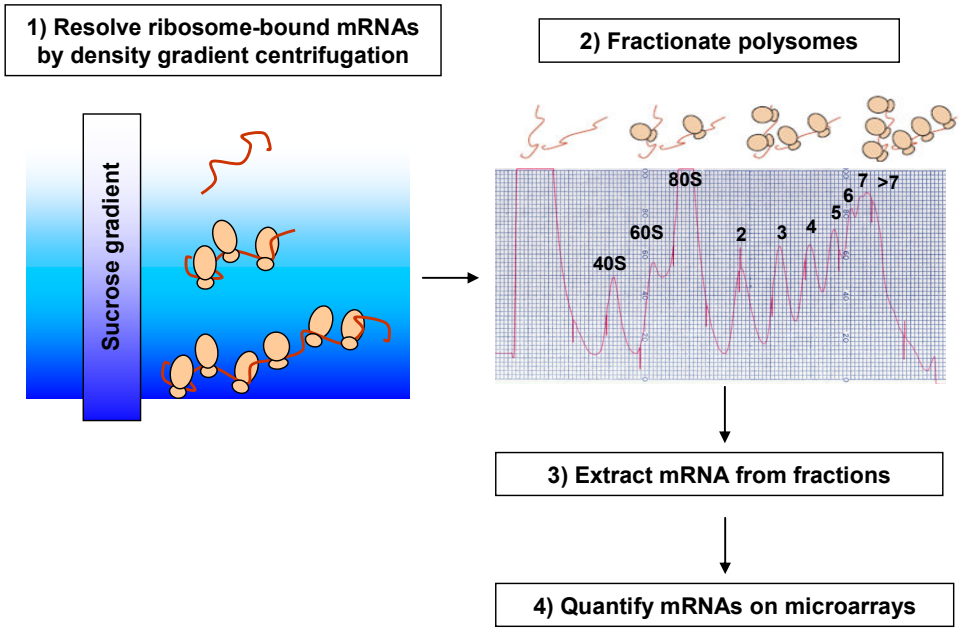


Figure 1.11 Translational profiling
Genome-wide measurements of translation can be achieved by combining polysome profiling with microarray technology, which is referred to as translational profiling. mRNAs are resolved on a sucrose gradient by ultracentifugation according to their density, which is determined by the number of associated ribosomes. After fractionation, mRNA from diverse fractions can then be extracted and quantified using microarrays.

In most studies where this technology is used to study translational regulation, the pool of mRNAs associated with polysomes is compared to the pool of un-translated

mRNAs or total mRNA preparations in order to define translationally regulated transcripts (Johannes et al. 1999; Kuhn et al. 2001; Kash et al. 2002; Rajasekhar et al. 2003; Qin and Sarnow 2004; Dinkova et al. 2005; Bushell et al. 2006; Iguchi et al. 2006; Spence et al. 2006; Thomas and Johannes 2007). Some studies, however, have used more than 10 fractions spaced along the polysome profile, which are then probed with microarrays to obtain higher-resolution data of changes in ribosome association for given mRNAs (Arava et al. 2003; Preiss et al. 2003; MacKay et al. 2004; Qin et al. 2007).

Using translational profiling, the effect on global and mRNA specific translational regulation has been examined in a variety of conditions. Examples are the exposure of cells to stress or changing environmental conditions such as hypoxia, treatment with rapamycin, heat shock, or change in carbon-source (Kuhn et al. 2001; Grolleau et al. 2002; Preiss et al. 2003; Thomas and Johannes 2007); the translational regulation during the mitotic cell cycle, meiosis, or during recovery from cell cycle arrest (Serikawa et al. 2003; Qin and Sarnow 2004; Iguchi et al. 2006); the dependence of mRNAs on specific translation initiation factors (Johannes et al. 1999; Dinkova et al. 2005); or translational regulation in response to oncogenic signaling or in transformed cells (Rajasekhar et al. 2003; Spence et al. 2006).

One of the first studies using translational profiling was conducted by Johannes et al. (1999): the requirement for cap-dependent translation initiation was examined by studying the association of mRNAs with polysomes in cells with reduced eIF4G concentrations, which was achieved by infecting the cells with poliovirus. Most of the examined mRNAs show the expected down-regulation in translation, whereas a small percentage remains associated with polysomes or even exhibits increased polysome association. These mRNAs are probably translated via IRES-mediated translational initiation and included mRNAs encoding immediate-early transcription factors and mitogen-acitvated regulators (Johannes et al. 1999). Another study conducted in *C. elegans* investigated the effect of the selective knock-out of one isoform of the cap-binding translation initiation factor eIF4E (Dinkova et al. 2005). Mutant worms show a mixture of phenotypic effects, reproduce more slowly and exhibit an egg laying defect. Using translational profiling, several mRNAs could be identified that show changes in their polysomal association without altered total mRNA levels. Interestingly, these mRNAs are enriched for genes with functions related to egg

laying, providing a possible explanation for the observed phenotype (Dinkova et al. 2005).

Kuhn et al. (2001) used translational profiling to measure the translational response in budding yeast cells to the transfer from a fermentable (glucose) to a non-fermentable (glycerol) carbon source. This shift results in a global down-regulation of translation. mRNAs encoding ribosomal proteins are strongly down-regulated in terms of total mRNA abundance as well as in their translational status, indicated by a diminished association with polysomal fractions. However, a few mRNAs show increased association with polysomes and most of these mRNAs also show increased abundances in their total mRNA levels. A similar connection between changes in total mRNA levels and polysome association was described in another study, which examined translational regulation in response to treatment with rapamycin and heat shock (Preiss et al. 2003). They found that mRNAs that show increased abundance in response to the treatment often also show increased translational efficiency. The same was true for mRNAs with decreased abundance. Furthermore, such a correlation between changes in total mRNA levels and translational efficiency has been observed in budding yeast in response to treatment with mating pheromone (MacKay et al. 2004). This coordination between changes in transcript levels and translation has been termed "potentiation" (Preiss et al. 2003). However, further studies will be required to determine whether potentiation happens through coordinated yet independent regulation of transcription and translation, or whether increased translation is a mere consequence of *de novo* transcription – for example *de novo* transcription could influence mRNP composition or could simply provide "new" and "intact" messages, which are then more efficiently translated.

Translational profiling has recently been used to study translational changes in the response to hypoxia (Thomas and Johannes 2007). When PC-3 cells are grown under hypoxic conditions, translation is globally down-regulated, concomitant with mTOR inactivation and phosphorylation of eIF2α (see above), and mRNAs encoding ribosomal proteins are found to be most sensitive to the global translational down-regulation. Again, several mRNAs were identified, which escape the translational down-regulation and still are associated with polysomal fractions under hypoxic conditions (Thomas and Johannes 2007). The authors suggest that translational regulation of these mRNAs might be initiated via cap-independent mechanisms. This

is another example of how certain mRNAs can be selectively translated in response to a specific stimulus, while most other cellular mRNAs are translationally down-regulated in this condition. Such sets of mRNAs could only be identified using genome-wide, unbiased approaches such as translational profiling, as their involvement in certain biological processes is unexpected and could not have been anticipated by traditional biological studies.

Another use of translational profiling was made by Arava et al. (2003): in this study, translational profiling was not used to look into translational regulation in response to changing conditions, but the authors give a comprehensive picture of translational efficiency in vegetatively growing budding yeast cells. mRNA extracted from 14 fractions across the polysomal profile are analyzed on microarrays, and the peak of the distribution for each mRNA along the profile is used to determine the average number of ribosomes associated with a given mRNA on a genome-wide scale. Several interesting findings could be made using this approach. For most mRNAs, 70-80% of the transcript was associated with polysomal fractions. Among the few mRNAs not associated with polysomal fractions several mRNAs were known to be translationally regulated. Furthermore, the authors could show that ribosomes are spaced well below the maximum packing capacity on most mRNAs, which corroborates the fact that translation initiation is the rate-limiting step in translation. The density of associated ribosomes varied strongly between transcripts and showed an inverse correlation to the length of the transcript.

Recently, Qin et al. (2007) also used a similar high-resolution translational profiling approach to study the extent of translational control during early *Drosophila* embryogenesis. One of their findings is that mRNAs that were known to be regionally translationally repressed in the early fly embryo such as Nanos, Hunchback or Caudal mRNA indeed only show a small portion of their transcript associated with polysomal fractions.

Alternative proteomic approaches to study translational regulation

At the moment, translational profiling should be the method of choice when examining translational regulation on a genome-wide scale, mainly due to the fact that the read-out of the assay is based on microarray measurements. Microarray technology has become robust, reliable and also affordable, and combined with proper

and careful analysis, translational profiling is a powerful tool to screen for translationally regulated mRNAs. However, recent advances in proteomic approaches will also be useful to study translational regulation. In 2 recent studies, the authors combined the measurement of absolute protein levels using proteomics and total mRNA levels using microarrays (Newman et al. 2006; Lu et al. 2007). Newman et al. (2006) used a collection of yeast strains, in which each protein is fused to green fluorescent protein (GFP) under the control of its own promoter. Using a flow cytometry approach, GFP abundance was measured for each strain grown in full medium or minimal medium and mRNA levels were measured using DNA microarrays in both conditions. Lu et al. (2007) used a mass spectometry approach together with a novel algorithm to make absolute measurement of protein levels (APEX, absolute protein expression measurements) in the same conditions. Both studies came to the conclusion that changes in protein levels between the conditions examined are largely due to changes in the abundance of the corresponding mRNAs, but certain mRNAs were identified, where the change in protein level could not be attributed solely to a change in mRNA level. These mRNAs are prime candidates for regulation at the translational level or at the level of protein stability.

There is a downside to these proteomic approaches: in the case of the GFP-tagged strain collection, the tag might interfere with translational regulation, which might be executed via sequence elements in the UTR, and mass spectometry approaches do not yet manage to identify every expressed protein in the cell and are biased towards highly abundant proteins. However, as these techniques improve, they will become an important tool for the genome-wide study of translational control.

mRNA decay

As mentioned above, mRNA turnover in the cell is regulated by multiple mechanisms (Wilusz et al. 2001; Parker and Song 2004). Deadenylation of the transcript is a key step in these regulatory mechanisms, and mRNAs are then decapped and degraded via the XRN1 exonuclease or, alternatively, mRNAs can be degraded without decapping by the exosome complex. In certain cases, mRNAs can be degraded via endonucleolytic mechanisms, such as degradation via the RNAi machinery (Tomari and Zamore 2005). Furthermore, nonsense-mediated decay (NMD) serves as a mRNA-quality control mechanism to degrade faulty mRNAs with a premature stop

codon. These mRNAs are decapped and directly degraded without prior deadenylation (Fasken and Corbett 2005). mRNAs that are lacking proper stop codons are degraded without decapping by the exosome in a process called non-stop decay (Vasudevan et al. 2002).

Global mRNA stability is often measured by blocking transcription with drugs or by using mutants of RNA polymerase II. At several times after the transcription block, mRNA is isolated and probed on a microarray (Figure 1.12; Mata et al. 2005). Using this approach, genome-wide mRNA stability has been determined in various organisms such as yeast (Wang et al. 2002; Grigull et al. 2004), plants (Gutierrez et al. 2002) and human cell lines (Raghavan et al. 2002; Yang et al. 2003).

The picture emerging from these studies is that mRNA decay is a controlled process and that decay rates vary substantially between different transcripts. mRNA decay rates often also correlate among mRNAs that encode functionally related proteins or proteins of the same macromolecular complex (Wang et al. 2002). mRNAs encoding transcription factors, parts of the transcriptional machinery, proteins involved in ribosome-biogenesis and the translational machinery have in general fast decay rates, whereas mRNAs encoding central metabolism proteins have slower decay rates (Wang et al. 2002; Yang et al. 2003; Grigull et al. 2004; McCarroll et al. 2004). It was suggested that the fast decay rates for mRNAs involved in the transcriptional and the translational process might be advantageous for fast regulation of these central gene expression processes in response to changing environmental conditions. However, it has to been mentioned that the transcriptional shut-down itself, and the use of drugs or mutants of RNA polymerase II in these experiments can also trigger a general stress response in the cell (Grigull et al. 2004). Thus, the fast decay of mRNAs involved in transcription and translation might happen rather as a response to the stress, and decay rates for the same mRNAs might actually be much slower in un-stressed vegetatively growing cells.

For many mRNAs, the fast decay rate correlates with the presence of ARE elements in their 3' UTR, but not all fast decaying mRNAs have ARE elements (Raghavan et al. 2002; Yang et al. 2003). However, no strong correlation between mRNA stability and other mRNA features such as ORF length, mRNA abundance or ribosome density seems to exist (Wang et al. 2002).

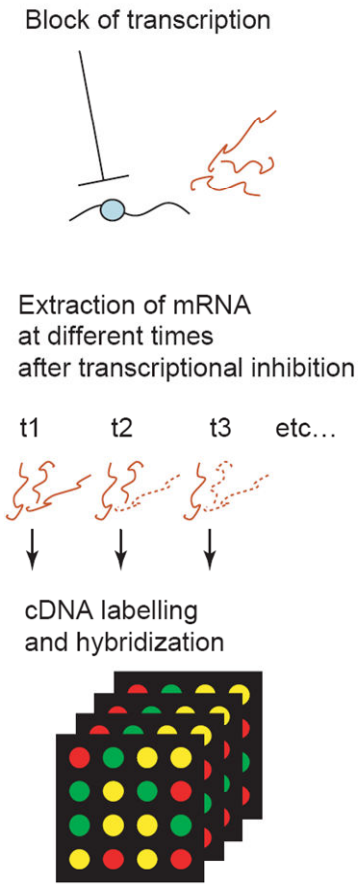


Figure 1.12 Genome-wide measurements of mRNA half-lives
Transcription is blocked using using drugs or mutants of RNA polymerase II. At different times after the transcriptional block, transcripts are isolated and quantified using DNA microarrays. mRNA half-lives can then be deduced from these data. This figure is taken from Mata et al. (2005).

In a recent study, Shock et al. (2007) determined the global decay rates of mRNAs in various stages during the intra-erythrocytic development cycle of *Plasmodium falciparum*, the pathogen causing human malaria. Interestingly, as the parasite passes through the examined intra-erythrocytic developmental stages, decay rates decrease globally for essentially all examined mRNAs, which suggests that post-transcriptional regulation might be the main mechanism of gene regulation in *Plasmodium falciparum*. Such genome-wide regulation of mRNA decay rates has not yet been described for any other organism.

Insights into the global regulation of mRNA decay also comes from measuring total mRNA levels in cells deleted for factors involved in mRNA degradation. An example is the measurement of global effects in yeast or mammalian cells compromised for

NMD function (He et al. 2003; Mendell et al. 2004). Apart from the involvement in quality control of mRNAs, a new aspect of this pathway could be detected through these global studies: several hundred mRNAs were found to be induced as a consequence to NMD switch-off, and they were enriched for mRNAs with specific functions. In mammalian cells, many of the enriched mRNAs are involved in amino acid metabolism (Mendell et al. 2004). As NMD requires translation and amino acid depletion inhibits translation, the authors suggest that the abundance of these transcripts is regulated by NMD to couple their mRNA levels to amino acid availability. Inhibition of translation and NMD might increase the abundance of these transcripts in order to turn on amino acid biosynthesis (Mendell et al. 2004). Thus, these genome-wide studies revealed that NMD not only functions in ensuring quality control of mRNAs but also acts as a more general regulator of gene expression.

In another recent genome-wide approach, Hollien et al. (2006) could show that the inositol-requiring enzyme 1 (IRE-1), which is involved in activating the unfolded protein response (UPR) as a consequence of accumulation of mis-folded proteins in the endoplasmatic reticulum (ER), is involved in the specific and immediate degradation of a subset of mRNAs during the UPR. IRE-1 is involved in the detection of unfolded proteins in the ER and subsequently activates a transcription factor, X-box–binding protein 1 (XBP-1), through endonucleolytic cleavage of its mRNA. In this study, IRE-1 or XBP-1 were depleted by RNAi in *Drosophila* S2 cells, in which the UPR has been induced. Global mRNA levels from these cells were then measured using DNA microarrays. A subset of mRNAs could be identified, whose repression is solely dependent on IRE-1, and not on XBP-1, and IRE-1 mediates the degradation of these mRNAs, based both on their localization to the ER membrane and on the amino acid sequence they encode (Hollien and Weissman 2006).

RNA-binding proteins and their target mRNAs

Central to virtually all aspects of post-transcriptional gene expression regulation – from mRNA processing and export to mRNA decay and translation – is the interplay between mRNAs and RNA-binding proteins (RBPs). Some RBPs bind most of the transcripts in the cell (e.g. PABP), whereas others bind only to a small set of specific mRNAs in order to exert a specialized function in determining these mRNAs' post-transcriptional fate (Hieronymus and Silver 2004; Mata et al. 2005; Moore 2005;

Keene 2007). Furthermore, RBPs most likely can also act in a combinatorial way, as each mRNA can be bound by several RBPs. In budding yeast, there are about 600 proteins estimated to have RNA-binding capacity, and this number is probably even higher in mammalian cells (Maris et al. 2005; Moore 2005).

Much insight into gene expression regulation via RBPs has come from the genome-wide identification of their targets via "RBP Immunoprecipitation followed by chip analysis" (RIP-chip, Figure 1.13): RBPs are immunopurified together with their associated RNAs, via an epitope-tag or via an antibody against the RBP of interest. The RNAs are then isolated from the immunoprecipitate, purified, labelled and then hybridized onto microarrays. In one of the first studies to employ this technology, Tenenbaum et al. (2006) used cDNA-filter arrays containing ~600 murine genes to identify mRNAs associated with the RBPs HuB, PABP and eIF4E, which all are involved in the regulation of translation. Even though only a few mRNAs were analyzed, each RBP bound a different subset of mRNAs, with PABP being associated with many mRNAs and HuB only associated with a few mRNAs. Furthermore, the authors found that the pattern of association of mRNAs with HuB is significantly altered after cells were induced to differentiate by treatment with retinoic acid.

One of the most comprehensive studies using RIP-chip was conducted by Gerber et al. (2004), who identified targets of all 5 members of the Pumilio family of RBPs in budding yeast (Puf1p-Puf5p). Forty to 220 mRNAs were found to be associated with each of the five Puf proteins, and the subset of mRNAs bound to each of the RBPs were enriched for common functional groups or subcellular localization. Puf1p and Puf2p associate with mRNAs encoding membrane-associated proteins; Puf3p nearly exclusively binds mRNAs that encode mitochondrial proteins; Puf4p associates with nucleolar ribosomal RNA-processing factors; and Puf5p associates with mRNAs encoding chromatin modifiers and components of the spindle pole body. Furthermore, distinct sequence elements in the 3' UTR of mRNAs bound by Puf3p, Puf4p and Puf5p could be identified (Gerber et al. 2004). A similar sequence motif was identified in mRNAs that co-immunoprecipitate with the *Drosophila* Pumilio protein (Gerber et al. 2006). Many of the mRNAs associated with Pumilio in *Drosophila* also encode functionally related proteins; however, these mRNAs are not related to the mRNAs associated with Puf3p in budding yeast (Gerber et al. 2006).

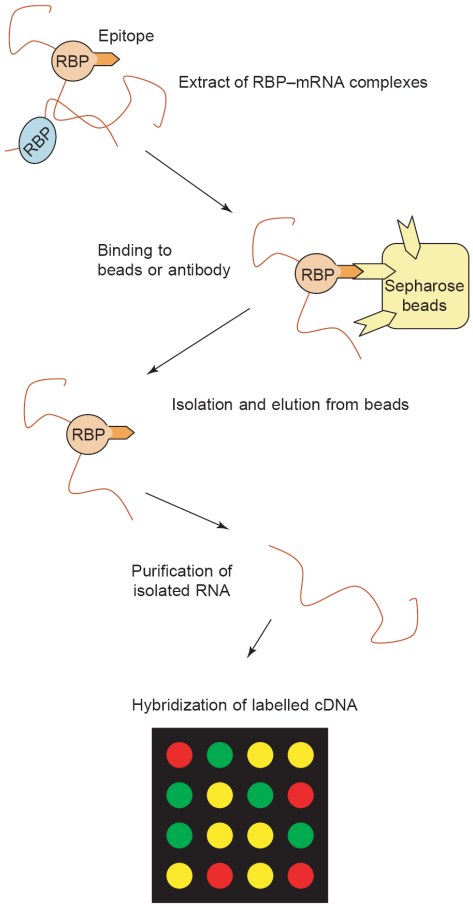


Figure 1.13 Genome-wide determination of mRNA targets of RNA-binding proteins (RBPs)
Targets of RBPs can be determined globally by "RBP Immunoprecipitation followed by chip analysis" (RIP-chip). RBPs are immunopurified together with their associated mRNAs, via an epitope-tag or via an antibody against the RBP of interest. The mRNAs are then isolated from the immunoprecipitate, purified, labelled and then hybridized onto microarrays. This figure is taken from Mata et al. (2005).

RIP-chip approaches have also been used to identify global targets of RBPs involved at other levels of post-transcriptional gene expression regulation such as splicing (Gama-Carvalho et al. 2006), nuclear mRNA export (Hieronymus and Silver 2003; Kim Guisbert et al. 2005), mRNA decay (Duttagupta et al. 2005), and poly(A) tail length control (Beilharz and Preiss 2007). Common to these studies is the finding that RBPs involved in a common process often share mRNA targets, but on top of that, each RBP seems to have unique targets; and mRNAs targeted by a certain group of RBPs often share functional specificity. Furthermore, RIP-chip studies also provided clues to unexpected functions of RPBs. An example is the identification of mRNAs

associated with the yeast La protein (Lhp1p). Lhp1p is involved in the biogenesis of non-coding RNAs transcribed by RNA polymerase III, and thus many non-coding mRNAs were identified as targets of this RBP (Inada and Guthrie 2004). However, Lhp1p was also found to bind a subset of coding mRNAs such as *HAC1* mRNA, which encodes a transcription factor required for the UPR. Follow-up experiments indicate that Lhp1p might play a role in the translational regulation of *HAC1* mRNA (Inada and Guthrie 2004 387).

Recently, RIP-chip approaches are also employed to measure translation on a global scale. In this case, the RBP is an epitope-tagged ribosomal subunit and polyribosomal complexes are immunopurified, which correspond to mRNAs bound to ribosomes. The feasibility of these approaches was first shown in budding yeast (Inada et al. 2002). The ribosomal protein Rpl25p was epitope-tagged and immunopurification via the epitope-tag yielded intact polysomal fractions. Zanetti et al. (2005) used a similar approach with epitope-tagged ribosomal protein RPL18 in *Arabidopsis* to isolate polyribosomes. The authors furthermore probed the mRNA from these immunopurified complexes with DNA microarrys and compared the data to total cellular mRNA samples. Their data show that for most genes the mRNAs are associated with polysomal complexes with an average level of association of 62%, which is slightly below the number of ribosome association determined for yeast mRNAs by translational profiling (Arava et al. 2003). This technology could become a powerful tool to study translational regulation in varying conditions or different cellular subtypes.

Aim of this thesis

The work of this thesis is based on a simple question: "What are the global patterns of translational regulation in fission yeast?"

Underlying this question was the fact that in recent years a wealth of genome-wide data was generated in fission yeast to describe changes in mRNA levels in a variety of conditions such as the response so stress (Chen et al. 2003), during the mitotic cell cycle (Rustici et al. 2004), and during meiosis and sporulation (Mata et al. 2002; Mata and Bähler 2006). Work from these studies gave a comprehensive overview of transcriptional regulation in the conditions examined. However, no genome-wide approaches had been conducted in fission yeast to examine gene expression regulation at the post-transcriptional levels – such as at the level of translation.

Therefore, the first aim of this work was to establish translational profiling in fission yeast and to measure translational rates on a global scale in vegetatively growing cells. These data could then be compared to genome-wide data sets of mRNA features and to genome-wide data sets of other levels of gene expression regulation.

A second aim of this study was to identify mRNAs that are specifically regulated at the level of translation in response to environmental and genetic perturbations such as cellular stress or in mutant yeast strains. Using translational profiling in combination with measurements of changes in total mRNA levels with microarrays, mRNAs can be identified that are regulated solely at the level of mRNA abundance, solely at the level of translation, or regulated at both levels. These data should provide a global view of the extent of translational regulation and identify candidates for translational regulation, which can be examined in follow-up studies.

Chapter 2

Materials and methods

Materials and methods

S. pombe strains

Most experiments were performed with the wild-type *972 h*$^-$ strain, with the exception of the experiments performed to study the translational response to the deletion of *rmt3*, where the following strains were used: FBY14 (*ade6M210 leu1-32 ura4-D18 his3-D1*) and FBY18 (*ade6M216 leu1-32 ura4-D18 his3-D1 rmt3::ura4*). Furthermore, induction and shut-off experiments of *pom1* and *rpb4* under the control of various *nmt1* promoters were performed with these strains: JB150 *(kanMX6-41nmt1-pom1*), JB151 (*kanMX6-3nmt1-pom1*), JB172 (*h*$^-$ *kanMX6-3nmt1-3HA-pom1*), JB175 (*h*$^-$ *kanMX6-41nmt1-3HA-pom1*), JB178 (*h*$^-$ *kanMX6-81nmt1-3HA-pom1*), JB394 (*h*$^-$ *kanMX6-3nmt1-rpb4*), JB395 (*h*$^-$ *kanMX6-41nmt1-rpb4*), and JB396 (*h*$^-$ *kanMX6-81nmt1-rpb4*).

S. pombe growth conditions

Cells were grown in full medium (supplemented yeast extract medium YES) or in Edinburgh minimal medium (EMM) (Moreno et al. 1991) at 32°C, except for FBY14 and FBY18, which were grown at 30°C, to a concentration of 0.3 - 0.7 OD_{600}.

For repression of genes under the control of the *nmt1* promoter, the corresponding strains were grown in EMM medium supplemented with 15 μM thiamine. To induce expression from the *nmt1* promoter, strains were washed and re-suspended in EMM medium lacking thiamine.

For the stress experiments, cells were grown in YES at 32°C. Oxidative stress was induced by the addition of H_2O_2 (SIGMA) to a final concentration of 0.5 mM, DNA-damage was induced by the addition of MMS (Fluka) to a final concentration of 0.02 % v/v, and heat stress was induced by moving the culture flask with the growing cells from 32°C to 39°C in a water bath.

Translational profiling

High-resolution translational profiling

Polysome fractionation, RNA extraction and microarray hybridizations. Cycloheximide was added to a final concentration of 100 µg/ml for 5 minutes (min) before harvesting aliquots of 50 OD_{600} of cells by centrifugation at 4ºC and washing in polysome lysis buffer (20 mM Tris-HCl pH 7.5, 50 mM KCl, 10 mM MgCl, 1 mM DTT, 100 µg/ml cycloheximide, 200 µg/ml heparin). Cells were resuspended in 100 µl polysome lysis buffer with 40 U/ml RNasin (Promega) and complete protease inhibitor cocktail (Roche). Cells were lysed in 1.5 ml tubes by using a Fastprep cell disruptor with 1 g of chilled glass beads (BioSpec Products). After lysis, 400 µl of lysis buffer was added and a hole was poked in the bottom of the tube to separate the lysate by centrifugation into another 1.5 ml tube. The lysate was cleared by two centrifugations at 4ºC. Aliquots corresponding to 25 OD_{260} units in 350 µl were loaded onto an 11 ml linear 10-50% (w/v) sucrose gradient, which was prepared with the Gradient Master (Biocomp) from 10% and 50% sucrose solutions (w/v) prepared with polysome lysis buffer. The gradients were separated by centrifugation for 160 min at 35,000 rpm in a SW 40Ti rotor (Beckman). The gradients were then fractionated by upward displacement with 55% (w/v) sucrose, and fractions of ~900 µl were collected directly into tubes containing 2 ml of 8 M Guanidium-HCl using an Isco fractionation system. Corresponding fractions from 3 gradients were pooled and 5 *in vitro*-transcribed *Bacillus subtilis* mRNAs (Lyne et al. 2003) were added for normalization in the following amounts: Trp 0.09 ng/µl, Dap 0.45 ng/µl, Lys 0.9 ng/µl, Phe 4.5 ng/µl, and Thr 9 ng/µl. After addition of an equal volume of 100% ethanol, RNA from each fraction was precipitated overnight at -20ºC and centrifuged at 4ºC for 90 min. Further purification of the RNA by phenol:chloroform extraction and LiCl-precipitation was performed as described by Arava et al. (2003). RNA from each pellet was then resuspended in 20 µl of DEPC-treated H_2O. 10 µl of the RNA from each fraction was used for microarray analysis and labelled using oligo(dT)-primers and the SuperScript™ Direct cDNA Labeling System (Invitrogen) and Cy3/Cy5-dCTP (Amersham). Polysomal RNA from each fraction was hybridized against 20 µg of total RNA extracted using the hot-phenol method. Half the amount of bacterial mRNAs indicated above was added to each aliquot of total RNA before labeling. Hybridization to microarrays, slide-washing and scanning of microarrays

was performed as described (see below). Polysome profiling was performed in triplicate from three independent biological repeats, including a dye swap.

Analysis of high-resolution translational profiling data. Data from high-resolution translational profiling cannot be normalized on the assumption that the overall RNA levels from the competitive hybridization are similar between the two RNA samples. Spiked bacterial mRNAs were therefore used to normalize for different amounts of RNA in the individual fractions. Probes for the bacterial mRNAs on the microarray were first normalized locally to obtain an average signal ratio of 1. Normalization based on signal intensities was then done as described (see below). Fraction 10 in the 3rd repeat was a clear outlier showing signal intensities roughly twice as high as in the other 2 repeats and higher signals than its neighbouring fractions. Thus, all ratios for this fraction were divided by a correction factor, which was calculated based on the best correlation to the other repeats. Data and conclusions presented here did not change if the 3rd experimental repeat was omitted, but the number of mRNAs included in the data set was reduced to 3020. To be included for further analysis, mRNAs had to fulfil the following criteria: 1) there had to be microarray data for all 12 fractions from at least 2 out of the 3 repeats, excluding 1012 mRNAs, and 2) the Pearson correlation between profiles for the same gene from the different repeats had to be $\geq$0.7 (if present in all 3 repeats) or at $\geq$0.75 (if present in only 2 repeats), excluding 352 mRNAs. These criteria were fulfilled by 3598 out of the 4962 nuclear encoded protein-coding genes.

Translational properties were then determined to estimate translational efficiencies for different mRNAs. Translation profiles were calculated as the percentages of a given mRNA in each of the 12 fractions such that the total over all fractions is 100%. Ribosome occupancy for a given mRNA was calculated by adding up the percentages of this mRNA in fractions 5 to 12, which are associated with ribosomes. For the mean number of associated ribosomes, we calculated the percentages of a given mRNA for each fraction associated with ribosomes such that the total of fractions 5 to 12 was 100%. The percentage of mRNA in each fraction was then multiplied with the corresponding estimate for associated ribosomes, and these values were added up to estimate the mean number of ribosomes bound to this mRNA. (Associated ribosome numbers for each fraction were estimated by plotting the defined peaks from the polysome profile, containing 1 to 8 ribosomes, against the relative distance from the

start of the profile and fitting an exponential curve; this curve was used to determine ribosome numbers at the beginning and end of a given fraction, which were averaged to produce the mean number of ribosomes associated with this fraction.) Ribosome densities represent the mean number of ribosomes associated with each mRNA divided by its ORF length. All values were determined individually for each biological repeat and then averaged.

Medium-resolution profiling

Polysome fractionation, RNA extraction and microarray hybridizations. To study the translational response to environmental stress, medium-resolution translational profiling was performed. Preparation of cell lysates and polysome fractionation was essentially done as described for the high-resolution translational profiling, except that cycloheximide was added directly when cells were harvested. Twelve fractions were collected during polysome fractionation. Fractions 1-3, 4-6, 7-9, and 10-12 were united respectively into 4 pools. RNA from each pool was precipitated overnight at -20ºC after the addition of an equal volume of 100% ethanol. After centrifugation at 4ºC for 90 min, the pellet was air dried, and dissolved in 100 µl DEPC-treated H_2O. The RNA was then purified using RNeasy columns (Qiagen) and eluted with 30 µl DEPC-treated H_2O. 10 µl of the RNA from each of the 4 pools was used for microarray analysis and labelled using a mix of oligo(dT)-primers and random hexameres and the SuperScript™ Direct cDNA Labeling System (Invitrogen) and Cy3/Cy5-dCTP (Amersham). RNA from each pool was hybridized against labelled genomic DNA as reference (see below). Two (heat stress, DNA damage) or three (oxidative stress) complete biological repeats including a dye swap were performed.

Analysis of medium-resolution translational profiling data. Microarray-data from each of the 4 pools were normalized using our standard normalization script, which removes spots with unreliable or low signal and locally adjusts the median of ratios to 1 within a sliding window (see below). For each mRNA, where data was obtained in all 4 pools, translation profiles were calculated as the percentages of a given mRNA in each of the 4 pools such that the total over all pools is 100%. Two approaches were used to identify altered translational profiles comparing the stress condition and the corresponding control. First, the total difference between the two corresponding

Chapter 3

From transcription to translation: global translational properties of fission yeast mRNAs and integration with other genome-wide data sets on gene expression

From transcription to translation: global translational properties of fission yeast mRNAs and integration with other genome-wide data sets on gene expression

This chapter will provide a global view of translational efficiency of mRNAs in vegetatively growing fission yeast cells measured by translational profiling. Furthermore, other genome-wide data sets on various aspects of gene expression regulation such as mRNA steady-state levels, poly(A) tail length of mRNAs, mRNA half-lives and transcriptional efficiency and the connections between these diverse layers of gene expression regulation will be presented. Data from this chapter have been published in Molecular Cell (Lackner et al. 2007).

Introduction

It is important to recognize that gene expression can be regulated at multiple levels, and cells need to coordinate different regulatory processes to function properly. Transcriptional rates, mRNA features such as poly(A) tail length, association with RNA-binding proteins and mRNA half-lives as well as translational rates all make a contribution to regulating gene expression in the cell (Hieronymus and Silver 2004; Mata et al. 2005). There is increasing evidence that these processes at the complex interplay between DNA, RNA and the regulatory apparatus are integrated with each other (Maniatis and Reed 2002; Orphanides and Reinberg 2002; Proudfoot et al. 2002; Moore 2005). Most data supporting this idea have been generated through numerous in-depth studies focussing on single genes which are regulated at several levels.

Complementary to the single gene approach, large-scale approaches have given us new insights into gene expression regulation from a genome-wide perspective. For many of these studies, microarrays were mostly used to measure mRNA steady-state levels for expression profiling (Lockhart and Winzeler 2000). Recently, sophisticated variations of microarray-based approaches made genome-wide measurements of additional aspects of gene expression possible (Hieronymus and Silver 2004; Mata et al. 2005). Many of these approaches were pioneered in the budding yeast *Saccharomyces cerevisiae*. Examples include genome-wide studies on mRNA half-

lives (Wang et al. 2002; Grigull et al. 2004), RNA-binding proteins (Gerber et al. 2004), and translation (Arava et al. 2003; Preiss et al. 2003; MacKay et al. 2004). These global data sets provide supplementary and unique views on specific aspects of gene expression and allow the discovery of unexpected connections; for example, translational profiling revealed that long mRNAs are less densely associated with ribosomes than are short mRNAs (Arava et al. 2003).

While traditional studies can address multiple aspects of regulation for one or a few genes, genome-wide studies typically are restricted to one aspect of regulation. It is not clear to what degree different regulatory levels of gene expression are inter-connected at a global scale, and whether any global patterns are conserved during evolution. For a comprehensive understanding of gene expression, it will be important to obtain and integrate global data sets covering as many regulatory aspects as possible, given that the cell itself regulates and coordinates multiple levels of gene expression.

This work was aimed at gaining insights into key aspects of gene expression in the fission yeast *Schizosaccharomyces pombe* on a genome-wide scale with a focus on translation. To this end, a detailed analysis of genome-wide translational properties was complemented by a range of other large-scale data for context and comparisons. Besides using established methods to determine translational profiles, mRNA steady-state levels and mRNA half-lives, novel microarray-based approaches were applied to estimate poly(A) tail lengths and transcription rates. The integrated analyses further incorporated publicly available data on *S. pombe* ORF lengths (Wood et al. 2002) and protein levels (Matsuyama et al. 2006). The systematic and quantitative data sets from this multi-dimensional approach, all acquired using a standardized growth condition and coherent methodology, helped to uncover global connections and trends that would not be apparent from studies involving only a few genes, and they revealed remarkably widespread relationships between multiple layers of gene expression. Furthermore, these data provided us with a valuable basis for the measurement of changes in translation efficiency in cells under different conditions such as after the exposure to stress (see Chapters 4 and 5).

Establishing polysome fractionation

To obtain data on translation in fission yeast, we wanted to determine the association of ribosomes with mRNAs on a global scale. To this end, polysome profiling combined with microarray analysis was used: Cycloheximide is added to the cells, which blocks translation and "freezes" the ribosomes on the mRNA. Cell lysates are then subjected to ultracentrifugation on a sucrose gradient to resolve mRNA-ribosome particles according to density, which corresponds to the number of bound ribosomes. The gradient is then fractionated by upward displacement with 55% sucrose and RNA absorbance is measured at 254 nm. RNA can then be extracted from the collected fractions, which correspond to mRNAs with increasing numbers of bound ribosomes, and probed on microarrays.

At the early stages of this study, 5-45% sucrose gradients were used to fractionate mRNA-ribosome particles and ultracentrifuge runs were done at 39,000 rpm for 160 min. A representative polysome profile is depicted in Figure 3.1A. To confirm that the peaks in Figure 3.1A correspond to the indicated ribosomal subunits (40S, 60S), monosome (80S) and polysome fractions (2 and more bound ribosomes), the distribution of ribosomal RNA (rRNA) along the profile was determined.

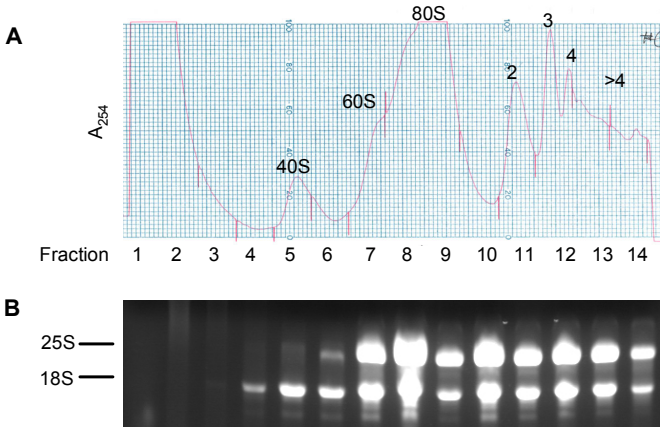


Figure 3.1 Polysome profile of ribosomes isolated from *S. pombe* and resolved by velocity sedimentation through a 5-45% sucrose gradient
(A) The positions of free small (40S) and large (60S) ribosomal subunits, monosomes (80S), and polysomes (2–4 ribosomes and above) are indicated in the profile.
(B) RNA was extracted from each fraction and an aliquot of each fraction was resolved using a 1% agarose gel and stained with ethidium bromide. The 2 most prominent bands correspond to 25S and 18S ribosomal RNA.

RNA was extracted from 14 fractions equally spaced along the profile (see Chapter 2), and equal amounts of RNA from each fraction were loaded onto a 1% agarose gel and stained with ethidium bromide, which is sufficient to visualize rRNAs given that they are the most abundant RNA species in growing cells. The occurrence of 18S RNA, which is part of the 40S ribosomal subunit, and 25S RNA, which is part of the 60S ribosomal subunit corresponded well with the peaks in the polysome profile (Figure 3.1B).

It was also important to confirm that mRNA was efficiently extracted from these fractions and that the amount of a given mRNA in the individual fractions would correspond to its translational efficiency. Actin is a very abundant protein in the cell (Futcher et al. 1999; Lu et al. 2007), and it could be assumed that *actin* mRNA is efficiently translated and most of its mRNA should be associated with the heavy polysome fractions. To test this, aliquots of mRNA extracted from each fraction were resolved on a 1% formaldehyd-agarose gel and analyzed by Northern blotting with a probe specific for *act1* mRNA (see Chapter 2). As expected, most *act1* mRNA was associated with fractions 12-14 of the polysome profile (Figure 3.2).

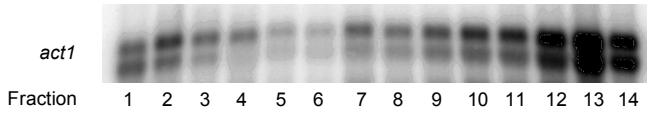


Figure 3.2 Association of *actin* mRNA across the polysome profile
Aliquots of each fraction were resolved on a 1% formaldehyd-agarose gel and analysed by Northern Blotting with a probe specific for actin. Most *act1* mRNA could be found associated with polysomal fractions. The 2 bands of different size probably belong to 2 transcripts with alternative 3' UTRs (Mertins and Gallwitz 1987).

In the 5-45% sucrose gradients, we were able to obtain single peak resolution of up to 4 ribosomes bound to mRNA in the polysome fractions (Figure 3.1A, Figure 3.3 top panel). To enhance this resolution, we used various gradients with differing sucrose concentrations for the polysome profiling and also varied the time and speed for the ultracentrifugation step. Best results were obtained using a 10-50% sucrose gradient and running the gradients at 35,000 rpm for 160 min. Using these conditions, we could resolve up to 8 ribosomes bound to mRNA as a singleton peak (Figure 3.3). Thus, unless otherwise indicated, polysome profiling in this study was done using these conditions.

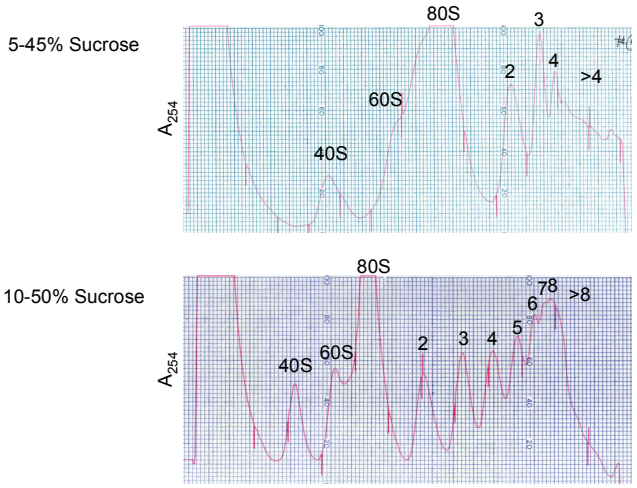


Figure 3.3 Comparison of polysome profiles obtained using sucrose gradients with different concentrations
Polysome profile of ribosomes isolated from *S. pombe* and resolved by velocity sedimentation through a 5-45% sucrose gradient run at 39,000 rpm for 160 minutes (top panel) or a 10-50% sucrose gradient run at 35,000 rpm for 160 minutes (bottom panel). The positions of free small (40S) and large (60S) ribosomal subunits, monosomes (80S), and polysomes (2–8 ribosomes and above) are indicated in the profile.

Genome-wide translational profiling

To determine the translational characteristics of mRNAs in fission yeast at a genome-wide scale, we prepared polysome profiles and hybridized microarrays with twelve mRNA fractions representing different numbers of associated ribosomes (Figure 3.4A). Normalization of the microarray data was done based on spiked-in bacterial mRNAs to correct for different RNA amounts in each fraction (see Chapter 2). Using this approach, we obtained high-resolution translational data for vegetative *S. pombe* cells growing exponentially in minimal medium at 32ºC.

Figure 3.4B provides examples of translation profiles from three independently repeated experiments. There was high reproducibility between these experiments. We verified that transcripts peaked in the expected fractions. For instance, the non-coding *rrk1* RNA (RNase P K-RNA; Krupp et al. 1986) peaked in fraction 2, reflecting an absence of associated ribosomes as expected for an RNA that is not translated. The *fba1* mRNA, encoding fructose-bisphosphate aldolase, peaked in fraction 11, reflecting an association with many ribosomes for most of the mRNA. Consistent with this, Fba1p is highly expressed and within the top 1% with respect to protein

levels (Hwang et al. 2006). The 78-nucleotide *rpl4101* is the shortest mRNA in *S. pombe* and is therefore not expected to be associated with many ribosomes; accordingly, it peaked around fraction 6, which corresponds to the binding of a single ribosome (Figure 3.4A,B). *Actin* mRNA showed a similar distribution throughout the fractions measured using microarrays (Figure 3.4B) or Northern blotting (Figure 3.2). Furthermore, these profiles obtained by microarrays corresponded well with independent profiles obtained by quantitative PCR in another study (Bachand et al. 2006).

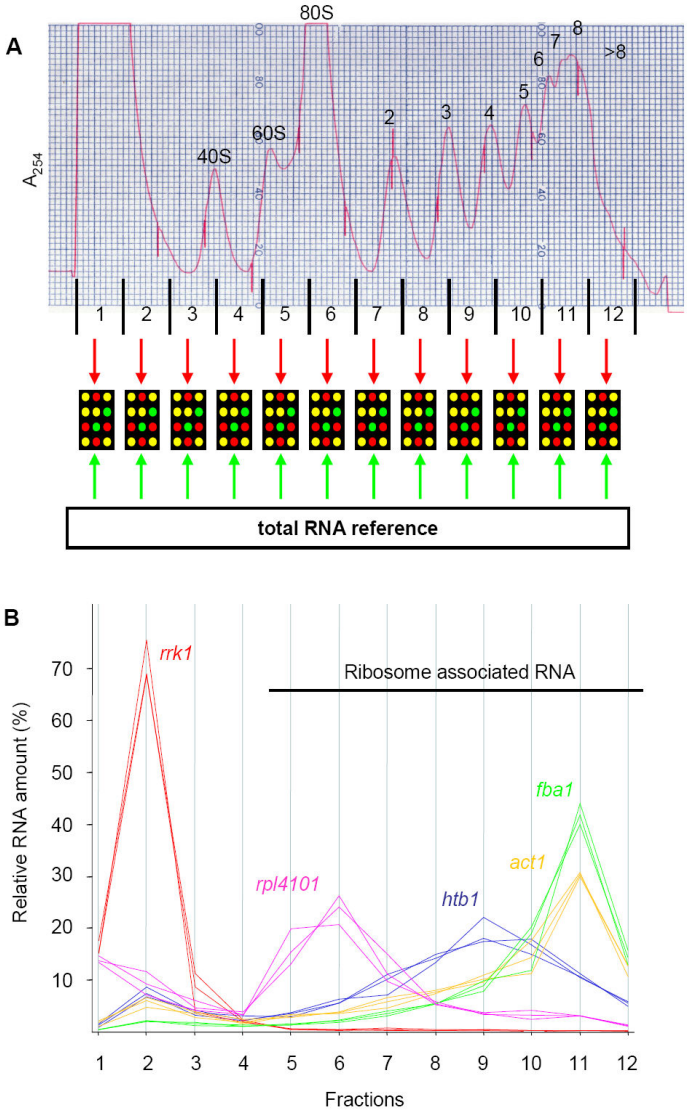


Figure 3.4 High-resolution polysome profiling
(A) Polysome profile of ribosomes isolated from vegetatively growing *S. pombe* cells and resolved by velocity sedimentation through a 10-50% sucrose gradient. The positions of free small (40S) and large (60S) ribosomal subunits, monosomes (80S), and polysomes (2–8 ribosomes and above) are indicated in the profile. RNA extracted from 12 fractions equally

spaced throughout the profile (bottom) was labelled and hybridized against a total RNA reference on microarrays containing all *S. pombe* genes.
(B) Translation profiles for selected transcripts obtained by microarray analysis, showing the relative RNA amounts for a given transcript contained in each of the 12 fractions. Fractions associated with ribosomes are indicated. Different transcripts are colour-coded, and polysome profiles from three independent biological repeats are shown for *rrk1* (RNase P K-RNA), *rpl4101* (encoding ribosomal protein), *htb1* (encoding histone H2B), *fba1* (encoding fructose-biphosphate aldolase) and *act1* (encoding actin).

Figure 3.5A shows average translational profiles for selected groups of transcripts. The profile of all mRNAs that provided translational data showed a peak in fraction 3 (reflecting free mRNA) along with a broad peak covering fractions 7-11 (reflecting polysomes of different sizes). Introns that were included on the microarrays peaked in fraction 3, which is not associated with ribosomes, as expected given that translation occurs on spliced mRNA. Conversely, mRNAs associated with Gene Ontology (GO) terms for translational regulation were associated with many ribosomes as expected for these highly expressed genes (Hwang et al. 2006). A list of 377 mRNAs encoding secreted proteins, which are translated on the endoplasmic reticulum membrane, showed an almost identical average translation profile to the one for all mRNAs (Figure 3.5B), indicating that the ribosome distribution for this specialized group is similar.

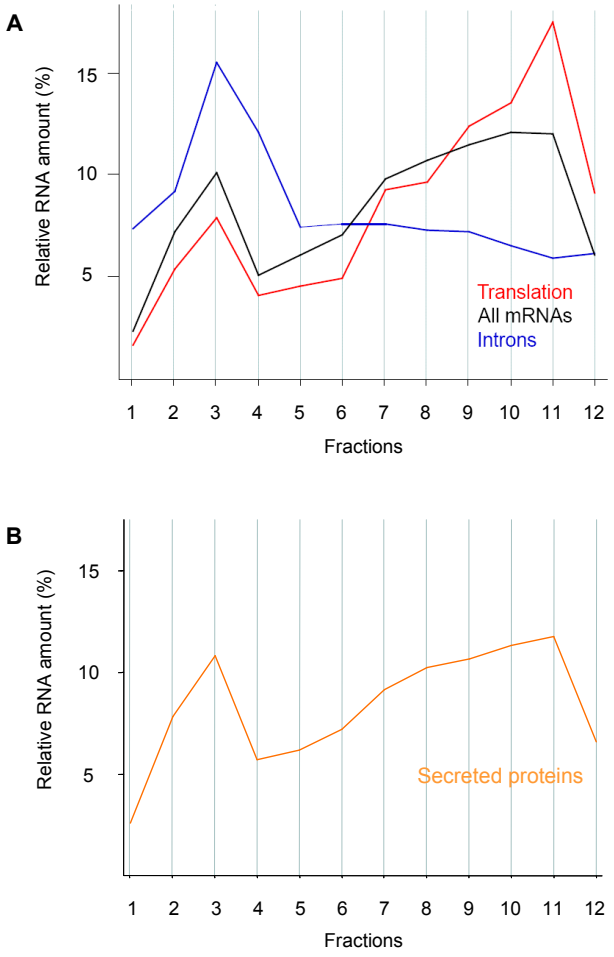


Figure 3.5 Average translation profiles for selected groups of RNAs
(A) Average translation profiles for selected groups of RNAs, plotted as in Fig. 3.4B for one experiment. All mRNAs, the 3505 high-confidence mRNAs with complete profiles in this experiment; Introns, 11 long introns included on the microarray; and Translation, 62 mRNAs associated with the GO terms “translational intiation,” “translational elongation,” or “translational termination”.
(B) Average translation profiles from the same experiment as in **(A)** for 377 mRNAs encoding secreted proteins.

Global translational properties of mRNAs

Although polysome profiles for almost all mRNAs were obtained, for further analysis we focussed on a conservative, high-confidence set of 3598 (72.5%) out of the 4962 nuclear encoded protein-coding genes. For a mRNA to be included in the high-confidence it had to fulfil the following criteria: (1) microarray data had to be available for at least 2 out of the 3 experiments for all 12 fractions, and (2) there had to be a minimum correlation of the profiles from different repeats (see Chapter 2 for details). Most of the excluded mRNAs were not or only weakly expressed under the condition used, which could be seen by looking at the disribution of these excluded genes according to relative expression levels measured using Affymetrix chips (Figure 3.6). These genes were also most enriched for GO terms related to meiosis ($P < 4e^{-24}$). From the translation profiles of the mRNAs included in the analysis, we determined different properties reflecting translational efficiency (see Chapter 2); these data on translational properties described below are provided in supplementary Table S1, which can be downloaded from http://www.sanger.ac.uk/PostGenomics/S_pombe/projects/translation/.

Ribosome occupancy indicates the percentage of a given type of mRNA that is associated with one or more ribosomes as opposed to free mRNA. The average ribosome occupancy was 77.3% with a relatively small standard deviation (SD) of 7.0%. This suggests that during exponential growth the majority of high-confidence mRNAs from most genes are engaged in translation, although a substantial fraction of >20% of mRNAs is not associated with any ribosomes.

The mean number of ribosomes bound to a given mRNA was calculated based on a weighted average by using the relative amount of the mRNA associated with each fraction and the number of ribosomes corresponding to that fraction. Only fractions associated with ribosomes were included for this (Figure 3.4A, B; fractions 5-12) so that the mean ribosome number is independent of ribosome occupancy. On average, 4.1 ribosomes were associated with mRNAs with a surprisingly small SD of 0.6. If the mRNAs not associated with ribosomes were also taken into account, this value was lowered to 3.6 ribosomes. As expected, the mean number of associated ribosomes generally increased as a function of open reading frame (ORF) length (Figure 3.7A), but this correlation breaks down for mRNAs with a length of over ~1.2 kilobases (kb)

and is more pronounced for shorter mRNAs such as mRNAs encoding ribosomal proteins (Figure 3.7B).

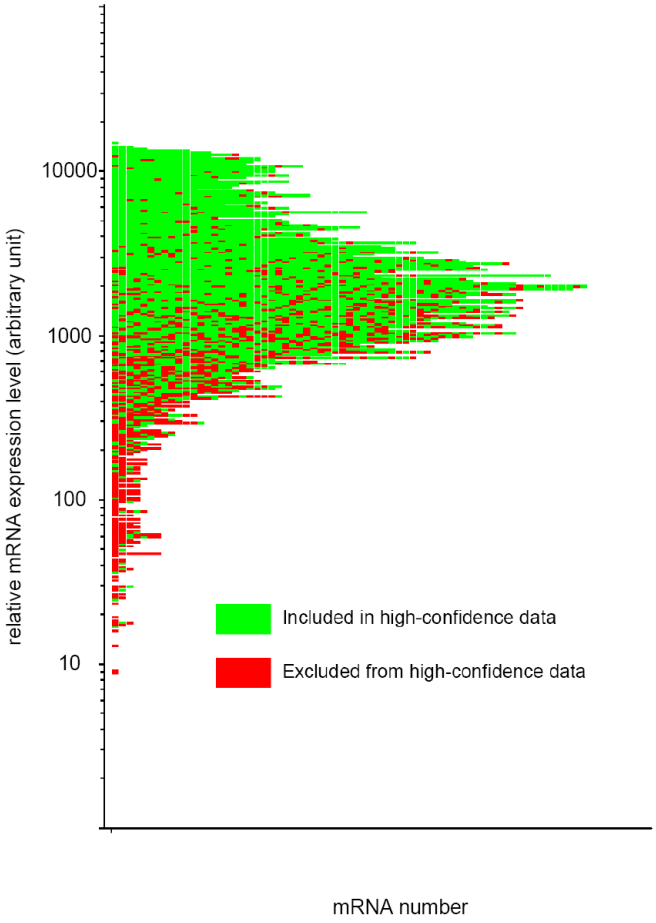


Figure 3.6 Distribution of mRNA levels for protein-coding genes included or excluded from high-confidence translational profiling data
Histogram showing the mRNA levels for 4818 protein-coding genes that provided signal data on Affymetrix chips. The average of two independent experiments is shown. Green: genes included in the high-confidence data set from the translational profiling experiments (3567 genes with measurable chip signals). Red: genes not included in the high-confidence data set (1251 genes with measurable chip signals).

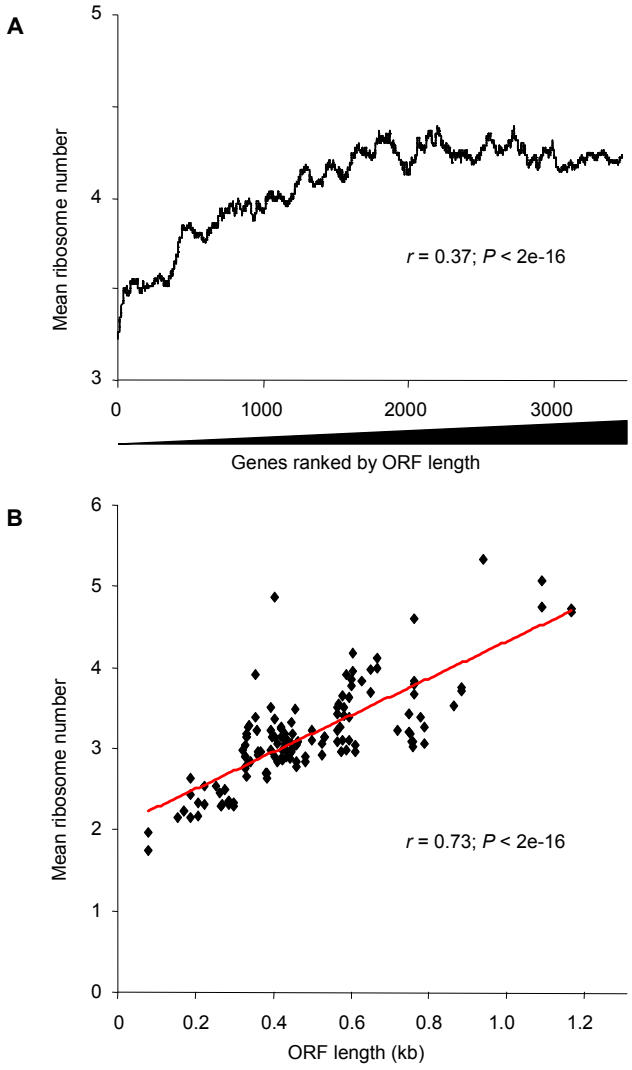


Figure 3.7 Correlation between ORF length and mean number of associated ribosomes
(A) Graph showing moving averages (100-gene window) of mean ribosome number as a function of genes ranked by ORF length (n = 3598), along with the corresponding Spearman rank correlation.
(B) Scatter plot of ORF length against mean number of associated ribosomes for mRNAs encoding ribosomal proteins (n = 134). The red line represents the linear trend-line for this correlation. The corresponding Spearman rank correlation between ORF length and mean ribosome number is also shown.

Arguably, the ribosome density is a better measure than the mean ribosome number to estimate translational efficiency as it normalizes for different mRNA lengths that influence the numbers of bound ribosomes (Figure 3.7A) (Arava et al. 2003; Beyer et al. 2004). The average ribosome density for all mRNAs was 4.5 ribosomes per kilobase of ORF, with a relatively large SD of 3.1 ribosomes per kb. On average, the

mRNAs thus contained one ribosome roughly every 222 nucleotides. Given that a eukaryotic ribosome occupies ~35 nucleotides of mRNA (Wolin and Walter 1988), the average density determined here is only about 1/6 of the maximal packing density. This is consistent with initiation being the rate-limiting factor during translation.

The sequence context of the AUG start codon influences the rate of translational initiation (Kozak 1991). To corroborate that high ribosome occupancy and density in our data reflect efficient translational initiation rather than slow elongation or ribosome stalling, we compared ribosome occupancy and density with the "AUG context adaptation index" (AugCAI), a measure for the effectiveness of the AUG context to promote translational initiation (Miyasaka 1999; Miyasaka 2002). Data for the AugCAI in fission yeast were calculated by Samuel Marguerat. Basically, mRNAs are assigned a score depending on the overlap with a consensus sequence around the AUG start codon derived from the 100 most abundant mRNAs, with a higher score for higher translation initiation efficiency. This analysis provided a consensus sequence for optimal translational initiation in *S. pombe* and revealed significant correlations between the AugCAI on one hand and ribosome occupancy and density on the other (Figure 3.8). This provides independent evidence that the translational profiling data are measures of translational efficiency.

We next looked for poorly and strongly translated mRNAs. Of the 3598 high-confidence mRNAs, only 57 showed ribosome occupancies of less than 60%, and 99 showed densities of less than one ribosome/kb on average. Just one mRNA (*urb2*, predicted role in ribosome biogenesis) was present in both of these groups. The 20% of mRNAs showing the highest ribosome occupancy were most enriched for transcripts repressed during stress ($P \sim 8e^{-30}$; Chen et al. 2003) and for those associated with the GO terms 'metabolism' and 'biosynthesis' ($P \sim 1e^{-30}$-$2e^{-31}$). The 20% of mRNAs with the lowest ribosome occupancy were diverse and showed no strong enrichment for any particular GO terms or functional groups. The 20% of mRNAs showing the highest ribosome density were most enriched for GO terms such as 'ribosome', 'organelle', and several terms related to mitochondria ($P \sim 1e^{-12}$ - $7e^{-42}$) and for transcripts containing introns ($P \sim 5e^{-17}$), which is notable given that introns can enhance translation in mammals (Nott et al. 2003). The 20% of mRNAs showing the lowest ribosome density were most enriched for genes with GO terms such as 'ATP-binding', 'hydrolase activity', 'signal transduction', and related terms ($P \sim 2e^{-10}$-$4e^{-26}$). The mRNAs with low ribosome density were also strongly enriched for the

longest mRNAs, while those with high ribosome density were enriched for the shortest mRNAs. This suggested a connection between mRNA length and ribosome density as analysed below.

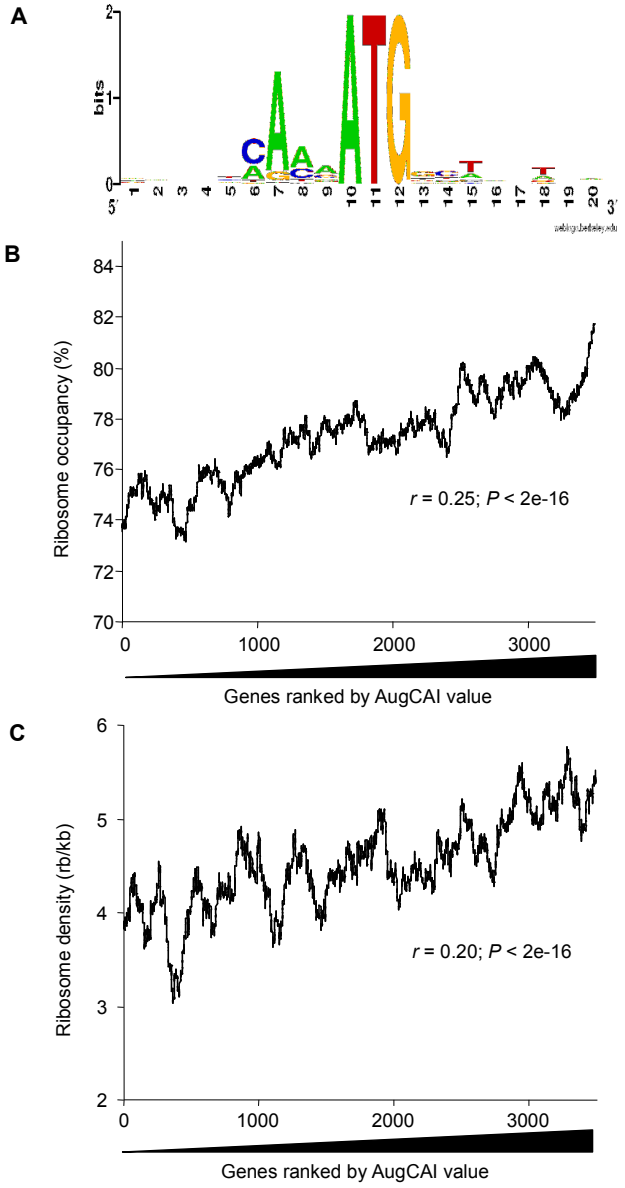


Figure 3.8. Correlations of AugCAI values with translation efficiency
(A) Consensus sequence for optimal translation initiation derived from the 100 most abundant mRNAs. The WebLogo tool was used for visualization (weblogo.berkeley.edu). Based on this consensus sequence, AugCAI values were calculated for all mRNAs.
(B) Graph showing moving averages (100-gene window) of ribosome occupancy as a function of genes ranked by AugCAI values (n = 3593), along with the corresponding Spearman rank correlation.
(C) Graph showing moving averages (100-gene window) of ribosome density as a function of genes ranked by AugCAI values (n = 3593), along with the corresponding Spearman rank correlation.

Short mRNAs are more efficiently translated

Whereas the mean ribosome numbers varied by less than 4 fold (1.8 to 6.8 ribosomes per mRNA), the ORF lengths varied more than 180 fold (78 to 14154 bp; Wood et al. 2002). Accordingly, the ribosome numbers showed only modest increase relative to ORF length, and they did not increase above ~4.3 ribosomes on average for mRNAs longer than ~1200 bp (Figure 3.7). Consistent with this, the group of mRNAs with the lowest ribosome densities was highly enriched for the longest mRNAs, and the SD for ribosome density was much larger than for ribosome numbers (see above). These observations indicate that the range of associated ribosomes does not keep up with the range of ORF lengths, and ORF length is therefore expected to be a major factor determining ribosome density. There was indeed a strong inverse correlation between ORF length and ribosome density (Figure 3.9A). Short mRNAs were much more tightly packed with ribosomes than were long mRNAs. This inverse correlation was evident over the whole range of ORF sizes and ribosome densities. A similar inverse correlation was obtained when using mRNA lengths instead of ORF lengths based on 198 mRNAs for which untranslated regions (UTRs) are available from *S. pombe* GeneDB ($r = -0.9$; $P < 2e^{-16}$).

We were concerned that this inverse correlation might reflect a systematic artefact of the translational profiling approach. A bias could arise from underestimating the numbers of ribosomes in the more poorly resolved higher fractions where single-peak resolution for polysomes cannot be achieved (Figure 3.4A). We observed a similar negative correlation, however, when using only the relatively short mRNAs encoding ribosomal proteins (Figure 3.9B; Figure 3.7B); these mRNAs showed defined peaks in the well-resolved fractions of the polysome profiles where ribosome numbers can be determined with confidence (Figure 3.4A, fractions 6-10). To further exclude a possible error due to underestimating ribosomes, we associated double the originally estimated number of ribosomes associated with fraction 12 (~20 ribsomes instead of 10), which slightly increased the ribosome number for transcripts that stronlgy peaked in this very heavy polysome fraction. This re-analysis resulted in a similar negative correlation between ribosome density and ORF length (Figure 3.10), suggesting that underestimation of ribosome numbers for long and strongly translated mRNAs is not the cause of the negative correlation between ORF length and ribosome density. Moreover, there was a significant inverse relationship between the AugCAI and ORF

length (r = -0.15; $P < 2e^{-16}$), providing independent evidence for a link between ORF length and translational efficiency.

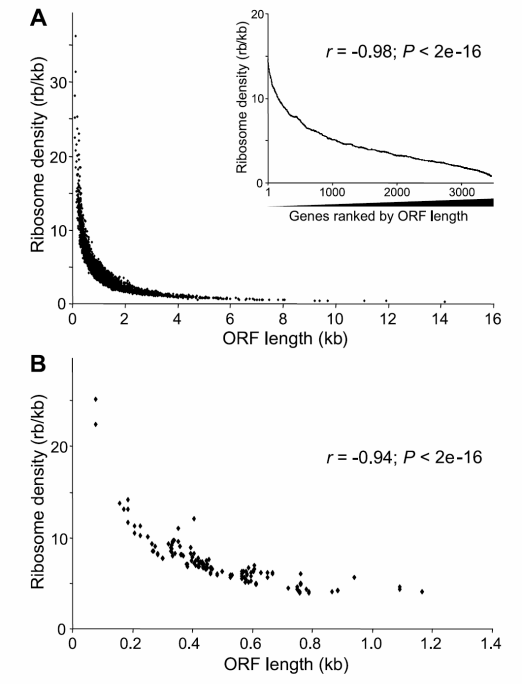


Figure 3.9 Inverse correlation between ribosome density and ORF length
(A) Scatterplot of ribosome density plotted against ORF length for the high-confidence set of protein-coding genes (n = 3598). The small inset graph shows moving averages (100-gene window) of ribosome density as a function of genes ranked by ORF length. The corresponding Spearman rank correlation between ribosome density and ORF length is also shown.
(B) Scatterplot of ribosome density plotted against ORF length as in (A) but showing only the mRNAs encoding ribosomal proteins (n = 134), along with the corresponding Spearman rank correlation.

We also observed a significant inverse correlation between ORF length and ribosome occupancy, although much less pronounced than for ribosome density (r = -0.27; $P < 2e^{-16}$). Together, these data raise the possibility that ORF length is a major factor for translational efficiency. We therefore expected that long proteins should be present in lower levels in the cell than short proteins due to differences in translational efficiency. To test this hypothesis, we took advantage of recent data on expression levels of nearly all *S. pombe* proteins (Matsuyama et al. 2006). For this study, all ORFs were cloned into the same vector, integrated into the same genomic site, and transcribed under the control of the same promoter. These data should therefore be minimally affected by differences in transcription or by differences in regulation of mRNA stability and translation via UTR sequences, as the lengths and sequences of

the ORFs are the only remaining factors that could influence translational efficiency, which (along with protein turnover) will determine protein levels. Protein levels in this study were then determined using a protein-based array technique, where tagged proteins were detected with an anti-His-tag antibody, and as internal reference α-tubulin was simultaneously quantified with an anti-α-tubulin antibody. The ribosome densities showed a significant positive correlation with protein levels from this study, while ORF length negatively correlated with protein levels on a global scale as predicted from our translational profiling data (Figure 3.11). For proteins present at lower levels, the correlations with ribosome density and ORF length were less evident, possibly due to increased noise. The protein levels also showed a significant correlation with ribosome occupancy ($r = 0.31$; $P < 2e^{-16}$) and a weak correlation with ribosome numbers ($r = 0.17$; $P < 2e^{-16}$). This is consistent with the intuition that ribosome density and ribosome occupancy are better measures for translational efficiency than the number of ribosomes associated with mRNAs. We conclude that ORF length is an important factor for translational efficiency and protein levels.

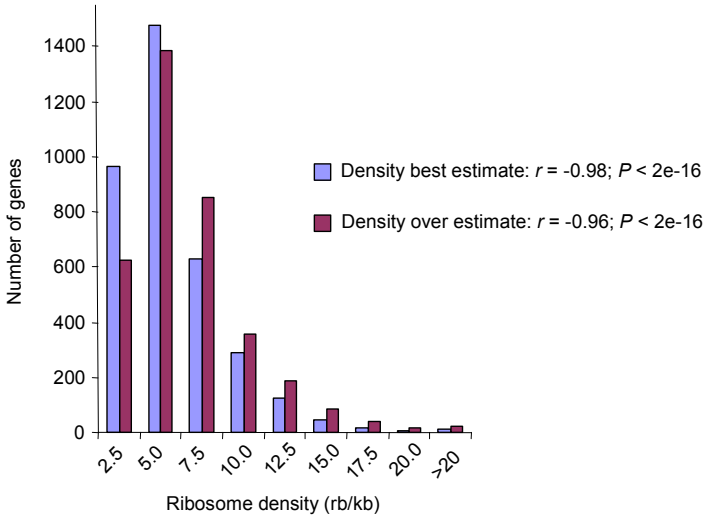


Figure 3.10 Overestimation of ribosome number for fraction 12 does not affect negative correlation between ribosome density and ORF length
Histogram using bins of different ribosome densities (upper bin limits given on X axis). Blue: distribution of ribosome densities calculated as described in Chapter 2. Purple: distribution of ribosome densities calculated the same way, except that the number of ribosomes associated with mRNAs in fraction 12 were 2 fold overestimated. The corresponding Spearman rank correlations between ORF length and ribosome densities calculated in both ways are also shown.

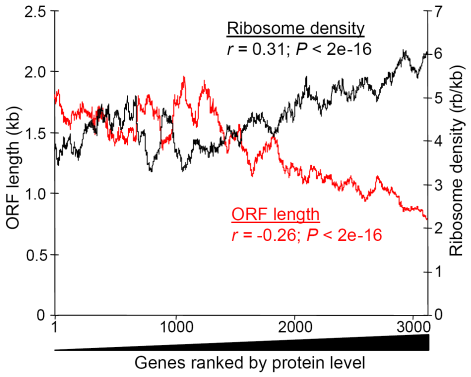


Figure 3.11 Correlations between ORF length/ribosome density and protein level
Graph showing moving averages (100-gene window) of ribosome density (black) and ORF length (red) as a function of genes ranked by protein level (n = 3265). The corresponding Spearman rank correlations between protein level and ribosome density (n = 3265) and between protein level and ORF length (n = 4434) are also shown.

Genome-wide measurement of poly(A) tail length

The lengths of the poly(A) tails of mRNAs are thought to determine the efficiency of translational initiation based on single-gene studies (Preiss and Hentze 1998; Sachs 2000; Wickens et al. 2000; Stevenson and Norbury 2006). We therefore wondered whether translational efficiency might be reflected in poly(A) tail lengths on a genome-wide scale. To obtain global data on polyadenylation we used a technique called polyadenlyation state array (PASTA) analysis: mRNAs are fractionated using a poly-U sepharose column and eluted at five different temperatures. The resulting five fractions correspond to mRNAs with different ranges of poly(A) tail length and were then hybridized to microarrays using total eluate as a reference (Figure 3.12). The fractionation of the mRNAs according to their length was done in collaboration with Traude Beilharz and Thomas Preiss (Victor Chang Cardiac Research Institute, Sydney, Australia), who have established this technique in the budding yeast *Saccharomyces cerevisiae* (Beilharz and Preiss 2007). The five fractions contained distinct, but partially overlapping distributions of poly(A) tail lengths (Figure 3.13).

Sizes of the poly(A) tail length ranged from ~10 to 80 nucleotides (nt) with the following peak sizes for each fraction: 12°C, ~10 nt; 25°C, ~22 nt; 30°C, ~30 nt; 35°C, ~40 nt; and 45°C, ~57 nt. These polyadenylation data revealed a continuous distribution of poly(A) tail lengths, both for specific mRNAs and between different mRNAs. There was minor cross-contamination of long-tailed mRNAs in the first 2 elution fractions. These transcripts may have bound non-specifically to the matrix or through poly(A) runs within the body of the transcript. Furthermore, short A-tract fragments were inefficiently precipitated in the bulk end-labelling experiment and as a consequence there is only weak signal on the gel from the first fraction (Figure 3.13). Nevertheless, the poly(A) profiles for different mRNAs were enriched for distinct sizes.

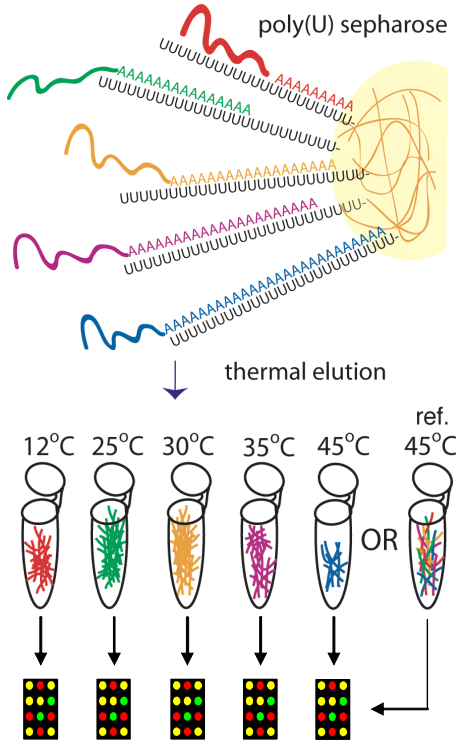


Figure 3.12 Experimental layout for polyadenylation state array (PASTA) mRNAs are fractionated using a poly(U) sepharose column and eluted at different temperatures. The resulting fractions contain mRNA with increasing poly(A) tail length, which are then hybridized onto a DNA microarray against total eluate as reference. Figure adapted from Beilharz et al. (2007).

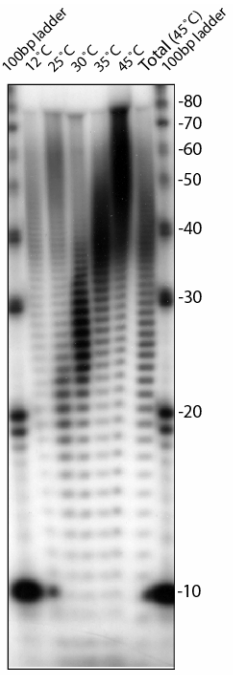


Figure 3.13 mRNAs fractionated using poly(U)-sepharose chromatography
Gel of poly(A) tail length tracts for mRNAs eluted from a poly(U) sepharose column at increasing temperatures as indicated on top, showing mRNAs with increasing poly(A) tail length from *S. pombe*. Aliquots of each mRNA fraction were end labeled with [^{32}P]-pCp, digested with RNases A and T1n, and poly(A) fragments analyzed by denaturing PAGE. Nucleotide numbers corresponding to the 100 bp ladder are indicated at right.

A modified RT-PCR assay, termed ligation–mediated poly(A) test (LM-PAT) (Salles and Strickland 1995; Beilharz and Preiss 2007) was used to verify the poly(A) profiles derived from the PASTA analysis (Figure 3.14). In this assay, PCR products between a gene-specific primer at the end of the ORF or within the 3' UTR and an anchored primer at the end of the poly(A) tail are generated. These PCR products reflect the poly(A) tail length of a specific mRNA. It should be mentioned that the use of oligo(dT)$_{12\text{-}18}$ mix and (dT)$_{12}$-anchor primes introduces a certain laddering of the products. Especially the shortest band represents all short tails (~7-22 nt) that can be bound by a (dT)$_{12}$-anchor but that cannot accommodate additional oligo(dT)$_{12\text{-}18}$ primers.

The distribution of poly(A) tails for mRNAs representing different tail lengths showed good agreement between the PASTA analysis and the LM-PAT-assay (Figure 3.15): *for3* mRNA, encoding formin, was enriched for short poly(A) tails in the PASTA analysis (Figure 3.15A), and consequently PCR products from the LM-PAT assay

could only be detected in the first fractions (Figure 3.15B). mRNAs encoding the ribosomal proteins *rps27* and *rpl14* showed peaks in the fractions enriched for poly(A) tails of medium length in both assays (Figure 3.15A,B). *Hhf1* and *hhf2*, two almost identical mRNAs encoding histones, peaked in the fractions enriched for long poly(A) tails (Figure 3.15A,B). Note that histone mRNAs in yeast are polyadenylated in contrast to most higher eukaryotes (Fahrner et al. 1980; Butler et al. 1990).

Moreover, mitochondrially encoded mRNAs, which lack long poly(A) tails in fission yeast (Schäfer et al. 2005), showed the expected peak in the first fraction in the PASTA analysis (Figure 3.16).

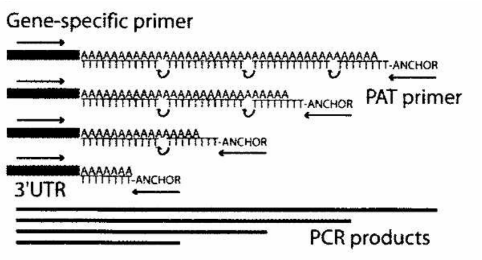


Figure 3.14 Experimental layout of LM-PAT assay

mRNAs are incubated with oligo(dT)$_{12-18}$ primers in the presence of T4-DNA ligase at 42°C followed by ligation of an oligo dT$_{12}$-anchor primer, thus covering the full length of poly(A) tails of mRNAs. cDNA is then synthesised from the ligated primers. Aliquots of this cDNA are used in PCR reactions to amplify a region between a site at the 3' end of the ORF of the mRNA under study and the anchor region. This figure is taken from Beilharz et al. (2007).

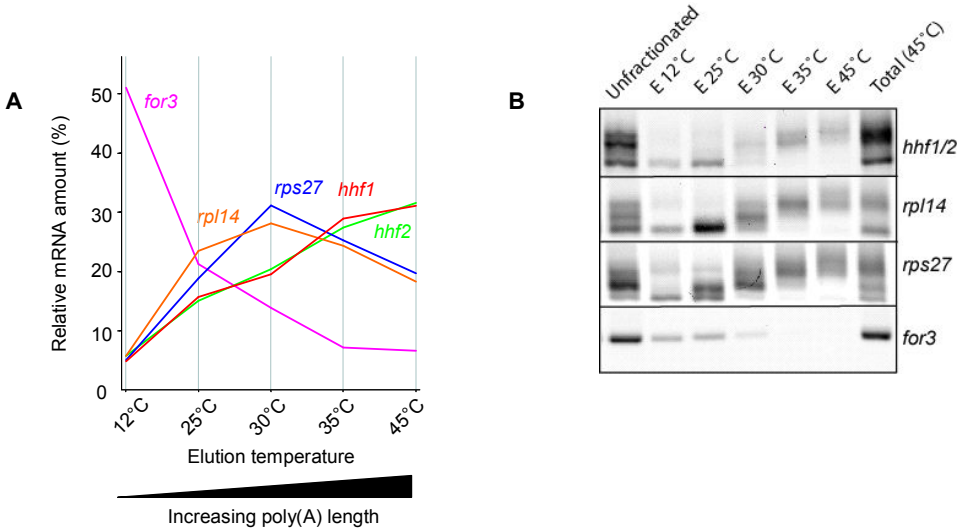


Figure 3.15 Examples of poly(A) tail length determination by LM-PAT assays and PASTA analysis
(A) Profiles of poly(A) tail length distribution for 5 mRNAs as determined by microarray-based PASTA analysis: *hhf1* and *hhf2* (almost identical mRNAs encoding histones, enriched for long poly(A) tails), *rpl14* and *rps27* (mRNAs encoding ribosomal proteins, enriched for poly(A) tails of medium length), and *for3* (mRNA encoding formin, enriched for short poly(A) tails). The curves show the relative amounts of RNA for a given mRNA in each of the five fractions as an average for two independent biological repeats. Different mRNAs are colour-coded.
(B) Distribution of poly(A) tail length determined using LM-PAT assays for the same mRNAs as in **(A)**. Fractions eluted from poly(U) sepharose column at increasing temperatures (corresponding to increasing tail lengths) as indicated on top.

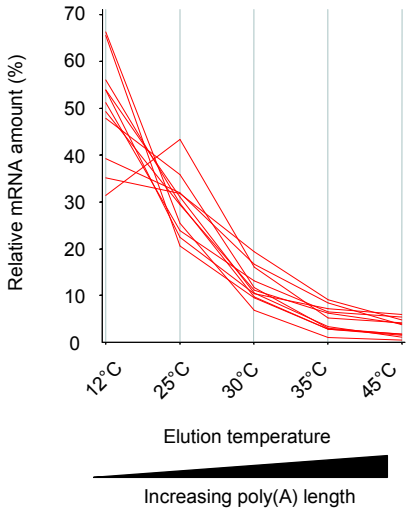


Figure 3.16 Poly(A) tail profiles for mitochondrially encoded mRNAs determined by PASTA analysis
Profiles of poly(A) tail length distribution for 11 mitochondrially encoded mRNAs (*SPMIT.01, SPMIT.02, SPMIT.03, SPMIT.04, SPMIT.05, SPMIT.06, SPMIT.07, SPMIT.08, SPMIT.09, SPMIT.10, SPMIT.11*) as determined by microarray-based PASTA analysis. The curves show the relative amounts of RNA for a given mRNA in each of the five fractions as an average of two independent biological repeats.

mRNAs with long poly(A) tails are more efficiently translated

For the further analyses, only mRNAs for which data could be obtained in all five fractions of two repeated experiments in the PASTA analysis were included. In total, 2795 protein-coding mRNAs fulfilled these criteria of which 2575 were also included in the translational profiling data set. The 20% of mRNAs with the longest tails were most enriched for transcripts repressed during environmental stress ($P \sim 1e^{-15}$; Chen et al. 2003) and for GO terms such as 'biosynthesis', 'cytoplasm', and 'ribosome' ($P \sim 2e^{-16}$ - $2e^{-27}$). The 20% of mRNAs with the shortest tails were most enriched for genes containing predicted nuclear localisation signals ($P \sim 1e^{-18}$) and for GO terms such as 'nuclear lumen', 'nucleolus', 'RNA metabolism', and 'ribosome biogenesis and assembly' ($P \sim 3e^{-8}$ - $3e^{-13}$).

The mRNAs were ranked by relative poly(A) tail length using a weighted average of the relative amounts of mRNA associated with each fraction (Table S1; Chapter 2). Integration of these data with the translational data revealed that poly(A) tail lengths significantly increased with increasing ribosome density (Figure 3.17). Moreover, poly(A) tail lengths increased with decreasing ORF lengths (Figure 3.17), consistent with the strong inverse correlation between ORF length and ribosome density (Figure 3.9). These data were corroborated by genome-wide binding data for the poly(A) binding protein Pab1p using RIP-chip: Pab1p tended to be most enriched in precipitated mRNAs with long poly(A) tails according to the PASTA analysis, and ORF lengths showed a strong inverse correlation with Pab1p enrichment (Juan Mata, unpublished data; see also Beilharz and Preiss 2007). Poly(A) tail lengths also correlated with ribosome occupancy ($r = 0.27$; $P < 2e^{-16}$) and with protein levels ($r = 0.21$; $P < 2e^{-16}$). Together, these data reveal a genome-wide connection between ORF length, poly(A) tail length, and translational efficiency: short mRNAs tend to have long poly(A) tails and are more efficiently translated than longer mRNAs that tend to have shorter poly(A) tails. These connections are ultimately reflected at the protein levels and are most evident for the highly expressed proteins (Figure 3.11).

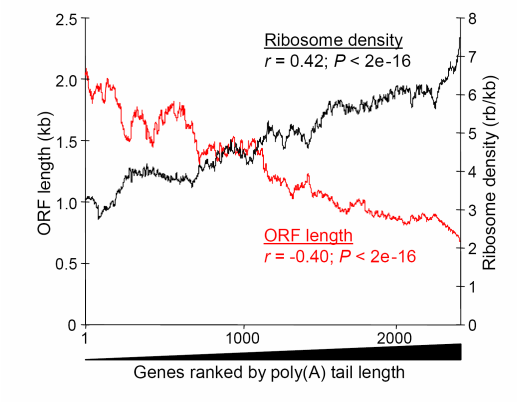


Figure 3.17 Correlations between ORF length and ribosome density and poly(A) tail length Moving averages (100-gene window) of ribosome density (black) and ORF length (red) as a function of 2576 genes ranked by poly(A) tail length. The Spearman rank correlations between poly(A) tail length and ribosome density (n = 2576) and between poly(A) tail length and ORF length (n = 2714) are also shown.

Abundant mRNAs are more efficiently translated

Steady-state mRNA levels are another important determinant of gene expression. mRNA levels in exponentially growing cells were estimated from the absolute hybridization signal intensities using Affymetrix chips (Table S1). These signal intensities should reflect mRNA abundance quite accurately, given that there are several different probes for each mRNA on the array, which should minimize the influence of differential efficiencies of hybridization to different probes. Furthermore, these data were in good agreement with independent data for mRNA levels obtained by hybridizing mRNA against a genomic DNA reference on our in-house DNA microarrays (data not shown). The 10% most abundant mRNAs were most enriched for transcripts repressed during environmental stress ($P \sim 2e^{-86}$; Chen et al. 2003) and for GO terms such as 'ribosome', 'protein biosynthesis', 'cellular metabolism', and related terms ($P \sim 2e^{-55}$ to $1e^{-128}$). The 10% least abundant mRNAs were most enriched for transcripts induced during meiosis and stress ($P \sim 3e^{-15}$ to $7e^{-19}$; Mata et al. 2002; Chen et al. 2003), for *S. pombe* specific transcripts ($P \sim 1e^{-34}$; Mata and Bähler 2003), and for GO terms such as 'meiosis', and 'M-phase' ($P \sim 1e^{-18}$ to $1e10^{-25}$). Note, that mRNA levels did not correlate with ORF lengths ($r = -0.02$; $P = 0.11$) and there was also no correlation when transcript length was used instead of ORF

length (r = -0.06; P = 0.41; calculated using the 198 mRNAs for which 5' and 3' UTR length data are available in *S. pombe* GeneDB (www.genedb.org/genedb/pombe/index.jsp) (Figure 3.18). mRNA levels significantly correlated, however, with poly(A) tail lengths, with the most abundant mRNAs showing a tendency for longer tails (Figure 3.19A).

We then checked for relationships between mRNA levels and translational efficiency. The mRNAs with the lowest expression levels tended to be associated with fewer ribosomes than the mRNAs with the highest levels (Figure 3.19B). This raised the possibility that mRNA abundance is somehow coordinated with translational efficiency. Consistent with this, ribosome densities showed some correlation with mRNA levels (r = 0.14; $P < 2e^{-16}$). Stronger correlations throughout the entire population of mRNAs were apparent between ribosome occupancy and mRNA levels (Figure 3.19C). There was also a significant correlation between the AugCAI and mRNA levels (r = 0.22; $P < 2e^{-16}$). Taken together, these findings indicate a genome-wide coordination between mRNA levels and translational efficiency: more abundant mRNAs tend to be more efficiently translated as reflected by their higher ribosome occupancy and, to a lesser extent, higher ribosome density.

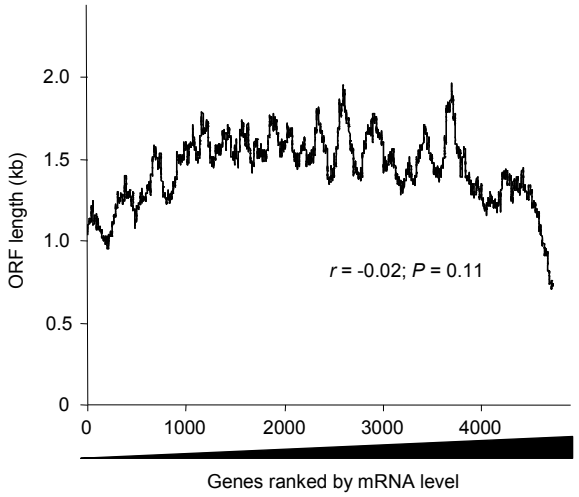


Figure 3.18 No correlation between mRNA levels and ORF length
Graph showing moving averages (100-gene window) of ORF length as a function of genes ranked by mRNA level (n = 4818). The corresponding Spearman rank correlation between ORF length and mRNA level is shown within the graph.

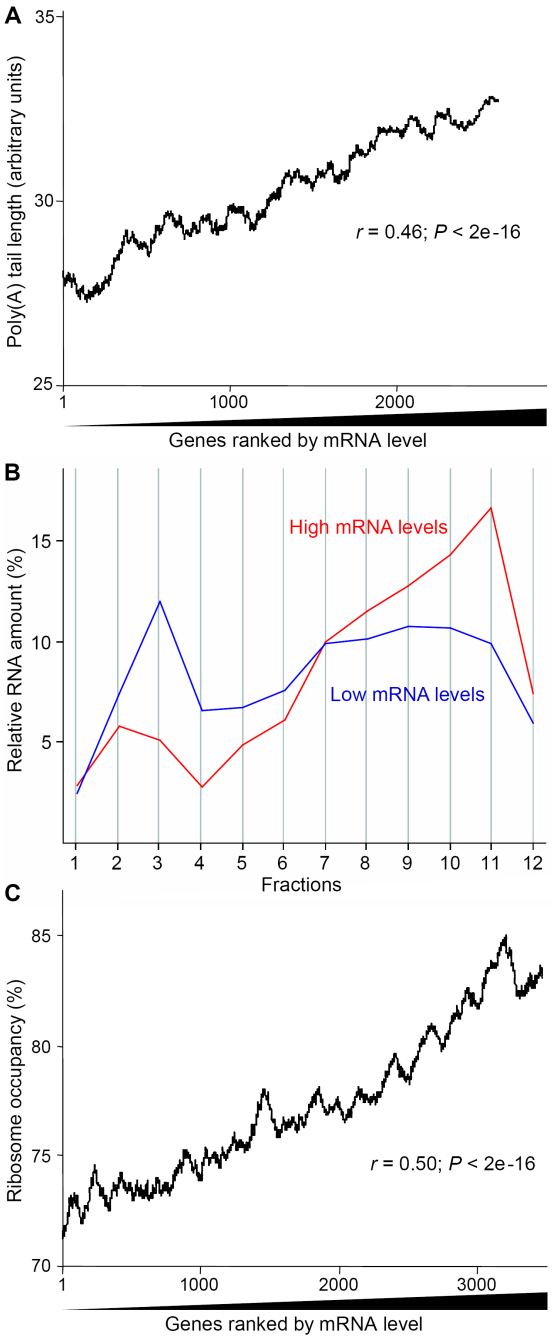


Figure 3.19 Correlations between mRNA level and poly(A) tail length and ribosome occupancy

(A) Graph showing moving averages (100-gene window) of poly(A) tail length as a function of genes ranked by mRNA level (n = 2688), along with the corresponding Spearman rank correlation.

(B) The curves show the average translation profiles of the mRNAs with the 500 highest (red) or 500 lowest (blue) levels. Curves are plotted as in Figure 3.4B.

(C) Graph showing moving averages (100-gene window) of ribosome occupancy as a function of genes ranked by mRNA level (n = 3567), along with the corresponding Spearman rank correlation.

Stable and highly transcribed mRNAs are more efficiently translated

The steady-state level of a given mRNA is determined by the rate of transcription and the rate of decay, both of which are controlled at genome-wide level (Mata et al. 2005). The correlation between translational efficiency and mRNA abundance could therefore reflect a connection between translation and mRNA stability and/or between translation and transcription.

Abundant mRNAs are expected to be more stable on average than less abundant mRNAs. To test whether mRNA stability is linked to translation, global mRNA half-lives were estimated by blocking transcription and measuring mRNA levels at different times after transcriptional shut-off. These experiments were performed by Samuel Marguerat. Cells were treated with the transcriptional inhibitor 1,10-phenanthroline, and mRNA was isolated before and at 4, 12, and 28 min after transcriptional shut-off and mRNA levels were measured using microarrays. Two lists of genes with short and long half-lives were generated from these data (see Chapter 2) (Figure 3.20; Table S1). These experiments provided reliable estimates on relative half-lives for the 868 least stable mRNAs, with half-lives ranging from ~10 to 96 min and a median of ~33 min. This group of unstable mRNAs is significantly enriched for genes with periodic expression during the cell cycle ($P \sim 6e^{-15}$; Rustici et al. 2004; Marguerat et al. 2006); these mRNAs peak in levels during a short phase of the cell cycle and are therefore expected to have short half-lives. The unstable mRNAs were also enriched for genes associated with the GO terms 'regulation of biological process', 'cell communication', 'signal transduction', and 'cell septum' ($P \sim 1e^{-5}$ - $2e^{-8}$). This probably reflects that mRNAs encoding regulatory proteins or proteins only required during a defined stage such as septation need to be tightly controlled. We also selected a similarly sized group of bona fide stable mRNAs whose expression levels were not altered 30 min after transcriptional shut-off (Figure 3.20). This group was most enriched for genes with the GO terms 'cytoplasm' and 'mitochondrial part' ($P \sim 5e^{-5}$ - $9e^{-8}$). As expected, mRNAs with short half-lives were significantly less abundant on average than mRNAs with longer half-lives (Figure 3.21A).

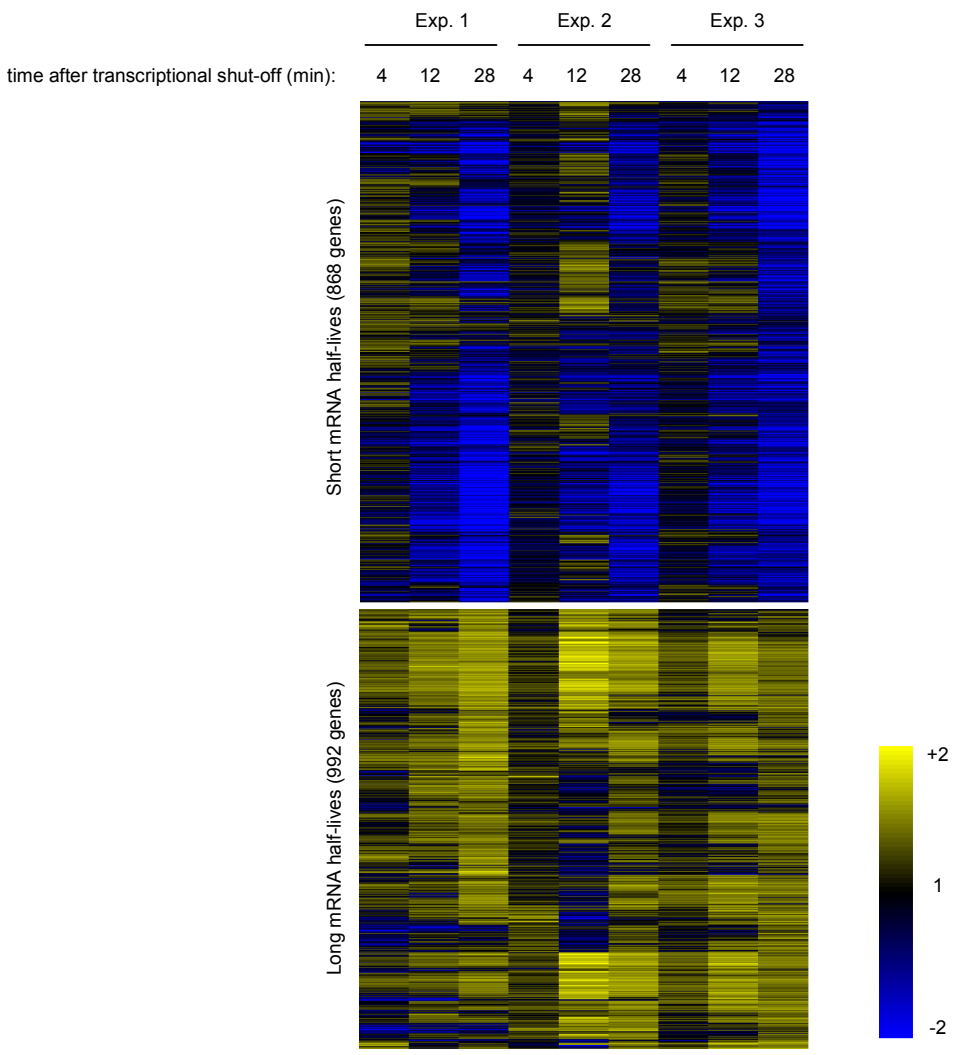


Figure 3.20 Determination of mRNAs with short and long half-lives
To estimate mRNA half-lives, cells were treated with the transcriptional inhibitor 1,10-phenanthroline, and mRNA was isolated before and at 4, 12, and 28 min after transcriptional shut-off. Two lists of genes with short and long half-lives were created from these data. The figure shows heat maps of these two gene lists, clustered using the Spearman correlation. Data from three independent biological experiments are shown. The columns represent experimental time points, and rows represent genes. The data of each array were normalized to the 50th percentile of the measurements taken from that array and colour-coded according to the ratios between experimental samples vs sample before transcriptional shut-off.

We then checked for relationships between mRNA stability and translational efficiency. The mRNAs with long half-lives showed significantly higher ribosome occupancies and densities on average than mRNAs with short half-lives (Figure 3.21B,C). Thus, stable mRNAs seem to be more efficiently translated than less stable mRNAs. Although translational efficiency correlated with both poly(A) tail length and mRNA stability, we did not detect any correlation between mRNA stability and poly(A) tail length (Figure 3.21D).

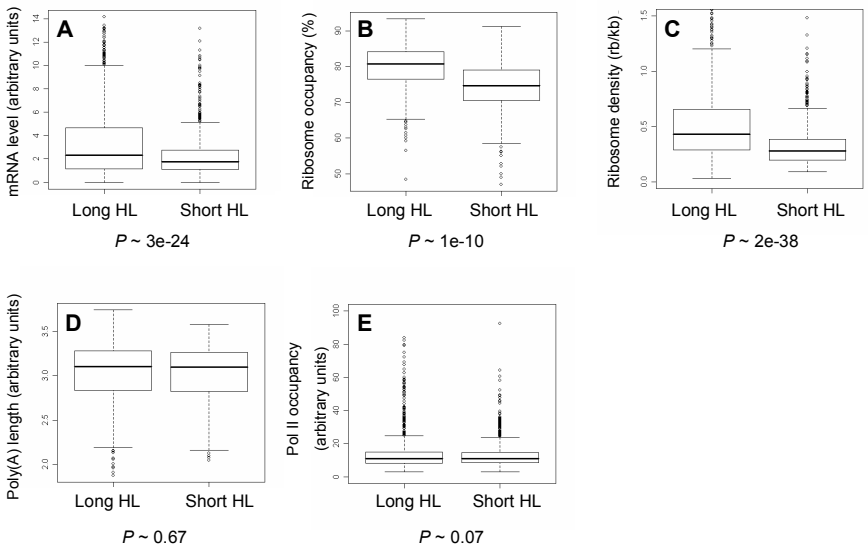


Figure 3.21 Correlations between mRNA half-lives and other gene expression properties

Boxplot showing mRNA levels **(A)**, ribosome occupancies **(B)**, ribosome densities **(C)**, poly(A) tail lengths **(D)**, and RNA polymerase II occupancies **(E)** for two groups of mRNAs with either long (long HL) or short (short HL) half-lives.

The box contains the middle 50% of the data. The upper edge (hinge) of the box indicates the 75th percentile of the data set, and the lower hinge indicates the 25th percentile. The range of the middle two quartiles represents the inter-quartile range. The thick black line indicates the median of the data. The ends of the vertical lines or "whiskers" indicate the minimum and maximum data values, unless outliers are present in which case the whiskers extend to a maximum of 1.5 times the inter-quartile range. The points outside the ends of the whiskers are outliers or suspected outliers.

The significance of the difference between the means of the two mRNA groups is given for each panel.

The relationship between mRNA stability and translational efficiency is consistent with data on single genes that indicate a connection between mRNA stability and translation (Sachs 2000; Wickens et al. 2000). This raises the possibility that mRNA stability may be the main determinant for the observed correlation between mRNA levels and translation (Figure 3.19). To test this, we needed to also estimate transcriptional efficiency, the other determinant of mRNA levels. The relative amount of RNA polymerase II associated with a given ORF provides an estimate for transcriptional efficiency (Sandoval et al. 2004). We therefore established a systematic approach to measure RNA polymerase II (Pol II) occupancy for all fission yeast ORFs using a chromatin immunoprecipitation followed by analysis on microarrays (Figure 3.22, Table S1; Material and Methods). These experiments were performed by Samuel Marguerat. Functional analysis of the 10% of genes that were either most or least associated with RNA polymerase II showed highly similar

enrichments for GO terms and functional groups as the 10% most or least abundant mRNAs, respectively. The mitochondrially encoded genes were a notable exception; they showed high mRNA levels but were strongly under-enriched in the polymerase II precipitations, consistent with these genes being transcribed by a different RNA polymerase (Schäfer et al. 2005). Transcriptional efficiency did not show any significant correlation with mRNA stability (Figure 3.21E). As expected, however, it was correlated with mRNA levels (Figure 3.23A).

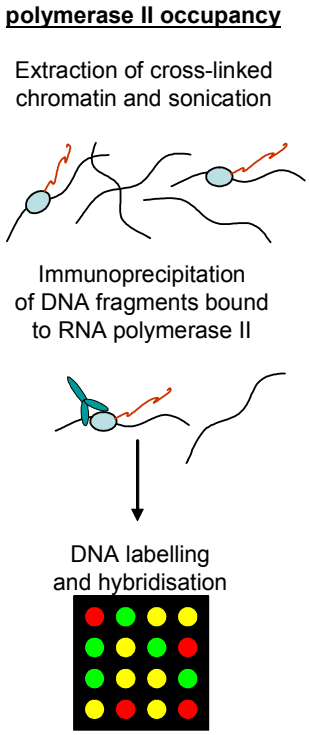


Figure 3.22 Experimental layout for estimating Pol II occupancy on a genome-wide scale
DNA and protein-complexes are crosslinked with formaldehyde, chromatin is extracted from the cells and sonicated. An immunoprecipitation using an antibody recognizing Pol II is performed. Immunoprecipitated DNA is labelled and probed on a microarray against input DNA. Genes that are more efficiently transcribed will have more Pol II bound and will be more strongly enriched in the immunoprecipitates.

We next checked for relationships between transcriptional and translational efficiencies. Pol II occupancy showed a correlation with ribosome occupancy (Figure 3.23B) and a marginal, albeit significant, correlation with ribosome density ($r = 0.11$; $P \sim 3e^{-11}$). Thus, both transcription and mRNA turnover are reflected at the level of translation: efficiently transcribed and stable mRNAs tend to be more efficiently translated.

Surprisingly, transcriptional efficiency was also correlated with poly(A) tail lengths (Figure 3.23C). This is in contrast to the apparent absence of any connection between mRNA stability and poly(A) tails (Figure 3.21D), but it is consistent with the correlation between mRNA levels and poly(A) tails (Figure 3.19A).

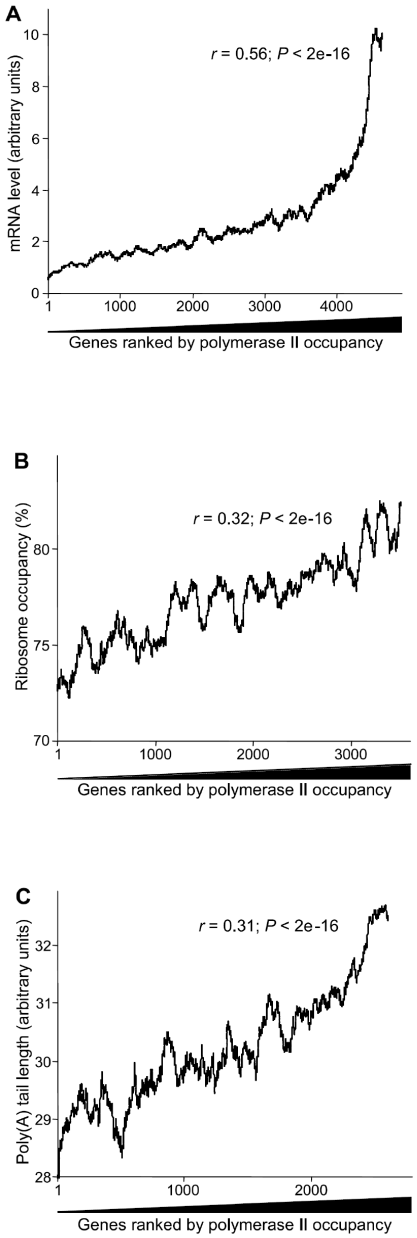


Figure 3.23 Correlations between Pol II occupancy and other gene expression properties
(A) Moving averages (100-gene window) of relative mRNA level as a function of 4724 genes ranked by Pol II occupancy, along with corresponding Spearman rank correlation.
(B) Moving averages (100-gene window) of ribosome occupancy as a function of 3598 genes ranked by Pol II occupancy, along with corresponding Spearman rank correlation.
(C) Moving averages (100-gene window) of poly(A) tail length as a function of 2713 genes ranked by Pol II occupancy, along with corresponding Spearman rank correlation.

Changes in mRNA polyadenylation in response to transcriptional switch-on

Given the correlation between poly(A) tail length and transcription (Figure 3.23C) and the finding that the carboxy-terminal domain (CTD) of Pol II is important for 3' end processing of the mRNA (Proudfoot and O'Sullivan 2002; Proudfoot et al. 2002), it was tempting to hypothesize that poly(A) tail lengths are actually determined by transcription rates. To test this hypothesis, we used LM-PAT assays to analyse polyadenylation for specific mRNAs that were transcribed at different rates using regulatable promoters. At first, *pom1* (Bähler and Nurse 2001) and *rpb4* (Sharma et al. 2006) were used as reporter genes. Pom1p is a cell cycle regulated kinase and essential for cell symmetry, Rpb4p is a subunit of RNA polymerase II. Both genes were regulated using *nmt1* promoters of different strength integrated into the genomic locus of the two genes. Expression from the *nmt1* promoter is induced by removal of thiamine from the medium. *3nmt1* is the strongest promoter, *41nmt1* is the intermediated promoter, and *81nmt1* is the weakest promoter (Basi et al. 1993). mRNAs before induction as well as 16 and 21 hours after induction were analyzed for poly(A) tail length by LM-PAT assays (Figure 3.24). Whereas mRNA levels increased for both mRNAs as expected, poly(A) tail length was not affected by the transcriptional up-regulation.

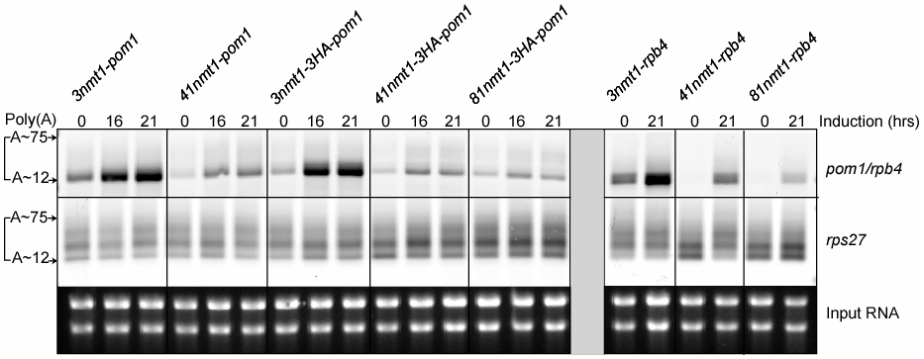


Figure 3.24 No changes in poly(A) tail length for mRNAs induced in expression using *nmt1* promoters with long induction time
The *pom1* (left panels) and *rpb4* (right panels) genes were transcriptionally induced by thiamine removal using regulatable *nmt1* promoters of different strength: *3nmt1*, strongest promoter; *41nmt1*, intermediate promoter; and *81nmt1*, weakest promoter. mRNAs before induction (0) as well as 16 and 21 hours after induction were analyzed for poly(A) tail length by LM-PAT assays. Both mRNAs showed short poly(A) tails independently of transcription rates. The longer-tailed *rps27* mRNA is included as a control (middle panels), and the input RNA is shown below.

These data indicate that the transcription rate does not influence poly(A) tail length for the tested mRNAs. A problem with this experiment and its interpretation might arise through the long induction time needed for expression from the *nmt1* promoter. Therefore we wanted to test whether the same result would be obtained looking at mRNAs induced for a short period only. To this end we made use of the promoter of *urg1* (uracil regulatable gene), which shows a fast induction time after addition of uracil to the medium (S. Watt, J. Mata, G. Burns and J. Bähler, manuscript in preparation). *pom1* under the regulation of this promoter, *urg1* under its own promoter and SPAC1002.17, which shows a similar short induction time under its own promoter after uracil addition, were tested by LM-PAT assay (Figure 3.25). A 30 min time-course of induction was followed by a 30 min time-course of repression 4 hours later. A weak band indicating some long-tailed form of *pom1* was present 5 min after induction, but from 10 min onwards the short-tailed form predominated. *Urg1* and SPAC1002.17 were present mainly in a long-tailed form 5 min after induction, and short-tailed forms appeared later after 15 and 30 min. However, these mRNAs seemed to have slower deadenylation kinetics, as long-tailed forms were still evident 30 min after induction, but were gone at the time of repression. Thus, when transcription was induced within a short time, a transient population of longer tailed mRNAs was apparent, which were then deadenylated with different kinetics depending on the particular mRNA.

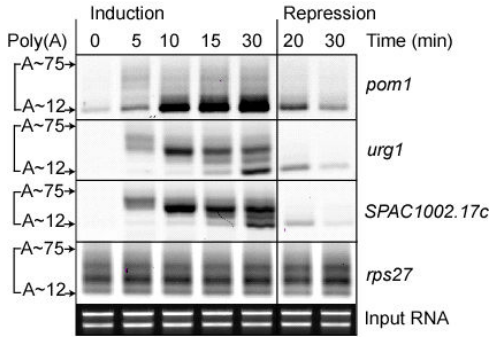


Figure 3.25 Transient changes in poly(A) tail length for mRNAs induced in expression using a promoter with short induction time
Transcription of *pom1* was induced under the control of the regulatable *urg1* promoter showing fast induction time. A 30-min timecourse of induction was followed by a 30-min timecourse of repression 4 hours later, and mRNAs were analyzed for poly(A) tail length by LM-PAT assays. Some long-tailed form of *pom1* mRNA is present at 5 min after induction, but from 10 min onwards the short-tailed form predominates. Corresponding LM-PAT assays from the same cells are also shown for *urg1* (under its own promoter) and SPAC1002.17c (which shows a similar short induction time under its own promoter). These mRNAs have slower deadenylation kinetics, and long-tailed forms are still evident 30 min after induction, but are gone at the time of repression. The unregulated *rps27* mRNA and input RNA are shown as controls.

We conclude that the transcription rate does not directly influence poly(A) tail lengths, although increased transcription can lead to transiently increased tail lengths before reaching steady-state conditions. Similar observations were made in budding yeast for mRNAs with predominantly long poly(A) tails measured by LM-PAT assay after replenishing stationary phase cells with fresh media (Beilharz and Preiss 2007).

Conclusion

Comparisons between our genome-wide data sets on key aspects of gene expression control, ranging from transcription to translation, highlight a remarkable degree of global interconnectivity between different layers of gene expression. For a summary of all correlations between the diverse data sets see Table 3.1.

Table 3.1 Summary of all correlations between the different genome-wide data sets on key aspects of gene expression

	Ribosome density	Ribosome occupancy	ORF length	Poly(A) tail	mRNA level	Pol II occupancy	Protein levels
Ribosome occupancy	**0.40** (< 2e-16)						
ORF length	**-0.98** (< 2e-16)	**-0.27** (< 2e-16)					
Poly(A) tail	**0.42** (< 2e-16)	**0.27** (< 2e-16)	**-0.40** (< 2e-16)				
mRNA level	**0.14** (< 2e-16)	**0.50** (< 2e-16)	**-0.02** (0.11)	**0.46** (< 2e-16)			
Pol II occupancy	**0.11** (8e-11)	**0.32** (< 2e-16)	**-0.05** (0.11)	**0.31** (< 2e-16)	**0.56** (< 2e-16)		
Protein levels	**0.31** (< 2e-16)	**0.31** (< 2e-16)	**-0.26** (< 2e-16)	**0.21** (< 2e-16)	**0.23** (< 2e-16)	**0.10** (2e-10)	
AugCAI	**0.20** (< 2e-16)	**0.25** (< 2e-16)	**-0.15** (< 2e-16)	**0.15** (2e-14)	**0.22** (< 2e-16)	**0.15** (< 2e-16)	**0.17** (< 2e-16)

This table shows the Spearman rank correlations between all data-sets, for which ranked lists were available. The corresponding *P* values are shown in brackets. Positive correlations with a *P* value smaller than 2e-16 are coloured green; negative correlations with a *P* value smaller than 2e-16 are coloured red.

The large network of correlations between all aspects of regulation suggests widespread coordination between multiple gene expression levels for coherent and efficient protein production. Some of these relationships may reflect direct mechanistic links (e.g., translational efficiency could influence mRNA stability), while others may reflect independent evolutionary selection at different levels of regulation (e.g., alignment of transcriptional and translational efficiencies). These rich data sets, all acquired under one standardized condition in a simple model organism,

are a basis to interpret global and specific regulation of gene expression in response to environmental or genetic perturbations. The findings presented here also provide a framework to gain comprehensive mechanistic insight into multi-layered gene expression programs and should advance a system-wide understanding of gene expression also in more complex organisms.

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

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

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.

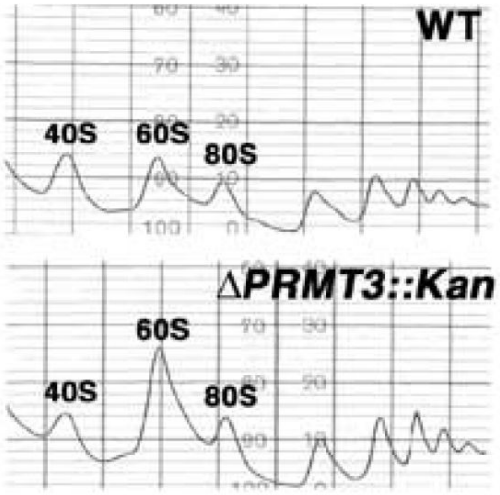


Figure 4.1 Imbalance in free 40S:60S ratio in *rmt3Δ* cells
Polysome profiles from wt (WT, top panel) and *rmt3Δ* (ΔPRMT3::Kan, bottom panel) cells. The position of free small (40S) and large (60S) ribosomal subunits and monosomes (80S) are indicated. This figure is taken from Bachand et al. (2004).

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.

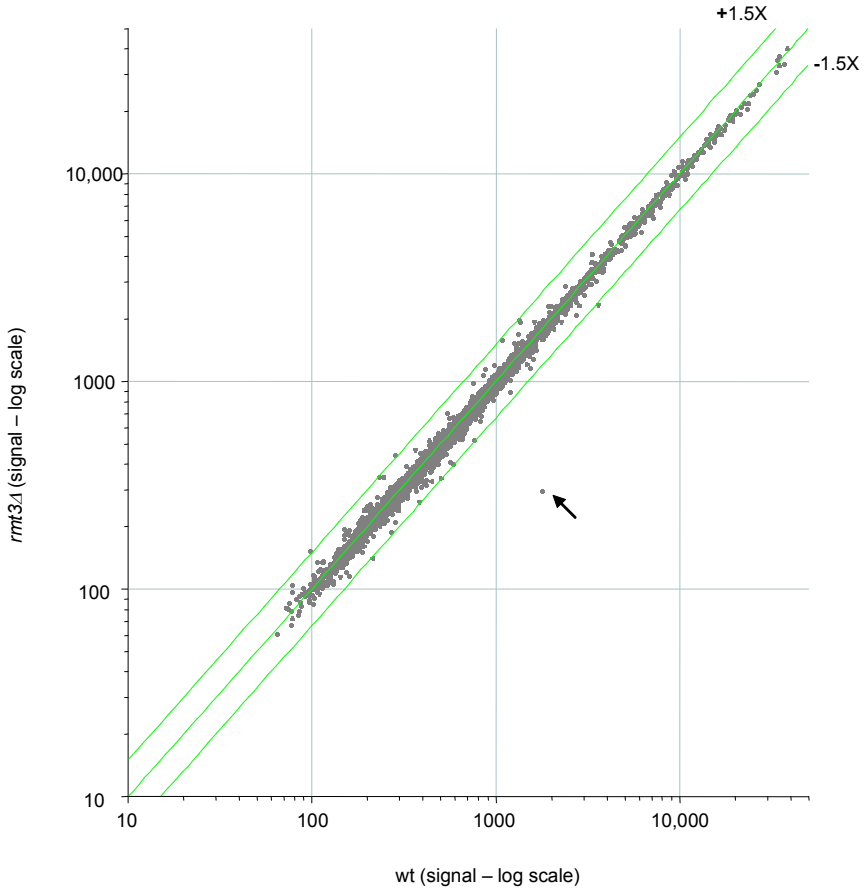


Figure 4.2 Genome-wide mRNA profiling comparing *rmt3Δ* and wt cells
Scatter blot of normalized mRNA levels from *rmt3Δ* and wt cells measured using DNA microarrays. Shown are the averaged data for 3 biological repeats. The green lines represent the 1.5 fold cut-off. The single mRNA indicated with an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

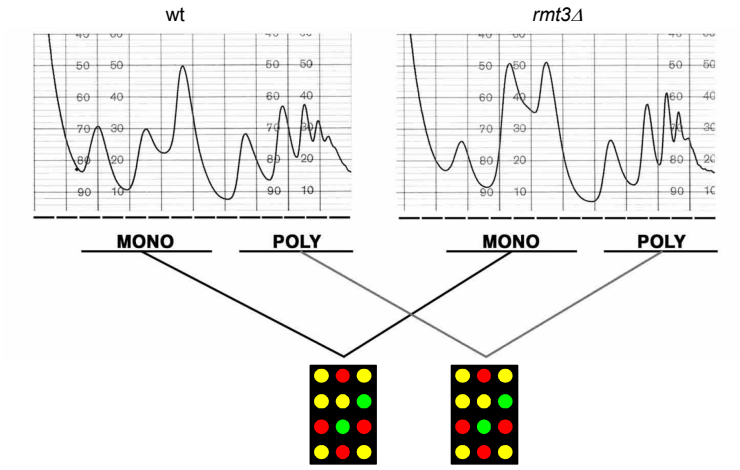


Figure 4.3 Experimental layout for translational profiling comparing monosomal and polysomal fractions between *rmt3Δ* and wt cells
Fractions from polysome profiles of *rmt3Δ* and wt cells were pooled as indicated into monosomal (MONO) and polysomal (POLY) fractions. mRNAs were extracted from these pools, 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).

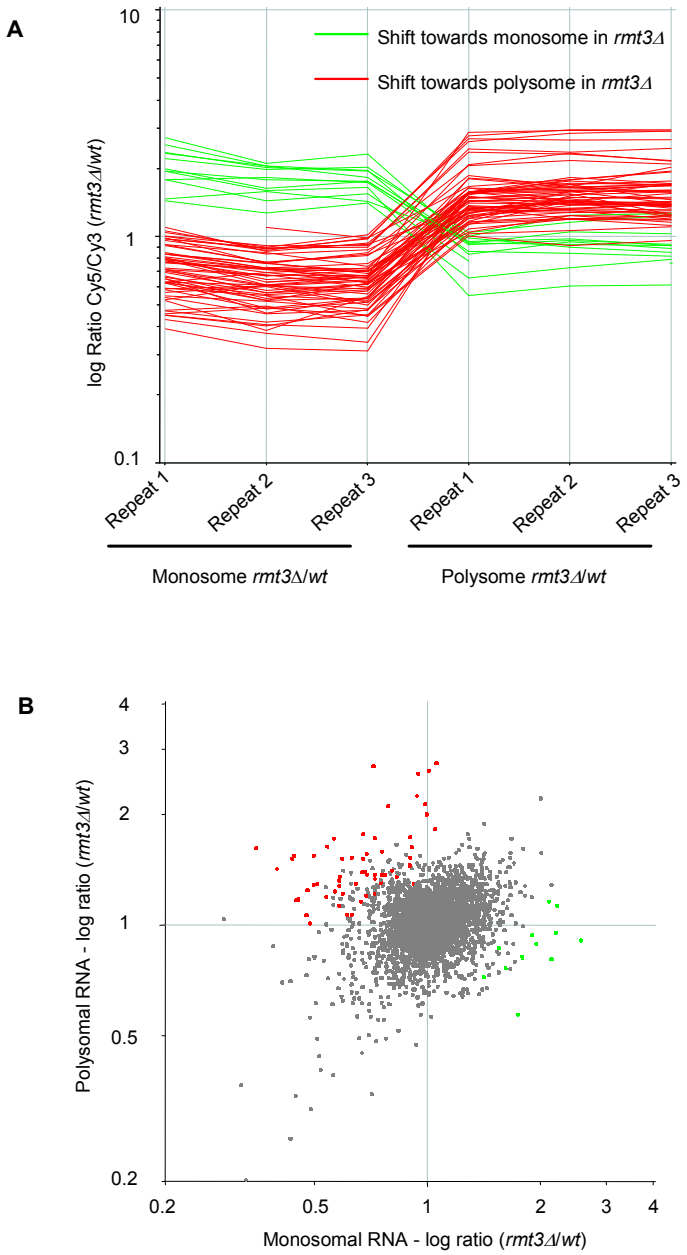


Figure 4.4 Translational changes in *rmt3Δ* cells
(A) Displayed are translational changes in *rmt3Δ* cells identified using translational profiling as outlined in Fig. 4.3 and identified using SAM (see main text). Shown are 3 independent biological repeats each comparing monosomal and polysomal fractions 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.

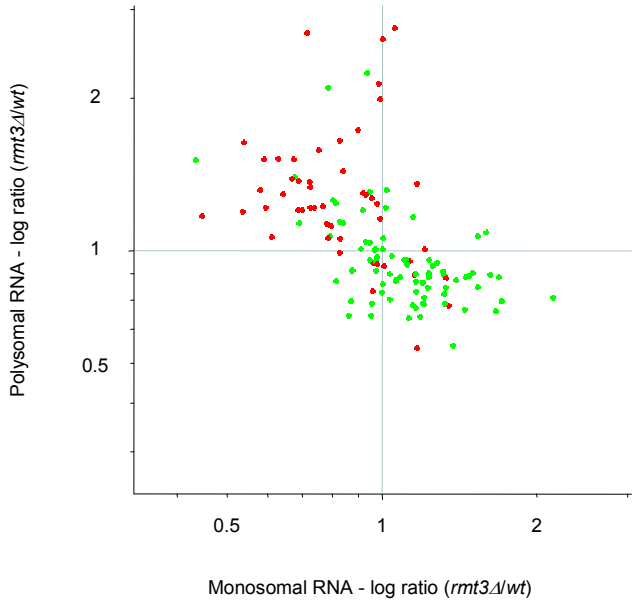


Figure 4.5 Polysomal and monosomal ratios for mRNAs encoding ribosomal proteins
Averaged monosomal and polysomal RNA ratios between *rmt3Δ* and wt cells displayed as in Fig. 4.4B for mRNAs encoding ribosomal proteins. Red dots: mRNAs encoding proteins of the 40S subunit. Green dots: mRNAs encoding proteins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

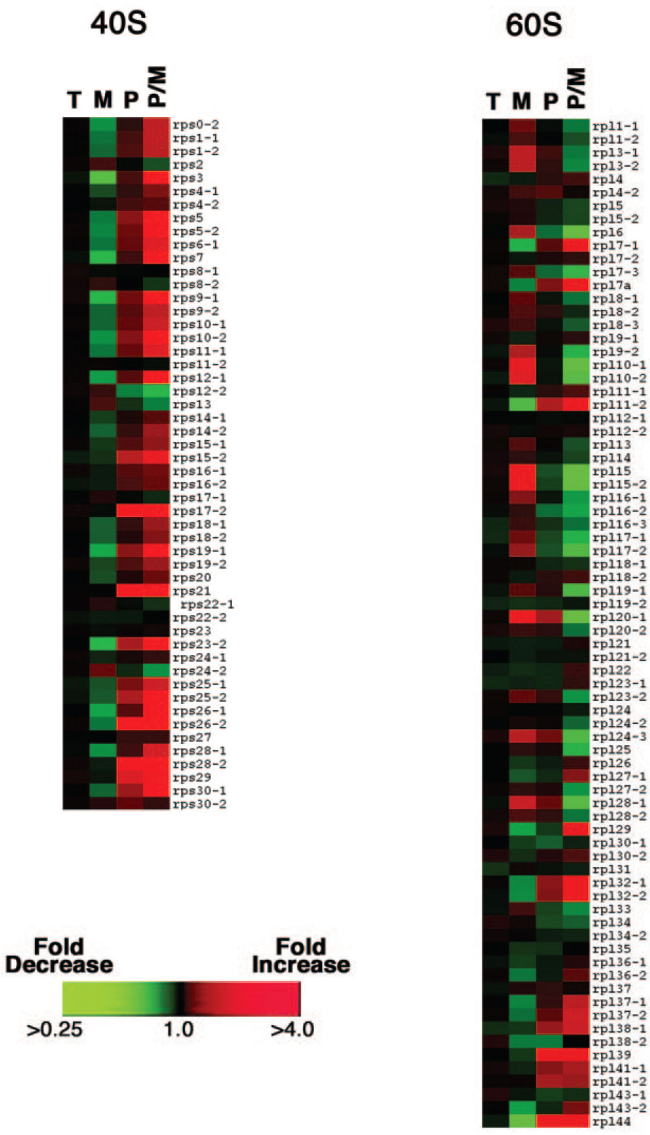


Figure 4.6 Gene expression changes of mRNAs encoding ribosomal proteins in *rmt3Δ* cells

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).

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).

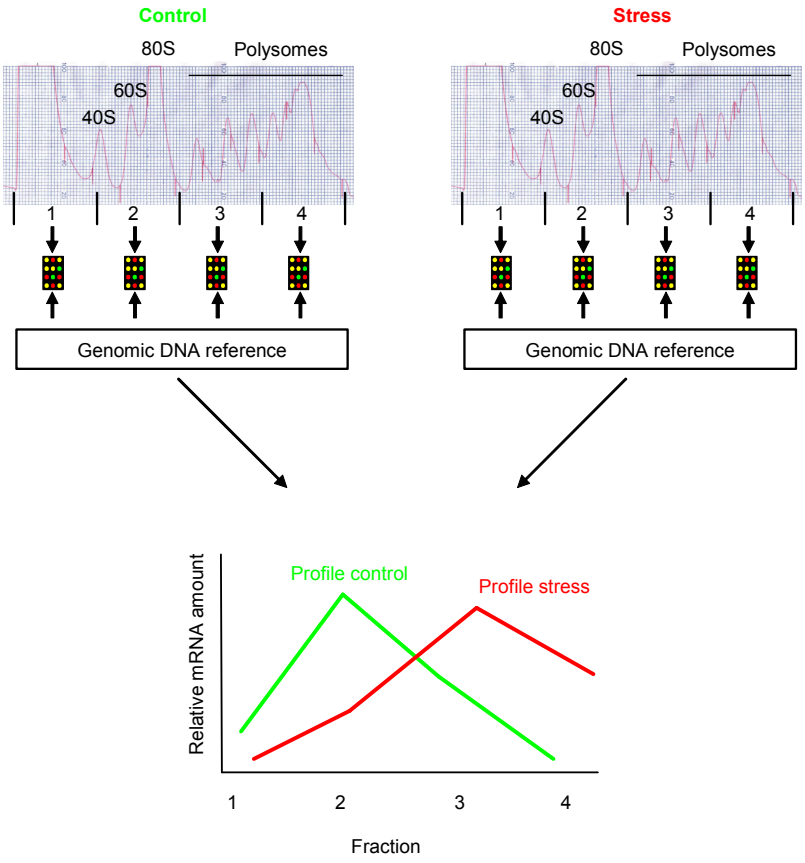


Figure 5.1 Experimental layout for medium resolution translational profiling under stress conditions
Polysome profiles were prepared from unstressed control cells and cells exposed to stress. mRNA was extracted from 4 fractions equally spaced throughout the profile, labelled and competitively hybridized 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.

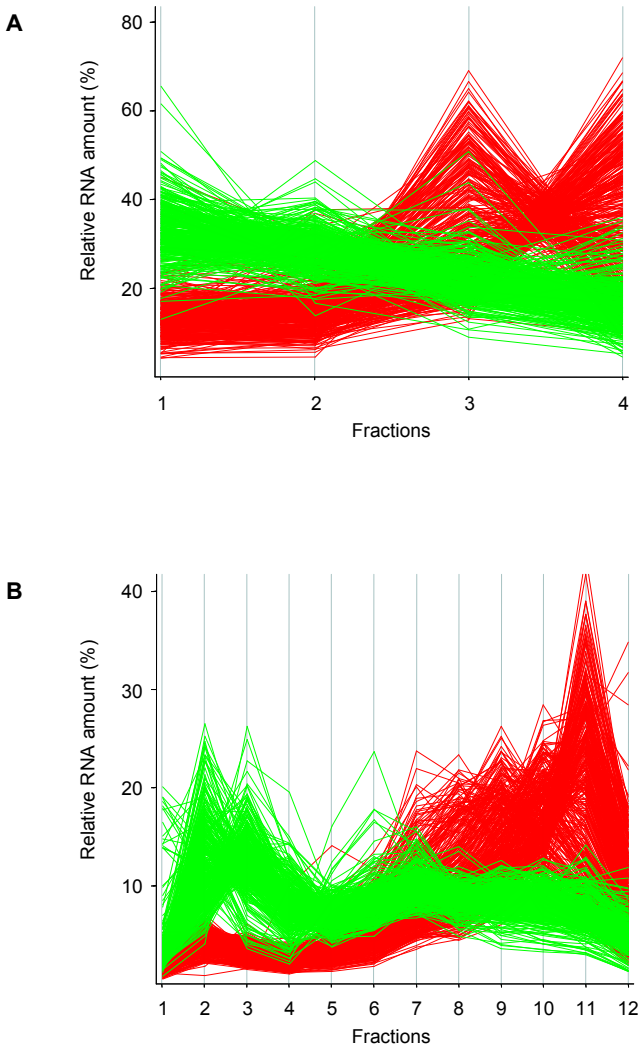


Figure 5.2 Comparison of the distribution of mRNAs with high and low ribosome occupancy between medium- and high-resolution translational profiling
Shown is the distribution of 10% of mRNAs with the highest ribosome occupancy (red lines) and 10% of mRNAs with the lowest ribosome occupancy (green lines) 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.

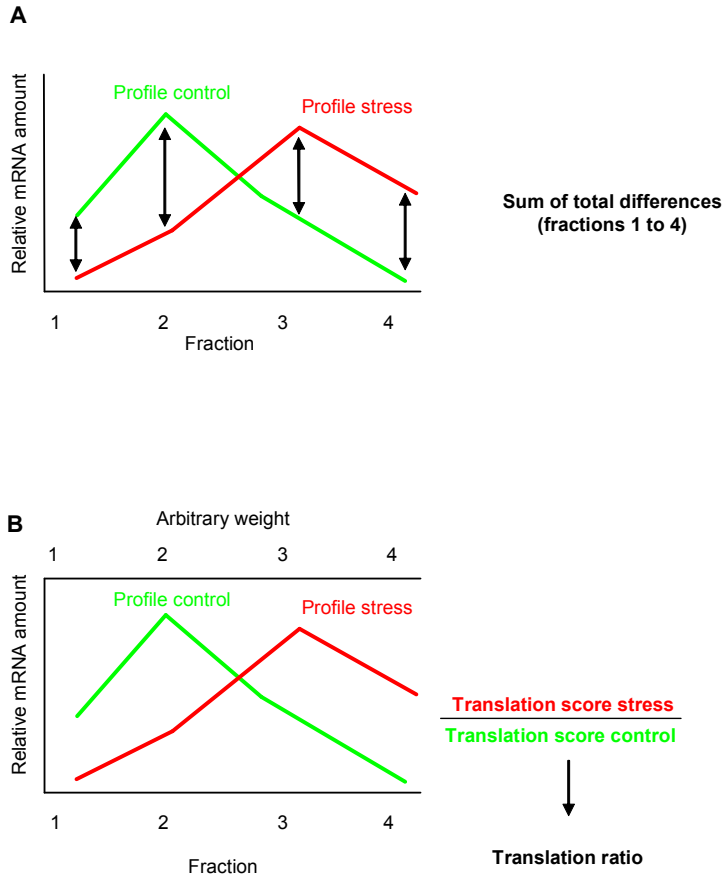


Figure 5.3 Outline of data analysis to define translationally regulated mRNAs
(A) The sum of total difference for a given mRNA between the translational profile under stress conditions and the translational profile in the control was calculated by summing the total difference in each fraction (indicated 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.

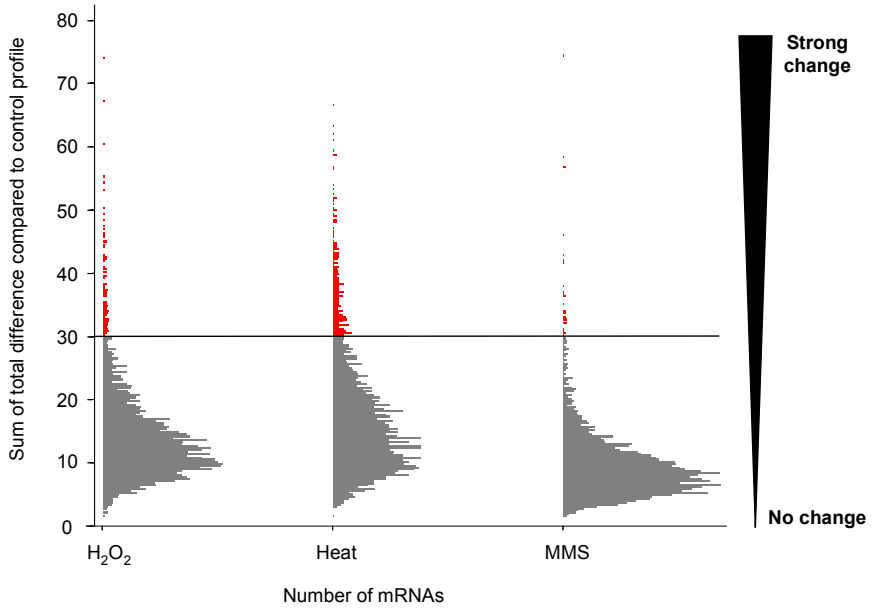


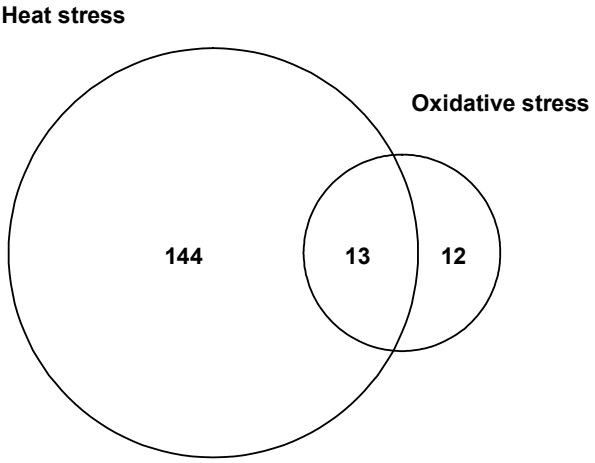
Figure 5.4 Sum of total difference between the translational profile in the stress conditions and in the control

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

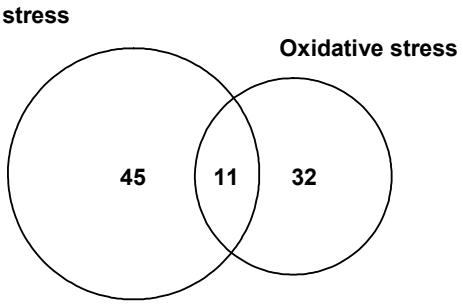


B

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.

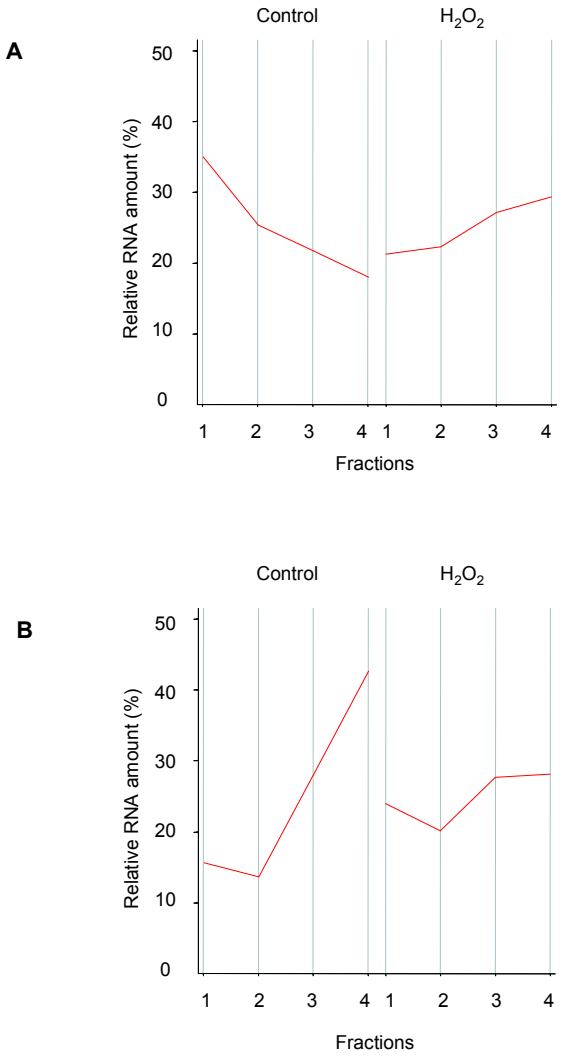


Figure 5.7 Average translation profiles for mRNAs translationally regulated under oxidative stress
(A) Average translation profile shown for 25 mRNAs identified to be translationally up-regulated after 15 min exposure to oxidative stress.
(B) Average translation profile shown for 43 mRNAs identified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

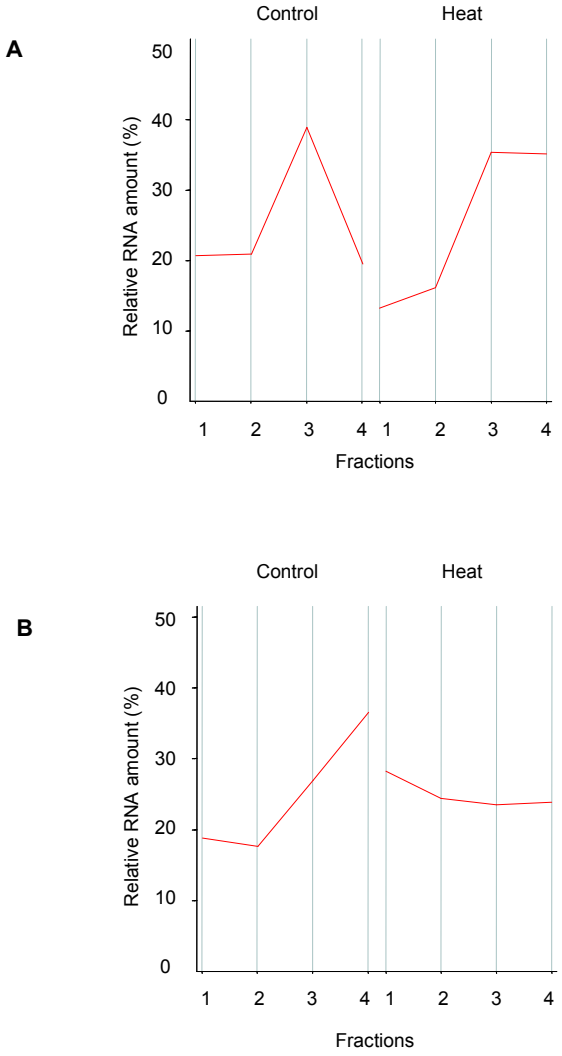


Figure 5.8 Average translation profiles for mRNAs translationally regulated under heat stress
(A) Average translation profile shown for 157 mRNAs identified to be translationally up-regulated after 15 min exposure to heat stress.
(B) Average translation profile shown for 56 mRNAs identified to be translationally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

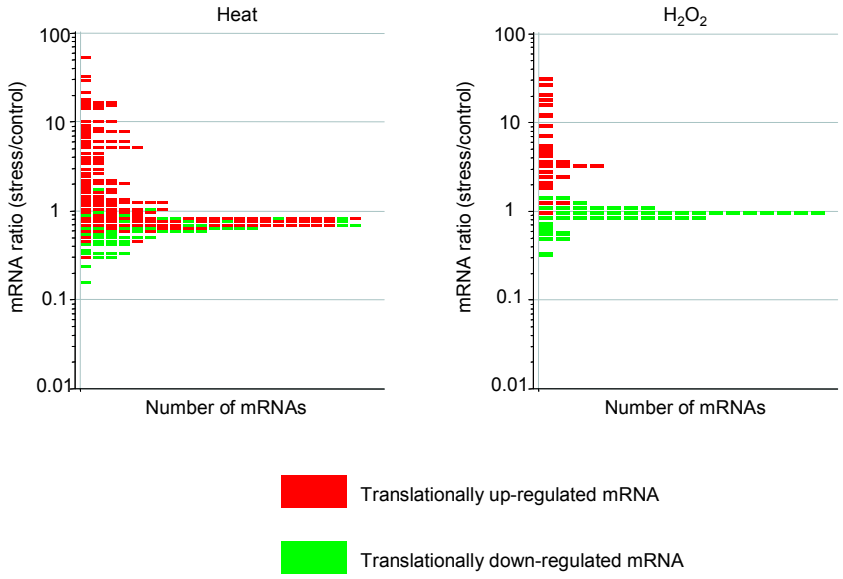


Figure 5.9 Changes in total mRNA levels for translationally regulated mRNAs in stress conditions
Changes in total mRNA abundance for mRNAs identified to be translationally regulated under heat or oxidative stress. mRNA levels are shown as ratios relative to control cells. The ratios represent the normalized 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.

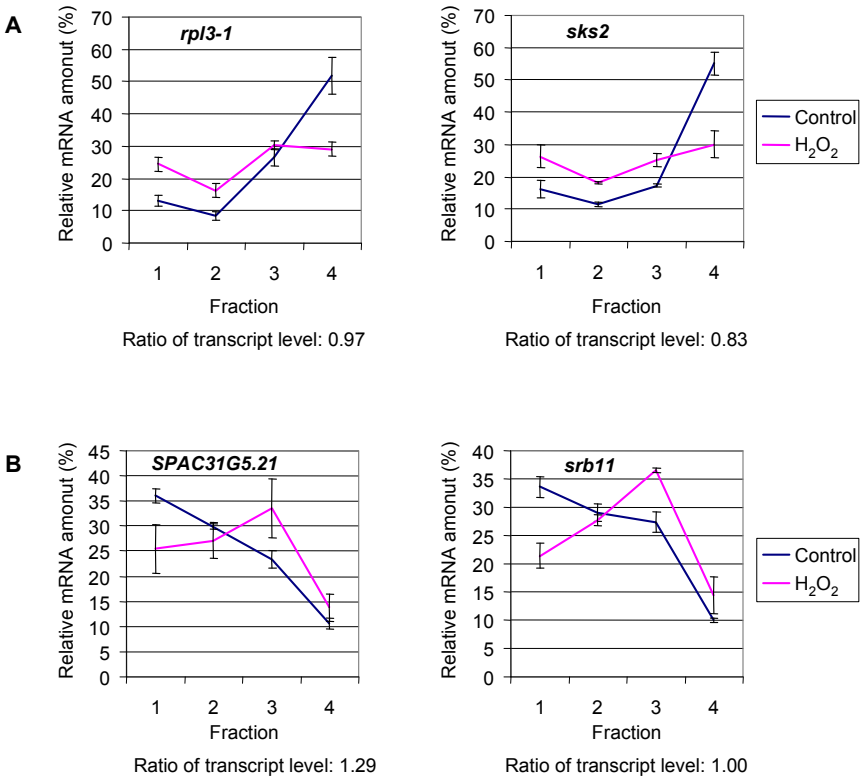


Figure 5.10 Example profiles of mRNAs that show translational regulation under oxidative stress, but are not regulated at the level of total mRNA abundance
(A) Translation profiles for mRNAs translationally down-regulated after 15 min exposure to oxidative stress. Shown are the averaged profiles from 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.

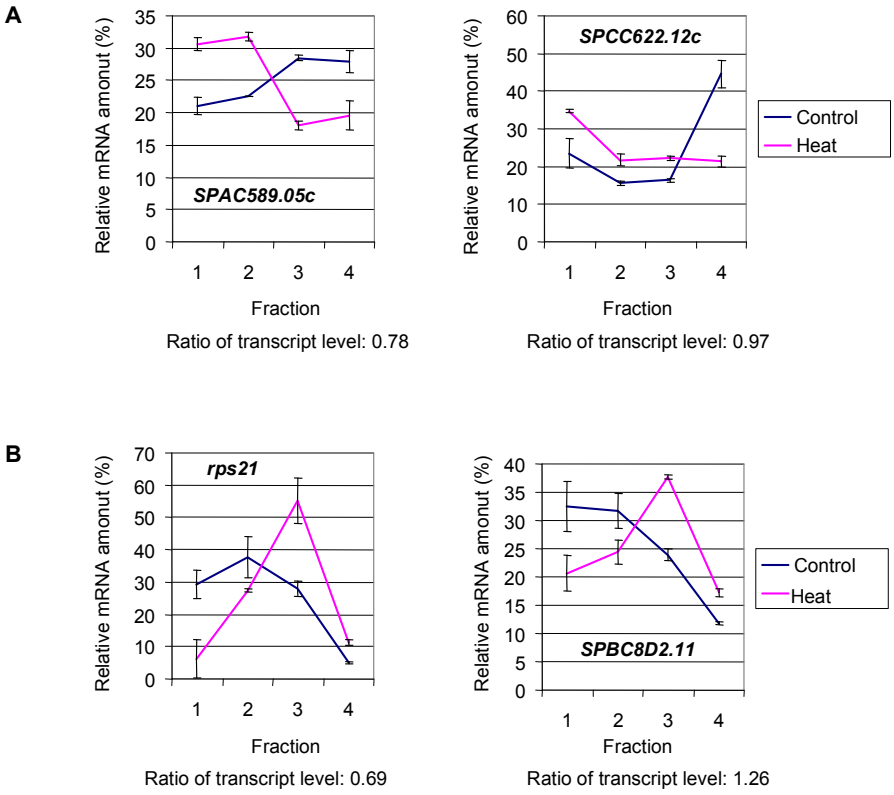


Figure 5.11 Example profiles of mRNAs that show translational regulation under heat stress, but are not regulated at the level of total mRNA abundance

(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.

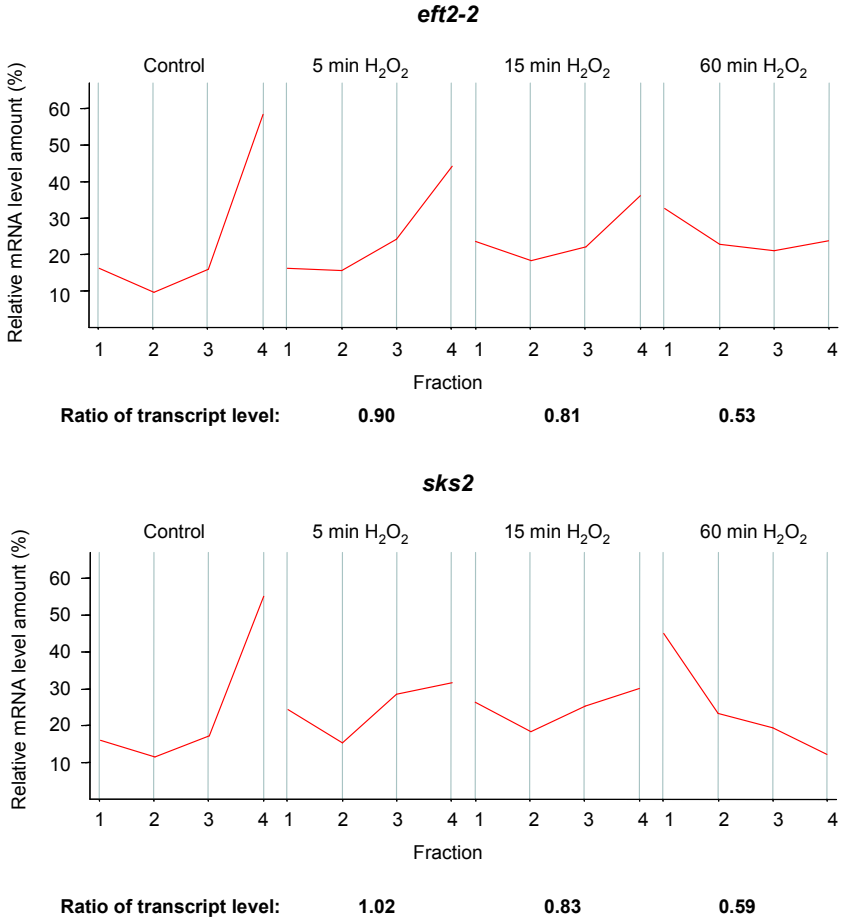


Figure 5.12 Translation profiles of down-regulated mRNAs after different times of exposure to oxidative stress
Shown are translational profiles for *eft2-2* (encoding translation elongation factor 2-2) and *sks2* (encoding a heat shock protein) after different times of exposure to oxidative stress. Translational 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.

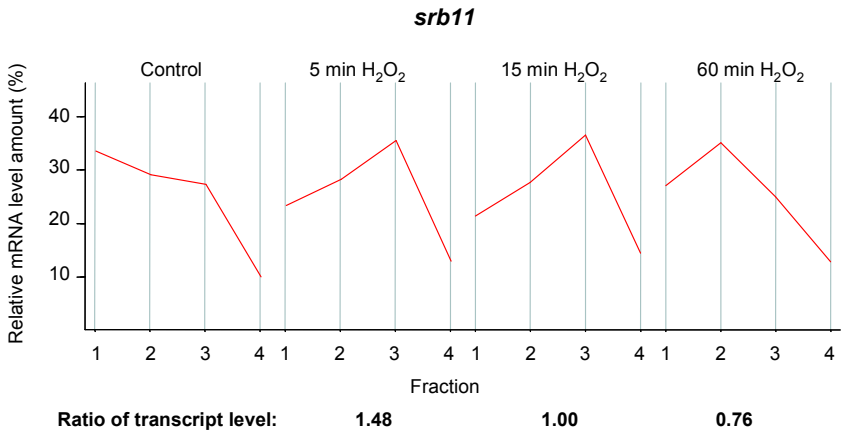


Figure 5.13 Translation profiles of an up-regulated mRNA after different times of exposure to oxidative stress
Translational profile for *srb11* (encoding a cyclin) after different times of exposure to oxidative stress. The translational profile for the control and the 15 min time point represent the 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.

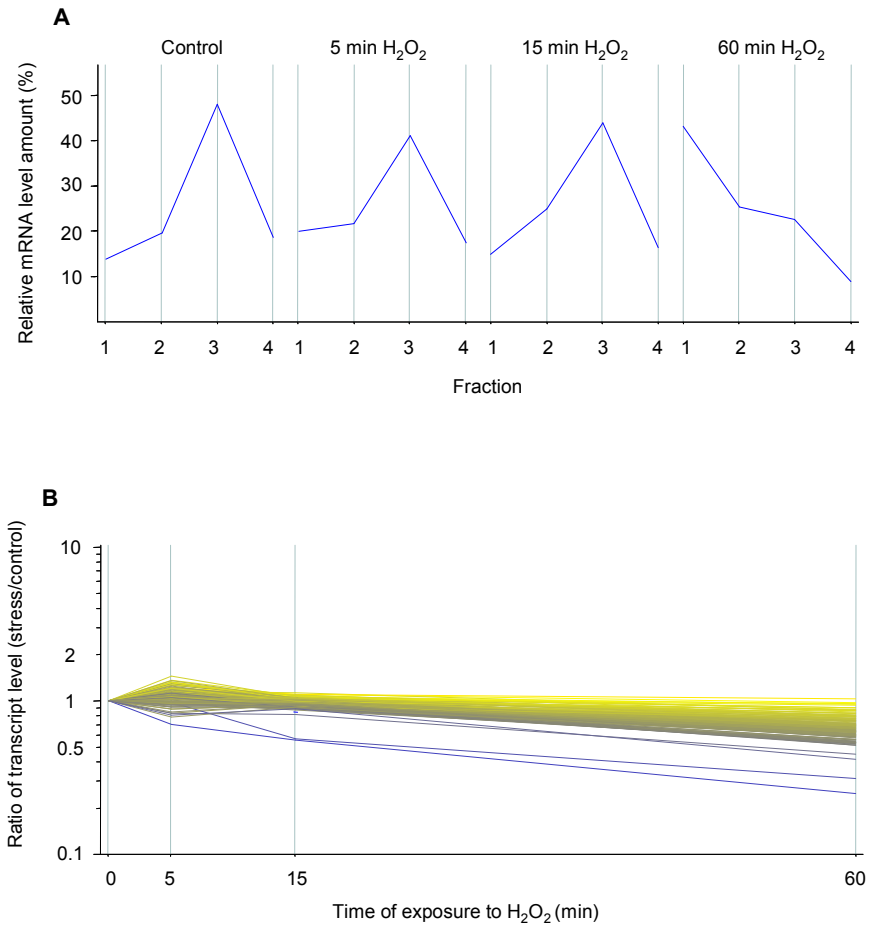


Figure 5.14 Translational regulation of mRNAs encoding ribosomal proteins under oxidative stress

(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.

Chapter 6

General discussion

General discussion

In this chapter, data from Chapters 3, 4 and 5 are discussed and put in context. A discussion on the global data set of translational efficiencies and its integration with other genome-wide data on gene expression (Chapter 3) will be followed by a discussion about translational control in a fission yeast mutant strain deleted for protein methyltransferase 3 (Chapter 4) and in response to environmental stress (Chapter 5). Furthermore, plans for future work are also described.

Global translational profiling and integration with other genome-wide data sets

Overview

The high-resolution translational profiling analysis provides a rich data source that gives comprehensive insight into translational properties of the great majority of all mRNAs in fission yeast, including estimates for ribosome occupancy and for average numbers and densities of associated ribosomes. These properties are different measures of translational efficiency. The 20% of mRNAs with the lowest ribosome densities showed a significant overlap with a list of orthologous genes reported to be poorly translated in budding yeast ($P \sim 5e^{-9}$; Law et al. 2005). This indicates that translational efficiency for a substantial number of mRNAs is conserved across evolution. Overall, numbers of bound ribosomes and average ribosome density are about 30% lower than those previously reported for budding yeast, while the ribosome occupancies are similar between the two yeasts (Arava et al. 2003; Lackner et al. 2007). Some of this discrepancy could be caused by differences in calculating the ribosome numbers between the two studies. Moreover, fission yeast was cultured in minimal medium, while budding yeast was cultured in rich medium that allows faster growth. Differences in growth rates are expected to lead to differences in global translational efficiency, which in turn would be reflected in ribosome numbers. In addition, fission yeast cells grow ~30% more slowly than budding yeast even in rich medium, and it is therefore possible that this difference is reflected (or even driven) by a generally higher translational efficiency in the latter.

To uncover global relationships between translational efficiency and other properties of gene expression, we have acquired further genome-wide data on transcriptional efficiency, and on mRNA polyadenylation, half-lives and steady-state levels in vegetative fission yeast cells grown under the standardized and highly controlled conditions used for translational profiling. These large-scale data sets have then been put in context with each other and with data on ORF length, AugCAI index and protein levels. This analysis reveals an extensive network of interactions between different aspects of gene expression. Figure 6.1 summarizes the widespread correlations between the independent data sets, highlighting a complex interplay between multiple gene expression layers.

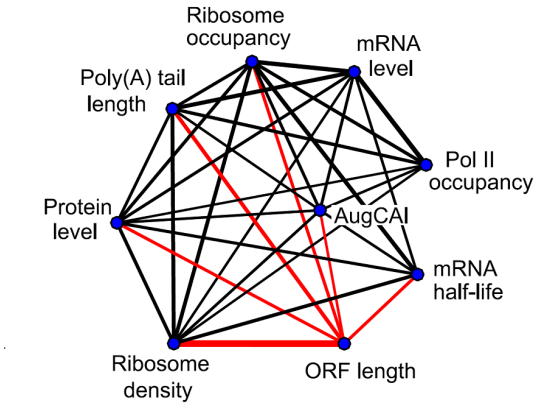


Figure 6.1 Summary of relationships between all aspects of gene expression
Weighted association map summarizing the relationships between the various aspects of gene expression analyzed here. The blue nodes represent the different data sets as labelled, black lines show significant positive correlations between connected data sets, and red lines show significant negative correlations. The weight of the lines reflects the absolute correlation value. This figure was created with the help of Falk Schubert.

Here I will just give a brief summary of the relationships between different aspects of gene expression, as some of the main connections will be discussed in more detail further below: the positive correlations between ribosome density, ribosome occupancy, AugCAI index and protein levels are expected as they are all independet measurements of translation efficiency. Furthermore, these correlations confirm the use of ribosome density and ribosome occupancy – derived from translational profiles – as valid measurments of translational efficiency. In the same way, a positive correlation between mRNA half-life and transcription (Pol II occupancy) with mRNA levels is also expected. Interestingly, there is no correlation between mRNA half-life

and transcription, which shows that mRNA decay is an mechanism that is regulated independently from transcription.

The length of the poly(A) tail has been thought to determine translational efficiency based on single-genes studies (Preiss and Hentze 1998; Sachs 2000; Wickens et al 2000), but a relation on a genome-wide scale was shown for the first time in this study. It is represented in Figure 6.1 by the connections between poly(A) tail length and ribosome density, ribosome occupancy, protein level, and AugCAI index.

The negative correlation between ORF length and translational properties (density, ribosome occupancy, protein level, and AugCAI index) will be discussed in more detail below, as will be the positive correlation between mRNA abundance, transcription and mRNA half-life with translational properties.

The most outstanding finding from this study is the identification of two basic properties of mRNAs that are coordinated with translational efficiency: length and abundance. Translation tends to be more efficient for shorter and more abundant mRNAs. Shorter and more abundant mRNAs also tend to have longer poly(A) tails, in accordance with small-scale data indicating that poly(A) tail length influences translational efficiency (Preiss and Hentze 1998; Sachs 2000; Wickens et al. 2000). Thus, mRNA length and mRNA levels are aligned on a genome-wide scale to both poly(A) tail length and translational efficiency. The lengths and levels of mRNAs, however, show no correlation with each other (Figure 3.18), suggesting that these two mRNA properties are connected with translation independently of each other. Notably, mRNA lengths correlate most with ribosome density (Figure 3.9), while mRNA levels correlate most with ribosome occupancy (Figure 3.19C). These two measures of translational efficiency may reflect distinct and partially independent mechanisms of translational control.

In a study conducted in budding yeast, the poly(A) tail length distribution of the budding yeast transcriptome has been surveyed in a similar way as reported here (Beilharz and Preiss 2007). Comparisons uncover intriguing parallels. In both yeasts, the mRNAs with long tails are enriched for GO terms related to ribosomal proteins, while the mRNAs with short tails are enriched for ribosomal biogenesis functions. Thus, although these two groups are similarly regulated with respect to mRNA levels (e.g., Jorgensen et al. 2002; Chen et al. 2003) and both contribute to ribosome function, they can be separated into distinct groups based on poly(A) tail length distribution. Another similarity is that long-tailed mRNAs are enriched for

cytoplasmic functions while short-tailed mRNAs are enriched for nuclear functions. It is remarkable that these features have been conserved over ~1 billion years of evolution, which strongly suggests that poly(A) tail lengths have functional importance. Comparisons of overall polyadenylation between budding and fission yeast reveal that the distribution of the poly(A) tail profile tends towards longer tails in fission yeast, while the maximal length is similar between the two yeasts (Figure 6.2). We speculate that this might be due to the absence of cytoplasmic poly(A) adenylases in budding yeast (Stevenson and Norbury 2006); these enzymes may re-adenylate some short-tailed transcripts in fission yeast.

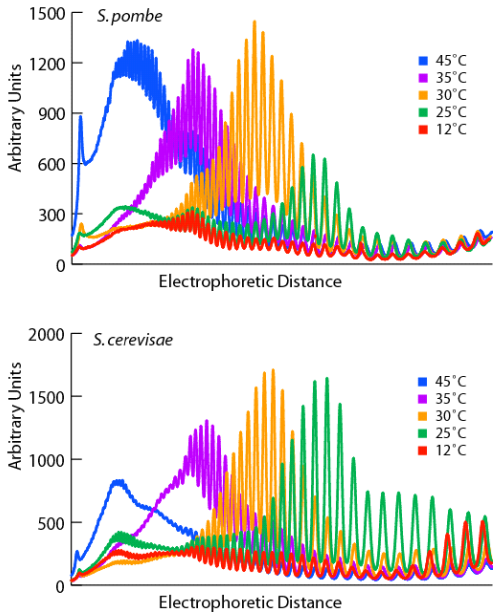


Figure 6.2 Comparison of poly(A) tail lengths between fission and budding yeast
Graph of poly(A) tail length tracts for *S. pombe* and *S. cerevisiae* showing relative intensity on the gel as a function of electrophoretic distance for all fractions, which have been eluted at different temperatures (as depicted in Figure 3.13). Each elution is enriched for a distinct population of poly(A) tail length. This figure was created with the help of Traude Beilharz.

mRNA length and translational efficiency

Available data suggest that the relationship between mRNA length and translational efficiency is conserved during evolution. Synonymous codon usage, which is thought to affect the accuracy or rate of translation, is negatively correlated with gene length in worm, flies, and plants (Duret and Mouchiroud 1999; Marais and Duret 2001). The AUG sequence context (AugCAI values), a measure for the efficiency of translational

initiation, also shows a negative relationship with gene length (Miyasaka 2002). Moreover Arava et al. (2003) have reported a strong inverse correlation between mRNA length and ribosome density for budding yeast. Here, we find that mRNA length is inversely correlated with several independent measures for translational efficiency such as ribosome density and occupancy, AugCAI, poly(A) tail length, mRNA half-life, and protein level (Figure 6.1).

It is not clear what causes the link between mRNA length and translational efficiency. Arava et al. (2003) have suggested three general classes of mechanisms to explain this correlation, based on translational initiation, elongation, or termination effects. An elegant follow-up study using ribosome density mapping for specific portions of mRNAs has indicated that differences in initiation rather than elongation or termination determine ribosome densities in mRNAs of different lengths (Arava et al. 2005). It is not known why initiation of translation would be more efficient for shorter mRNAs. One possibility is simply a higher likelihood for the formation of complex secondary structures in longer mRNAs which as a consequence could inhibit translation initiation (McCarthy 1998; Hershey and Merrick 2000). Alternatively or in addition, the mRNA closed-loop model (Sachs 2000; Kahvejian et al. 2001) suggests that interaction between the 5' UTR and the 3' poly(A) tail is important for translation initiation, and it could be easier for shorter mRNAs to achieve this conformation. It is well possible, however, that the mRNA length has no direct influence on translational efficiency but is an independently co-opted parameter (see below).

Our data suggest that poly(A) tail length is instrumental in the link between mRNA length and translation. An inverse correlation between mRNA length, ORF length, and UTR length on one hand and poly(A) tail length on the other has also been found in budding yeast (Beilharz and Preiss 2007). This study further shows that the 3' UTRs are the main determinant for poly(A) tail length control and are sufficient to influence translation rates. These data are most compatible with mRNA length being a co-opted parameter (see below). It will be interesting to unravel the intriguing and conserved global connections between ORF length, poly(A) tail length, and translational efficiency.

Integration of our findings with recent *S. pombe* ORFeome data (Matsuyama et al. 2006) confirm that the inverse relationship between ORF length and translational efficiency is ultimately reflected in protein levels. Shorter mRNAs tend to encode proteins present in higher levels in the cell, although the inverse correlation between

mRNA length and protein levels is weaker than the one between mRNA length and ribosome density (Figures 3.9 and 3.11). It is possible that the tendency of longer mRNAs to encode proteins of lower abundance reflects cellular parsimony. The synthesis of longer proteins is energetically more costly, and there could be evolutionary pressure for abundant proteins to become smaller. The highly abundant ribosomal proteins, for example, are all relatively small. Thus, mRNA length may be a co-opted parameter reflecting an overall goal for gene expression but without any direct mechanistic link to polyadenylation and translation. Notably, the tendency of short mRNAs to be highly expressed is only implemented at the translational level; no correlation between ORF length and transcription or between ORF length and mRNA levels is evident from our data (Figure 6.1). Highly expressed proteins also evolve more slowly, and the influence of expression level on the evolutionary rate appears to be influenced by the number of translation events rather than the cellular protein abundance (Drummond et al. 2005). In this work, Drummond et al. use comparative genomics of several sequenced yeast species and global data sets on expression levels and protein abundances in order to find the causal relationship between the slow evolutionary rate and high expression levels. The authors explain this relation with a "translational robustness hypothesis", where selections against the expression-level-dependent cost of misfolded proteins favours rare protein sequences, thus slowing down evolutionary rates of these sequences. Proteins with these sequences are supposed to be able to fold properly despite translation errors. In the light of these data, one could also speculate that the inverse correlation between translational efficiency and ORF length might be due to evolutionary pressure, which favours shorter proteins. Considering a constant rate of translation errors, shorter proteins should be less prone to misfolding due to mis-incorporation of the wrong amino acid than longer proteins.

mRNA abundance and translational efficiency

Unlike the lengths, the levels of mRNAs positively correlate with translational efficiency and with related but independent measures such as AugCAI, poly(A) tail lengths, and protein levels (Figure 6.1). Transcription rates (estimated from Pol II occupancy) and mRNA half-lives contribute to mRNA steady-state levels, and both of them also seem to contribute to the link between mRNA levels and translational efficiency, as both of them correlate with translational efficiency and related measures

(Figure 6.1). As expected, both transcriptional efficiency and mRNA half-lives also correlate with mRNA levels, but they do not correlate with each other (Figure 3.21E). This suggests that transcriptional efficiency and mRNA half-lives are coordinated with translation independently of each other. The connection between mRNA half-lives and translational efficiency is not unexpected given that translation inhibits mRNA decay (Parker and Song 2004). Our data indicate that more efficiently translated mRNAs are better protected from decay. Consistent with this, mRNA half-lives correlate positively with the AugCAI and with protein levels and negatively with ORF length (Figure 6.1). A global study on mRNA decay in budding yeast, however, did not detect correlations between mRNA half-lives and mRNA levels or ribosome densities (Wang et al. 2002). We speculate that this discrepancy reflects differences in methodology rather than biological differences between the two yeasts.

Transcriptional efficiency, but not mRNA half-life, correlates with poly(A) tail length. The study comparing these data sets in budding yeast has not detected any relationship between poly(A) tail length and mRNA half-lives either (Beilharz and Preiss 2007). Although poly(A) tail shortening is required for mRNA decay (Wilusz et al. 2001; Parker and Song 2004), the gradual shortening of poly(A) tails after transcription (Figure 3.25; Beilharz and Preiss 2007) may not be a rate-limiting step. This is consistent with findings that the half-lives of poly(A)$^+$ mRNAs are shorter than those of overall mRNAs (Wang et al. 2002), and that a high proportion of mRNAs seem to persist in short-tailed forms (this work; Beilharz and Preiss 2007). A previous study has identified mRNAs with short oligo(A) tails as an important intermediate for decay (Decker and Parker 1993). Together, these data indicate that, unlike for translational efficiency, the lengths of poly(A) tails do not affect mRNA half-lives.

Could the unexpected connection between transcriptional efficiency and poly(A) tail length reflect a direct mechanistic link between transcription and polyadenylation? It is known that transcription is integrated with mRNA processing at several steps, and polyadenylation requires interaction between the carboxy-terminal domain (CTD) of the largest Pol II subunit and polyadenylation factors (Proudfoot et al. 2002). This raises the possibility that high transcription rates promote long poly(A) tails, which in turn increase translational efficiency. In this scenario, the poly(A) tails would provide a link between mRNA levels and translational efficiency. However, our data on polyadenylation of mRNAs expressed at different levels do not support this idea, at least for the five genes tested. These experiments indicate that newly transcribed

mRNAs contain long poly(A) tails that are then deadenylated with different kinetics depending on the specific mRNAs; the final tail length is therefore not influenced by the transcription rate (Figure 3.24, 3.25). This view is consistent with detailed data from budding yeast, which indicate that the 3' UTRs are critical to determine deadenylation rates and ultimate poly(A) tail lengths (Beilharz and Preiss 2007).

The correlation between transcriptional and translational efficiency (and polyadenylation) could reflect independent evolutionary selection for efficient expression of proteins in high demand at these two distinct levels of gene expression. In this scenario, the correlation between transcription and translation would not be caused by any mechanistic link between the two. Consistent with this view, the mRNA levels in our data (based on genes expressed from their native promoters) correlate with the protein levels from the ORFeome study (Matsuyama et al. 2006; r = 0.23; $P < 2e^{-16}$). This finding is striking given that the protein levels have been determined after expressing all genes from the same promoter, and the mRNA levels of the ORFeome study do not correlate with the protein levels (Matsuyama et al. 2006). Overall, evolutionary selection thus seems to independently but congruently influence both transcriptional and translational control to optimize and fine-tune gene expression for production of required protein levels.

Data from budding yeast suggest similar correlations between mRNA levels and ribosome densities or ribosome occupancy, although these relationships have not been emphasized (Arava et al. 2003; Beilharz and Preiss 2004; Beyer et al. 2004). Several groups have reported that mRNAs that are more highly transcribed in different conditions also become more efficiently translated (Preiss et al. 2003; Serikawa et al. 2003; MacKay et al. 2004; Smirnova et al. 2005); this coordination between changes in transcription and in translation has been termed "potentiation". The dynamics of deadenylation discussed above provides an explanation for the potentiation phenomenon. Increased transcription would temporarily increase the proportion of long- versus short-tailed mRNAs, which in turn would lead to increased translation. This could provide an elegant way for the cell to link changes in transcription with corresponding changes in translation on a global scale.

Translational control in response to genetic perturbation and environmental stress

Translational changes in fission yeast cells deleted for *rmt3*

Using translational profiling, we could show that fission yeast cells deleted for the gene encoding protein methyltransferase 3 (*rmt3*) exhibit a translational up-regulation of many mRNAs encoding proteins of the small ribosomal (40S) subunit, whereas total mRNA levels for these mRNAs are not altered. Two mRNAs (*sui1*, *tif45*), which were found to be translationally up-regulated in the *rmt3* mutant and for which antibodies were available, were tested for changes in actual protein abundance by our collaborators. In both cases, a roughly 1.5 fold change in protein level was observed, reflecting the changes in the distribution across the polysome profile (Bachand et al. 2006). These data from immunoblotting validate the use of translational profiling to identify translationally regulated mRNAs.

Rmt3p methylates the ribosomal protein Rps2p, which is a constituent of the 40S subunit, and deletion of *rmt3* results in an imbalance between the free 40S and 60S subunit in the cell (Figure 4.1; Bachand and Silver 2004). Apart from the ribosomal imbalance, *rmt3* deletion mutants do not exhibt any other obvious phenotypes (Bachand and Silver 2004). Overexpression of Rps2p in an *rmt3* deletion mutant restores the 40S:60S imbalance and also the polysomal distributions for two tested mRNAs encoding 40S ribosomal proteins (*rps23-3*, *rps26-2*) back to wt levels (Bachand et al. 2006). This suppression of the *rmt3* deletion phenotype was not seen in response to the overexpression of other ribosomal proteins such as Rps3p or Rps7p. Together, these data suggest that the observed translational regulation happens as a specific response to the lack of methylation on Rps2p due to deletion of *rmt3*.

The interesting question remains why cells respond to the lack of methylated Rps2p with a translational up-regulation of many other mRNAs encoding 40S ribosomal proteins. One possible explanation is that methylation of Rps2p via Rmt3p is important in a specific – yet undefined – mechanism to ensure proper functioning of the 40S subunit. Loss of methylation could interfere with this function, but an up-regulation of translation of most mRNAs encoding 40S proteins can suppress the lack of methylated Rps2p and establish proper functioning ribosomes. However, this scenario is purely speculative, and further work will be needed to define the

underlying mechanism that detects the lack of methylation and/or problems with 40S function, and the mechanisms that exert the translational stimulation of mRNAs encoding 40S proteins.

Translational regulation in response to environmental stress

Translational profiling was used to identify translationally regulated mRNAs under environmental stress conditions. A medium resolution approach was applied, where mRNAs were fractionated into 4 fractions (untranslated mRNAs, mRNAs associated with the monosome, mRNAs associated with light polysomes, and mRNAs associated with heavy polysomes). Translation profiles were obtained from control cells and cells exposed to stress. Automated methods and visual inspection of the data was employed to define translationally regulated mRNAs.

From a data analysis point of view, it is to some extent difficult to define whether an mRNA is regulated at the transcriptional level and/or the translational level by using arbitrary cut-offs. Despite using a stringent cut-off of a 1.5 fold change to define regulation at the level of mRNA abundance, a change in mRNA abundance that is slightly below this cut-off could still be biologically relevant. It is even more difficult to define translational changes based on the changes in translational profiles, especially as not many previous data of this kind exists to use as a reference. However, by using stringent and conservative criteria to define our data sets, we are confident to have identified possible targets for translational regulation. Nevertheless, further work will be needed to verify the targets and validate the data. Such measurements will then also allow us to adjust the analysis and the cut-off for future work using the same approach.

Several trends are emerging from our analysis: the extent of translational changes is comparable to the extent of changes at the level of mRNA abundance, which have been described previously in the study by Chen at al. (2003). In this study, most mRNAs with altered abundance were identified during oxidative and heat stress, whereas much fewer mRNAs changed after the exposure to the DNA-damaging agent MMS. A similar trend is seen at the level of translational regulation, where basically no large changes in terms of translational regulation could be detected after 15 min exposure to MMS.

Furthermore, there was a certain amount of coordination between changes in mRNA levels and in translation. Many mRNAs regulated at the level of translation also showed regulation at the level of mRNA abundance, and many of these mRNAs were members of the core environmental stress response (CESR; Chen et al. 2003). As mentioned before, this potentiation has also been observed in gene expression changes in response to other conditions (Preiss et al. 2003; Serikawa et al. 2003; MacKay et al. 2004; Smirnova et al. 2005). This potentiation could reflect better "translatibility" of newly made transcripts in terms of mRNP composition and transcript integrity, instead of independent regulation of transcription, mRNA stability and translation. Again, the fact that newly transcribed mRNAs posses a longer polyA tail might contribute to this increased "translatibility" (see above; Beilharz and Preiss 2007; Lackner et al. 2007). Not all mRNAs show potentiation however, and there were mRNAs only regulated at either the translational level or at the level of mRNA abundance.

By using additional early and late time points for the oxidative stress response, we could identify several mRNAs which respond very rapidly at the level of translation while changes in mRNA abundance were only measured after a certain delay. This could reflect an immediate response by the cell, as changes in translation should have a more immediate influence on protein levels than changes in transcription. However, it also needs to be noted that translational profiling directly measures the association of mRNAs with the translation machinery and as such is more sensitive to picking up immediate changes. On the other hand, the measurement of total mRNA levels reflects the outcome of transcription minus decay and even immediate changes in either of these processes would only manifest themselves after a certain time (i.e. when mRNAs have undergone transcription, processing and splicing or when the amount of mRNA has been diminished by changes in mRNA half-life). Due to these differences in measurements, it is also difficult to compare the extent of translational regulation to regulation at the level of mRNA abundance in total numbers. However, using a 1.5 fold cut-off to define changes in transcript levels relative to control cells, and comparing these changes on the level of mRNA abundance to the non-curated and the curated data set of translational changes, roughly 4 to 10 times more mRNAs are regulated at the level of mRNA abundance compared to mRNAs regulated at the level of translation after 15 min exposure to stress. Further experiments will be needed to

explore the impact of translational changes and changes at the level of mRNA abundance on changes in actual protein levels.

What are the genes that are regulated at the translational level in stress conditions? Apart from CESR genes, which are also regulated at the level of mRNA abundance, the only other functional group of mRNAs enriched in the translational targets are those encoding ribosomal proteins. Many of these mRNAs show a down-regulation in translation already after 15 min exposure to oxidative stress, when mRNA levels are not significantly altered. In contrast, many ribosomal mRNAs showed an increase in ribosomal association after 15 min exposure to heat stress, despite a slight decrease of the levels of mRNAs. This up-regulation could be an initial response to the shift from 32°C to 39°C, as temperature equilibrium in the culture might not be achieved immediately and fission yeast shows the highest doubling times at 35.5°C. The up-regulation of translation of ribosomal proteins under heat shock might happen in response to a transient increase in growth triggered by increased temperature, as cell growth and proliferation are linked to ribosome biogenesis (Jorgensen and Tyers 2004).

The translational down-regulation of ribosomal mRNAs after 15 min exposure to oxidative stress is in line with a much stronger down-regulation at the level of translation of almost all mRNAs encoding ribosomal proteins after 60 min exposure to oxidative stress (Figure 5.14). However, polysome profiles from this time-point are still not significantly altered compared to control cells (data not shown), which suggest that these mRNAs are specifically down-regulated preceding a general shut-down of translation. Furthermore, despite a general trend of lowered mRNA abundance for ribosomal mRNAs at this late time-point, not all of them show this down-regulation at the mRNA level, whereas most of them were already strongly down-regulated at the level of translation. Apart from this work and regulation in response to the deletion of *rmt3* (see above), translational regulation for mRNAs encoding ribosomal proteins has been described in several other conditions using genome-wide approaches (Johannes et al. 1999; Kuhn et al. 2001; Thomas and Johannes 2007). Furthermore synthesis of the translational apparatus may be regulated at the translational level by terminal oligoyrimidine (TOP) motifs, which can be found in mammlian ribosomal mRNAs (Meyuhas 2000). Together, these data argue for an independent regulation of translation and transcription for mRNAs encoding

ribosomal proteins, which might be an important mechanism to quickly respond to the environmental changes in terms of modulating global translation rates.

Future work

Validation of translationally regulated mRNAs after exposure to stress

An important part of any future work will be the validation of targets of translational regulation in stress conditions, which were identified using translational profiling. To this end, genes will be epitope-tagged at their 3' end and expressed under their natural promoter from their genomic locus. Immunblotting in a time-course experiment of fission yeast cells exposed to stress will identify changes in actual protein levels. Furthermore, RT-PCR will be used to quantitatively determine the mRNA levels for the corresponding gene. Data from these experiments should (1) validate the translational regulation of the candidate mRNA and (2) provide information about the temporal order of the regulation (i.e. how long it takes until changes in the ribosome-association of a given mRNA manifest themselves in changes of actual protein levels). Candidate genes that are mainly regulated at the translational level will be deleted and deletion strains, if viable, will be tested for sensitivity or resistance to the corresponding stress.

It will also be interesting to screen translationally regulated mRNAs for sequence-specific elements in their 3' and 5' UTRs, which might be essential for the translational regulation. At the moment, UTRs in fission yeast have only been identified for few, mainly highly abundant, mRNAs. However, current projects in our lab are on the way to identify these UTRs for the whole transcriptome, and data from these projects can then be used for computational analysis of UTR sequences to look into possible connections with translational regulation.

Translational regulation in response to starvation

Another future project in the lab will employ translational profiling to examine translational control in different starvation conditions. My preliminary data from fission yeast cells starved for either glucose or nitrogen suggest two different modes

of starvation, which are in line with recent studies (Shimanuki et al. 2007): whereas cells stop to divide in both conditions, translation is completely shut down in cells starved for glucose, whereas many mRNAs are still associated with polysomal fractions in nitrogen-starved cells, which remain metabolically active and can survive for extended times.

Alternative methods to measure global translational regulation

Translational profiling is an effective and established method to measure translation on a genome-wide scale. However, polysome isolation by immunopurification using a RIP-chip approach could be an alternative and less labour-intensive method. To this end, I have conducted preliminary studies. Several ribosomal proteins were C-terminally tagged with a TAP-tag (tandem affinity purification). From one viable strain, which did not exhibit a growth phenotype, ribosomes were immunopurified via the tagged ribosomal protein, and associated mRNAs were isolated, labelled and hybridized on a microarray and compared to total RNA. mRNAs that showed an enrichment corresponded to mRNAs with high ribosome density, whereas under-enriched mRNAs corresponded to mRNAs with low ribosome density. Thus, this approach could be optimized and used in the future to study translational changes in various conditions. This RIP-chip approach would be especially advantageous in experiments with many time points, as less cellular material than for translational profiling and only one microarray is needed for each time point; for example, this approach could be employed to study translational regulation during the cell cycle. Nevertheless, further validation experiments and optimization steps will be necessary to fully establish this alternative approach.

References

Abaza, I., O. Coll, S. Patalano and F. Gebauer (2006). "Drosophila UNR is required for translational repression of male-specific lethal 2 mRNA during regulation of X-chromosome dosage compensation." Genes Dev **20**: 380-9.

Aguilera, A. (2005). "Cotranscriptional mRNP assembly: from the DNA to the nuclear pore." Curr Opin Cell Biol **17**: 242-50.

Allen, E., Z. Xie, A. M. Gustafson and J. C. Carrington (2005). "microRNA-directed phasing during trans-acting siRNA biogenesis in plants." Cell **121**: 207-21.

Andrei, M. A., D. Ingelfinger, R. Heintzmann, T. Achsel, R. Rivera-Pomar and R. Luhrmann (2005). "A role for eIF4E and eIF4E-transporter in targeting mRNPs to mammalian processing bodies." RNA **11**: 717-27.

Arasu, P., B. Wightman and G. Ruvkun (1991). "Temporal regulation of lin-14 by the antagonistic action of two other heterochronic genes, lin-4 and lin-28." Genes Dev **5**: 1825-33.

Arava, Y., F. E. Boas, P. O. Brown and D. Herschlag (2005). "Dissecting eukaryotic translation and its control by ribosome density mapping." Nucleic Acids Res **33**: 2421-32.

Arava, Y., Y. Wang, J. D. Storey, C. L. Liu, P. O. Brown and D. Herschlag (2003). "Genome-wide analysis of mRNA translation profiles in Saccharomyces cerevisiae." Proc Natl Acad Sci U S A **100**: 3889-94.

Askree, S. H., T. Yehuda, S. Smolikov, R. Gurevich, J. Hawk, C. Coker, A. Krauskopf, M. Kupiec and M. J. McEachern (2004). "A genome-wide screen for Saccharomyces cerevisiae deletion mutants that affect telomere length." Proc Natl Acad Sci U S A **101**: 8658-63.

Aslett, M. and V. Wood (2006). "Gene Ontology annotation status of the fission yeast genome: preliminary coverage approaches 100%." Yeast **23**: 913-9.

Babu, M. M., N. M. Luscombe, L. Aravind, M. Gerstein and S. A. Teichmann (2004). "Structure and evolution of transcriptional regulatory networks." Curr Opin Struct Biol **14**: 283-91.

Bachand, F. (2007). "Protein arginine methyltransferases: from unicellular eukaryotes to humans." Eukaryot Cell **6**: 889-98.

Bachand, F., D. H. Lackner, J. Bähler and P. A. Silver (2006). "Autoregulation of ribosome biosynthesis by a translational response in fission yeast." Mol Cell Biol **26**: 1731-42.

Bachand, F. and P. A. Silver (2004). "PRMT3 is a ribosomal protein methyltransferase that affects the cellular levels of ribosomal subunits." EMBO J **23**: 2641-50.

Bähler, J. and P. Nurse (2001). "Fission yeast Pom1p kinase activity is cell cycle regulated and essential for cellular symmetry during growth and division." EMBO J **20**: 1064-73.

Baird, S. D., M. Turcotte, R. G. Korneluk and M. Holcik (2006). "Searching for IRES." RNA **12**: 1755-85.

Barreau, C., L. Paillard and H. B. Osborne (2005). "AU-rich elements and associated factors: are there unifying principles?" Nucleic Acids Res **33**: 7138-50.

Barrera, L. O. and B. Ren (2006). "The transcriptional regulatory code of eukaryotic cells--insights from genome-wide analysis of chromatin organization and transcription factor binding." Curr Opin Cell Biol **18**: 291-8.

Bartel, D. P. (2004). "MicroRNAs: genomics, biogenesis, mechanism, and function." Cell **116**: 281-97.

Bashaw, G. J. and B. S. Baker (1997). "The regulation of the Drosophila msl-2 gene reveals a function for Sex-lethal in translational control." Cell **89**: 789-98.

Bashkirov, V. I., H. Scherthan, J. A. Solinger, J. M. Buerstedde and W. D. Heyer (1997). "A mouse cytoplasmic exoribonuclease (mXRN1p) with preference for G4 tetraplex substrates." J Cell Biol **136**: 761-73.

Basi, G., E. Schmid and K. Maundrell (1993). "TATA box mutations in the Schizosaccharomyces pombe nmt1 promoter affect transcription efficiency but not the transcription start point or thiamine repressibility." Gene **123**: 131-6.

Beckmann, K., M. Grskovic, F. Gebauer and M. W. Hentze (2005). "A dual inhibitory mechanism restricts msl-2 mRNA translation for dosage compensation in Drosophila." Cell **122**: 529-40.

Beilharz, T. H. and T. Preiss (2004). "Translational profiling: The genome-wide measure of the nascent proteome." Brief Funct Genomic Proteomic **3**: 103-11.

Beilharz, T. H. and T. Preiss (2007). "Widespread use of poly(A) tail length control to accentuate expression of the yeast transcriptome." RNA **13**: 982-97.

Bernstein, B. E., A. Meissner and E. S. Lander (2007). "The mammalian epigenome." Cell **128**: 669-81.

Berriz, G. F., O. D. King, B. Bryant, C. Sander and F. P. Roth (2003). "Characterizing gene sets with FuncAssociate." Bioinformatics **19**: 2502-4.

Bertone, P., M. Gerstein and M. Snyder (2005). "Applications of DNA tiling arrays to experimental genome annotation and regulatory pathway discovery." Chromosome Res **13**: 259-74.

Best, D. J. and D. E. Roberts (1975). "AS 89, Upper Tail Probabilities of Spearman's Rho." Applied Statistics **24**: 377-79.

Beyer, A., J. Hollunder, H. P. Nasheuer and T. Wilhelm (2004). "Post-transcriptional Expression Regulation in the Yeast Saccharomyces cerevisiae on a Genomic Scale." Mol Cell Proteomics **3**: 1083-1092.

Bhattacharyya, S. N., R. Habermacher, U. Martine, E. I. Closs and W. Filipowicz (2006). "Relief of microRNA-mediated translational repression in human cells subjected to stress." Cell **125**: 1111-24.

Binder, R., J. A. Horowitz, J. P. Basilion, D. M. Koeller, R. D. Klausner and J. B. Harford (1994). "Evidence that the pathway of transferrin receptor mRNA degradation involves an endonucleolytic cleavage within the 3' UTR and does not involve poly(A) tail shortening." EMBO J **13**: 1969-80.

Brengues, M., D. Teixeira and R. Parker (2005). "Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies." Science **310**: 486-9.

Brewer, G. (1991). "An A + U-rich element RNA-binding factor regulates c-myc mRNA stability in vitro." Mol Cell Biol **11**: 2460-6.

Bushell, M., M. Stoneley, Y. W. Kong, T. L. Hamilton, K. A. Spriggs, H. C. Dobbyn, X. Qin, P. Sarnow and A. E. Willis (2006). "Polypyrimidine tract binding protein regulates IRES-mediated gene expression during apoptosis." Mol Cell **23**: 401-12.

Butler, J. S., P. P. Sadhale and T. Platt (1990). "RNA processing in vitro produces mature 3' ends of a variety of Saccharomyces cerevisiae mRNAs." Mol Cell Biol **10**: 2599-605.

Cao, Q. and J. D. Richter (2002). "Dissolution of the maskin-eIF4E complex by cytoplasmic polyadenylation and poly(A)-binding protein controls cyclin B1 mRNA translation and oocyte maturation." EMBO J **21**: 3852-62.

Carballo, E., W. S. Lai and P. J. Blackshear (1998). "Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin." Science **281**: 1001-5.

Causton, H. C., et al. (2001). "Remodeling of yeast genome expression in response to environmental changes." Mol Biol Cell **12**: 323-37.

Chen, D., W. M. Toone, J. Mata, R. Lyne, G. Burns, K. Kivinen, A. Brazma, N. Jones and J. Bähler (2003). "Global transcriptional responses of fission yeast to environmental stress." Mol Biol Cell **14**: 214-29.

Cho, P. F., F. Poulin, Y. A. Cho-Park, I. B. Cho-Park, J. D. Chicoine, P. Lasko and N. Sonenberg (2005). "A new paradigm for translational control: inhibition via 5'-3' mRNA tethering by Bicoid and the eIF4E cognate 4EHP." Cell **121**: 411-23.

Chu, C. Y. and T. M. Rana (2006). "Translation repression in human cells by microRNA-induced gene silencing requires RCK/p54." PLoS Biol **4**: e210.

Cole, C. N. and J. J. Scarcelli (2006). "Transport of messenger RNA from the nucleus to the cytoplasm." Curr Opin Cell Biol **18**: 299-306.

Coller, J. and R. Parker (2005). "General translational repression by activators of mRNA decapping." Cell **122**: 875-86.

Collins, C. A. and C. Guthrie (2000). "The question remains: is the spliceosome a ribozyme?" Nat Struct Biol **7**: 850-4.

Conti, E. and E. Izaurralde (2005). "Nonsense-mediated mRNA decay: molecular insights and mechanistic variations across species." Curr Opin Cell Biol **17**: 316-25.

Costa-Mattioli, M., et al. (2005). "Translational control of hippocampal synaptic plasticity and memory by the eIF2alpha kinase GCN2." Nature **436**: 1166-73.

Cougot, N., S. Babajko and B. Seraphin (2004). "Cytoplasmic foci are sites of mRNA decay in human cells." J Cell Biol **165**: 31-40.

Cvijovic, M., D. Dalevi, E. Bilsland, G. J. Kemp and P. Sunnerhagen (2007). "Identification of putative regulatory upstream ORFs in the yeast genome using heuristics and evolutionary conservation." BMC Bioinformatics **8**: 295.

Daneholt, B. (1997). "A look at messenger RNP moving through the nuclear pore." Cell **88**: 585-8.

Decker, C. J. and R. Parker (1993). "A turnover pathway for both stable and unstable mRNAs in yeast: evidence for a requirement for deadenylation." Genes Dev **7**: 1632-43.

Dever, T. E. (2002). "Gene-specific regulation by general translation factors." Cell **108**: 545-56.

Dever, T. E., A. C. Dar and F. Sicheri (2007). The eIF2α Kinases. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 319-344.

Dever, T. E., L. Feng, R. C. Wek, A. M. Cigan, T. F. Donahue and A. G. Hinnebusch (1992). "Phosphorylation of initiation factor 2 alpha by protein kinase GCN2 mediates gene-specific translational control of GCN4 in yeast." Cell **68**: 585-96.

Dinkova, T. D., B. D. Keiper, N. L. Korneeva, E. J. Aamodt and R. E. Rhoads (2005). "Translation of a small subset of Caenorhabditis elegans mRNAs is dependent on a specific eukaryotic translation initiation factor 4E isoform." Mol Cell Biol **25**: 100-13.

Dong, J., H. Qiu, M. Garcia-Barrio, J. Anderson and A. G. Hinnebusch (2000). "Uncharged tRNA activates GCN2 by displacing the protein kinase moiety from a bipartite tRNA-binding domain." Mol Cell **6**: 269-79.

Doudna, J. A. and P. Sarnow (2007). Translation Initiation by Viral Internal Ribosome Entry Sites. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 129-153.

Drummond, D. A., J. D. Bloom, C. Adami, C. O. Wilke and F. H. Arnold (2005). "Why highly expressed proteins evolve slowly." Proc Natl Acad Sci U S A **102**: 14338-43.

Dubnau, J. and G. Struhl (1996). "RNA recognition and translational regulation by a homeodomain protein." Nature **379**: 694-9.

Dunand-Sauthier, I., C. Walker, C. Wilkinson, C. Gordon, R. Crane, C. Norbury and T. Humphrey (2002). "Sum1, a component of the fission yeast eIF3 translation initiation complex, is rapidly relocalized during environmental stress and interacts with components of the 26S proteasome." Mol Biol Cell **13**: 1626-40.

Dunand-Sauthier, I., C. A. Walker, J. Narasimhan, A. K. Pearce, R. C. Wek and T. C. Humphrey (2005). "Stress-activated protein kinase pathway functions to support protein synthesis and translational adaptation in response to environmental stress in fission yeast." Eukaryot Cell **4**: 1785-93.

Duncan, K., M. Grskovic, C. Strein, K. Beckmann, R. Niggeweg, I. Abaza, F. Gebauer, M. Wilm and M. W. Hentze (2006). "Sex-lethal imparts a sex-specific function to UNR by recruiting it to the msl-2 mRNA 3' UTR: translational repression for dosage compensation." Genes Dev **20**: 368-79.

Duret, L. and D. Mouchiroud (1999). "Expression pattern and, surprisingly, gene length shape codon usage in Caenorhabditis, Drosophila, and Arabidopsis." Proc Natl Acad Sci U S A **96**: 4482-7.

Duttagupta, R., B. Tian, C. J. Wilusz, D. T. Khounh, P. Soteropoulos, M. Ouyang, J. P. Dougherty and S. W. Peltz (2005). "Global analysis of Pub1p targets reveals a coordinate control of gene expression through modulation of binding and stability." Mol Cell Biol **25**: 5499-513.

Ehrenberg, M., V. Hauryliuk and T. A. A. Pöyry (2007). Translation Termination, the Prion [*PSI*$^{+}$], and Ribosomal Recycling. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 173-196.

Elroy-Stein, O. and W. C. Merrick (2007). Translation Initiation Via Cellular Internal Ribosome Entry Sites. Translational Control in Biology an Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 155-172.

Eystathioy, T., E. K. Chan, S. A. Tenenbaum, J. D. Keene, K. Griffith and M. J. Fritzler (2002). "A phosphorylated cytoplasmic autoantigen, GW182, associates with a unique population of human mRNAs within novel cytoplasmic speckles." Mol Biol Cell **13**: 1338-51.

Eystathioy, T., A. Jakymiw, E. K. Chan, B. Seraphin, N. Cougot and M. J. Fritzler (2003). "The GW182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLSm4 in cytoplasmic GW bodies." RNA **9**: 1171-3.

Fahrner, K., J. Yarger and L. Hereford (1980). "Yeast histone mRNA is polyadenylated." Nucleic Acids Res **8**: 5725-37.

Fasken, M. B. and A. H. Corbett (2005). "Process or perish: quality control in mRNA biogenesis." Nat Struct Mol Biol **12**: 482-8.

Ferraiuolo, M. A., S. Basak, J. Dostie, E. L. Murray, D. R. Schoenberg and N. Sonenberg (2005). "A role for the eIF4E-binding protein 4E-T in P-body formation and mRNA decay." J Cell Biol **170**: 913-24.

Filipowicz, W. (2005). "RNAi: the nuts and bolts of the RISC machine." Cell **122**: 17-20.

Forch, P. and J. Valcarcel (2003). "Splicing regulation in Drosophila sex determination." Prog Mol Subcell Biol **31**: 127-51.

Fraser, C. S. and J. A. Doudna (2007). "Structural and mechanistic insights into hepatitis C viral translation initiation." Nat Rev Microbiol **5**: 29-38.

Futcher, B., G. I. Latter, P. Monardo, C. S. McLaughlin and J. I. Garrels (1999). "A sampling of the yeast proteome." Mol Cell Biol **19**: 7357-68.

Gama-Carvalho, M., N. L. Barbosa-Morais, A. S. Brodsky, P. A. Silver and M. Carmo-Fonseca (2006). "Genome-wide identification of functionally distinct subsets of cellular mRNAs associated with two nucleocytoplasmic-shuttling mammalian splicing factors." Genome Biol **7**: R113.

Gasch, A. P. (2007). "Comparative genomics of the environmental stress response in ascomycete fungi." Yeast (in press).

Gasch, A. P., M. Huang, S. Metzner, D. Botstein, S. J. Elledge and P. O. Brown (2001). "Genomic expression responses to DNA-damaging agents and the regulatory role of the yeast ATR homolog Mec1p." Mol Biol Cell **12**: 2987-3003.

Gasch, A. P., P. T. Spellman, C. M. Kao, O. Carmel-Harel, M. B. Eisen, G. Storz, D. Botstein and P. O. Brown (2000). "Genomic expression programs in the response of yeast cells to environmental changes." Mol Biol Cell **11**: 4241-57.

Gasch, A. P. and M. Werner-Washburne (2002). "The genomics of yeast responses to environmental stress and starvation." Funct Integr Genomics **2**: 181-92.

Gatti, L., et al. (2004). "Global gene expression of fission yeast in response to cisplatin." Cell Mol Life Sci **61**: 2253-63.

Gebauer, F. and M. W. Hentze (2004). "Molecular mechanisms of translational control." Nat Rev Mol Cell Biol **5**: 827-35.

Gebauer, F., L. Merendino, M. W. Hentze and J. Valcarcel (1998). "The Drosophila splicing regulator sex-lethal directly inhibits translation of male-specific-lethal 2 mRNA." RNA **4**: 142-50.

Gerber, A. P., D. Herschlag and P. O. Brown (2004). "Extensive association of functionally and cytotopically related mRNAs with Puf family RNA-binding proteins in yeast." PLoS Biol **2**: E79.

Gerber, A. P., S. Luschnig, M. A. Krasnow, P. O. Brown and D. Herschlag (2006). "Genome-wide identification of mRNAs associated with the translational regulator PUMILIO in Drosophila melanogaster." Proc Natl Acad Sci U S A **103**: 4487-92.

Gherzi, R., K. Y. Lee, P. Briata, D. Wegmuller, C. Moroni, M. Karin and C. Y. Chen (2004). "A KH domain RNA binding protein, KSRP, promotes ARE-directed mRNA turnover by recruiting the degradation machinery." Mol Cell **14**: 571-83.

Gingras, A. C., B. Raught and N. Sonenberg (1999). "eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation." Annu Rev Biochem **68**: 913-63.

Giraldez, A. J., Y. Mishima, J. Rihel, R. J. Grocock, S. Van Dongen, K. Inoue, A. J. Enright and A. F. Schier (2006). "Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs." Science **312**: 75-9.

Goyer, C., M. Altmann, H. Trachsel and N. Sonenberg (1989). "Identification and characterization of cap-binding proteins from yeast." J Biol Chem **264**: 7603-10.

Gressner, A. M. and I. G. Wool (1974). "The phosphorylation of liver ribosomal proteins in vivo. Evidence that only a single small subunit protein (S6) is phosphorylated." J Biol Chem **249**: 6917-25.

Grigull, J., S. Mnaimneh, J. Pootoolal, M. D. Robinson and T. R. Hughes (2004). "Genome-wide analysis of mRNA stability using transcription inhibitors and microarrays reveals posttranscriptional control of ribosome biogenesis factors." Mol Cell Biol **24**: 5534-47.

Grolleau, A., J. Bowman, B. Pradet-Balade, E. Puravs, S. Hanash, J. A. Garcia-Sanz and L. Beretta (2002). "Global and specific translational control by rapamycin in T cells uncovered by microarrays and proteomics." J Biol Chem **277**: 22175-84.

Grskovic, M., M. W. Hentze and F. Gebauer (2003). "A co-repressor assembly nucleated by Sex-lethal in the 3'UTR mediates translational control of Drosophila msl-2 mRNA." EMBO J **22**: 5571-81.

Gu, M. and C. D. Lima (2005). "Processing the message: structural insights into capping and decapping mRNA." Curr Opin Struct Biol **15**: 99-106.

Gueydan, C., L. Droogmans, P. Chalon, G. Huez, D. Caput and V. Kruys (1999). "Identification of TIAR as a protein binding to the translational regulatory AU-rich element of tumor necrosis factor alpha mRNA." J Biol Chem **274**: 2322-6.

Gutierrez, R. A., R. M. Ewing, J. M. Cherry and P. J. Green (2002). "Identification of unstable transcripts in Arabidopsis by cDNA microarray analysis: rapid decay is associated with a group of touch- and specific clock-controlled genes." Proc Natl Acad Sci U S A **99**: 11513-8.

Haarer, B., S. Viggiano, M. A. Hibbs, O. G. Troyanskaya and D. C. Amberg (2007). "Modeling complex genetic interactions in a simple eukaryotic genome: actin displays a rich spectrum of complex haploinsufficiencies." Genes Dev **21**: 148-59.

Hanlon, S. E. and J. D. Lieb (2004). "Progress and challenges in profiling the dynamics of chromatin and transcription factor binding with DNA microarrays." Curr Opin Genet Dev **14**: 697-705.

Hao, S., et al. (2005). "Uncharged tRNA and sensing of amino acid deficiency in mammalian piriform cortex." Science **307**: 1776-8.

Harding, H. P., I. Novoa, Y. Zhang, H. Zeng, R. Wek, M. Schapira and D. Ron (2000). "Regulated translation initiation controls stress-induced gene expression in mammalian cells." Mol Cell **6**: 1099-108.

He, F., X. Li, P. Spatrick, R. Casillo, S. Dong and A. Jacobson (2003). "Genome-wide analysis of mRNAs regulated by the nonsense-mediated and 5' to 3' mRNA decay pathways in yeast." Mol Cell **12**: 1439-52.

Hellen, C. U. and P. Sarnow (2001). "Internal ribosome entry sites in eukaryotic mRNA molecules." Genes Dev **15**: 1593-612.

Hershey, J. W. B. and W. C. Merrick (2000). Pathway and Mechanism of Initiation of Protein Synthesis. Translational Control of Gene Expression. N. Sonenberg, J. W. B. Hershey and M. B. Mathews, Cold Spring Harbor Laboratory Press**:** 33-88.

Heyer, W. D., A. W. Johnson, U. Reinhart and R. D. Kolodner (1995). "Regulation and intracellular localization of Saccharomyces cerevisiae strand exchange protein 1 (Sep1/Xrn1/Kem1), a multifunctional exonuclease." Mol Cell Biol **15**: 2728-36.

Hieronymus, H. and P. A. Silver (2003). "Genome-wide analysis of RNA-protein interactions illustrates specificity of the mRNA export machinery." Nat Genet **33**: 155-61.

Hieronymus, H. and P. A. Silver (2004). "A systems view of mRNP biology." Genes Dev **18**: 2845-60.

Hinnebusch, A. G. (2005). "Translational regulation of GCN4 and the general amino acid control of yeast." Annu Rev Microbiol **59**: 407-50.

Hinnebusch, A. G., T. E. Dever and K. Asano (2007). Mechanism of Translation Initiation in the Yeast *Saccharomyces cerevisiae*. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 225-268.

Hinnebusch, A. G. and K. Natarajan (2002). "Gcn4p, a master regulator of gene expression, is controlled at multiple levels by diverse signals of starvation and stress." Eukaryot Cell **1**: 22-32.

Holcik, M. and N. Sonenberg (2005). "Translational control in stress and apoptosis." Nat Rev Mol Cell Biol **6**: 318-27.

Hollien, J. and J. S. Weissman (2006). "Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response." Science **313**: 104-7.

Humphreys, D. T., B. J. Westman, D. I. Martin and T. Preiss (2005). "MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function." Proc Natl Acad Sci U S A **102**: 16961-6.

Hwang, K. H., C. Carapito, S. Bohmer, E. Leize, A. Van Dorsselaer and R. Bernhardt (2006). "Proteome analysis of Schizosaccharomyces pombe by two-dimensional gel electrophoresis and mass spectrometry." Proteomics **6**: 4115-29.

Iacono, M., F. Mignone and G. Pesole (2005). "uAUG and uORFs in human and rodent 5'untranslated mRNAs." Gene **349**: 97-105.

Iguchi, N., J. W. Tobias and N. B. Hecht (2006). "Expression profiling reveals meiotic male germ cell mRNAs that are translationally up- and down-regulated." Proc Natl Acad Sci U S A **103**: 7712-7.

Inada, M. and C. Guthrie (2004). "Identification of Lhp1p-associated RNAs by microarray analysis in Saccharomyces cerevisiae reveals association with coding and noncoding RNAs." Proc Natl Acad Sci U S A **101**: 434-9.

Inada, T., E. Winstall, S. Z. Tarun, Jr., J. R. Yates, 3rd, D. Schieltz and A. B. Sachs (2002). "One-step affinity purification of the yeast ribosome and its associated proteins and mRNAs." RNA **8**: 948-58.

Ingelfinger, D., D. J. Arndt-Jovin, R. Luhrmann and T. Achsel (2002). "The human LSm1-7 proteins colocalize with the mRNA-degrading enzymes Dcp1/2 and Xrnl in distinct cytoplasmic foci." RNA **8**: 1489-501.

Jackson, R. J. (2005). "Alternative mechanisms of initiating translation of mammalian mRNAs." Biochem Soc Trans **33**: 1231-41.

Jackson, R. J. and N. Standart (2007). "How do microRNAs regulate gene expression?" Sci STKE **2007**: re1.

Jakymiw, A., S. Lian, T. Eystathioy, S. Li, M. Satoh, J. C. Hamel, M. J. Fritzler and E. K. Chan (2005). "Disruption of GW bodies impairs mammalian RNA interference." Nat Cell Biol **7**: 1267-74.

Jefferies, H. B., C. Reinhard, S. C. Kozma and G. Thomas (1994). "Rapamycin selectively represses translation of the "polypyrimidine tract" mRNA family." Proc Natl Acad Sci U S A **91**: 4441-5.

Johannes, G., M. S. Carter, M. B. Eisen, P. O. Brown and P. Sarnow (1999). "Identification of eukaryotic mRNAs that are translated at reduced cap binding complex eIF4F concentrations using a cDNA microarray." Proc Natl Acad Sci U S A **96**: 13118-23.

Jorgensen, P., J. L. Nishikawa, B. J. Breitkreutz and M. Tyers (2002). "Systematic identification of pathways that couple cell growth and division in yeast." Science **297**: 395-400.

Jorgensen, P. and M. Tyers (2004). "How cells coordinate growth and division." Curr Biol **14**: R1014-27.

Jurica, M. S. and M. J. Moore (2003). "Pre-mRNA splicing: awash in a sea of proteins." Mol Cell **12**: 5-14.

Kadonaga, J. T. (2004). "Regulation of RNA polymerase II transcription by sequence-specific DNA binding factors." Cell **116**: 247-57.

Kahvejian, A., G. Roy and N. Sonenberg (2001). "The mRNA closed-loop model: the function of PABP and PABP-interacting proteins in mRNA translation." Cold Spring Harb Symp Quant Biol **66**: 293-300.

Kash, J. C., D. M. Cunningham, M. W. Smit, Y. Park, D. Fritz, J. Wilusz and M. G. Katze (2002). "Selective translation of eukaryotic mRNAs: functional

molecular analysis of GRSF-1, a positive regulator of influenza virus protein synthesis." J Virol **76**: 10417-26.

Kedersha, N. and P. Anderson (2002). "Stress granules: sites of mRNA triage that regulate mRNA stability and translatability." Biochem Soc Trans **30**: 963-9.

Kedersha, N., et al. (2005). "Stress granules and processing bodies are dynamically linked sites of mRNP remodeling." J Cell Biol **169**: 871-84.

Keene, J. D. (2007). "RNA regulons: coordination of post-transcriptional events." Nat Rev Genet **8**: 533-43.

Kelley, R. L., I. Solovyeva, L. M. Lyman, R. Richman, V. Solovyev and M. I. Kuroda (1995). "Expression of msl-2 causes assembly of dosage compensation regulators on the X chromosomes and female lethality in Drosophila." Cell **81**: 867-77.

Kelley, R. L., J. Wang, L. Bell and M. I. Kuroda (1997). "Sex lethal controls dosage compensation in Drosophila by a non-splicing mechanism." Nature **387**: 195-9.

Kellis, M., B. W. Birren and E. S. Lander (2004). "Proof and evolutionary analysis of ancient genome duplication in the yeast Saccharomyces cerevisiae." Nature **428**: 617-24.

Kim, J. H. and J. D. Richter (2006). "Opposing polymerase-deadenylase activities regulate cytoplasmic polyadenylation." Mol Cell **24**: 173-83.

Kim Guisbert, K., K. Duncan, H. Li and C. Guthrie (2005). "Functional specificity of shuttling hnRNPs revealed by genome-wide analysis of their RNA binding profiles." RNA **11**: 383-93.

Kim, V. N. (2005). "MicroRNA biogenesis: coordinated cropping and dicing." Nat Rev Mol Cell Biol **6**: 376-85.

Kisselev, L. L. and R. H. Buckingham (2000). "Translational termination comes of age." Trends Biochem Sci **25**: 561-6.

Klann, E. and J. D. Richter (2007). Translational Control of Synaptic Plasticity and Learning and Memory. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 485-506.

Kouzarides, T. (2007). "Chromatin modifications and their function." Cell **128**: 693-705.

Kozak, M. (1989). "The scanning model for translation: an update." J Cell Biol **108**: 229-41.

Kozak, M. (1991). "Structural features in eukaryotic mRNAs that modulate the initiation of translation." J Biol Chem **266**: 19867-70.

Kozak, M. (1999). "Initiation of translation in prokaryotes and eukaryotes." Gene **234**: 187-208.

Kozak, M. (2002). "Pushing the limits of the scanning mechanism for initiation of translation." Gene **299**: 1-34.

Kramer, A. (1996). "The structure and function of proteins involved in mammalian pre-mRNA splicing." Annu Rev Biochem **65**: 367-409.

Krupp, G., B. Cherayil, D. Frendewey, S. Nishikawa and D. Soll (1986). "Two RNA species co-purify with RNase P from the fission yeast Schizosaccharomyces pombe." EMBO J **5**: 1697-703.

Kuersten, S. and E. B. Goodwin (2003). "The power of the 3' UTR: translational control and development." Nat Rev Genet **4**: 626-37.

Kuhn, K. M., J. L. DeRisi, P. O. Brown and P. Sarnow (2001). "Global and specific translational regulation in the genomic response of Saccharomyces cerevisiae to a rapid transfer from a fermentable to a nonfermentable carbon source." Mol Cell Biol **21**: 916-27.

Lackner, D. H., T. H. Beilharz, S. Marguerat, J. Mata, S. Watt, F. Schubert, T. Preiss and J. Bähler (2007). "A network of multiple regulatory layers shapes gene expression in fission yeast." Mol Cell **26**: 145-55.

Lal, A., K. Abdelmohsen, R. Pullmann, T. Kawai, S. Galban, X. Yang, G. Brewer and M. Gorospe (2006). "Posttranscriptional derepression of GADD45alpha by genotoxic stress." Mol Cell **22**: 117-28.

Lal, A., K. Mazan-Mamczarz, T. Kawai, X. Yang, J. L. Martindale and M. Gorospe (2004). "Concurrent versus individual binding of HuR and AUF1 to common labile target mRNAs." EMBO J **23**: 3092-102.

Lamphear, B. J., R. Kirchweger, T. Skern and R. E. Rhoads (1995). "Mapping of functional domains in eukaryotic protein synthesis initiation factor 4G (eIF4G) with picornaviral proteases. Implications for cap-dependent and cap-independent translational initiation." J Biol Chem **270**: 21975-83.

Laroia, G., B. Sarkar and R. J. Schneider (2002). "Ubiquitin-dependent mechanism regulates rapid turnover of AU-rich cytokine mRNAs." Proc Natl Acad Sci U S A **99**: 1842-6.

Law, G. L., K. S. Bickel, V. L. MacKay and D. R. Morris (2005). "The undertranslated transcriptome reveals widespread translational silencing by alternative 5' transcript leaders." Genome Biol **6**: R111.

Lee, J. H., T. V. Pestova, B. S. Shin, C. Cao, S. K. Choi and T. E. Dever (2002). "Initiation factor eIF5B catalyzes second GTP-dependent step in eukaryotic translation initiation." Proc Natl Acad Sci U S A **99**: 16689-94.

Lewis, B. P., C. B. Burge and D. P. Bartel (2005). "Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets." Cell **120**: 15-20.

Li, B., M. Carey and J. L. Workman (2007). "The role of chromatin during transcription." Cell **128**: 707-19.

Liao, B., Y. Hu and G. Brewer (2007). "Competitive binding of AUF1 and TIAR to MYC mRNA controls its translation." Nat Struct Mol Biol **14**: 511-8.

Liu, J., F. V. Rivas, J. Wohlschlegel, J. R. Yates, 3rd, R. Parker and G. J. Hannon (2005). "A role for the P-body component GW182 in microRNA function." Nat Cell Biol **7**: 1261-6.

Liu, J., M. A. Valencia-Sanchez, G. J. Hannon and R. Parker (2005). "MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies." Nat Cell Biol **7**: 719-23.

Llave, C., Z. Xie, K. D. Kasschau and J. C. Carrington (2002). "Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA." Science **297**: 2053-6.

Lockhart, D. J. and E. A. Winzeler (2000). "Genomics, gene expression and DNA arrays." Nature **405**: 827-36.

Lodish, H. F. and M. Jacobsen (1972). "Regulation of hemoglobin synthesis. Equal rates of translation and termination of - and -globin chains." J Biol Chem **247**: 3622-9.

Lu, P., C. Vogel, R. Wang, X. Yao and E. M. Marcotte (2007). "Absolute protein expression profiling estimates the relative contributions of transcriptional and translational regulation." Nat Biotechnol **25**: 117-24.

Luscombe, N. M., M. M. Babu, H. Yu, M. Snyder, S. A. Teichmann and M. Gerstein (2004). "Genomic analysis of regulatory network dynamics reveals large topological changes." Nature **431**: 308-12.

Lykke-Andersen, J. (2002). "Identification of a human decapping complex associated with hUpf proteins in nonsense-mediated decay." Mol Cell Biol **22**: 8114-21.

Lyne, R., G. Burns, J. Mata, C. J. Penkett, G. Rustici, D. Chen, C. Langford, D. Vetrie and J. Bähler (2003). "Whole-genome microarrays of fission yeast: characteristics, accuracy, reproducibility, and processing of array data." BMC Genomics **4**: 27.

MacDonald, C. C., J. Wilusz and T. Shenk (1994). "The 64-kilodalton subunit of the CstF polyadenylation factor binds to pre-mRNAs downstream of the cleavage site and influences cleavage site location." Mol Cell Biol **14**: 6647-54.

MacKay, V. L., et al. (2004). "Gene expression analyzed by high-resolution state array analysis and quantitative proteomics: response of yeast to mating pheromone." Mol Cell Proteomics **3**: 478-89.

Mager, W. H. and M. Siderius (2002). "Novel insights into the osmotic stress response of yeast." FEMS Yeast Res **2**: 251-7.

Mallory, A. C., B. J. Reinhart, M. W. Jones-Rhoades, G. Tang, P. D. Zamore, M. K. Barton and D. P. Bartel (2004). "MicroRNA control of PHABULOSA in leaf development: importance of pairing to the microRNA 5' region." EMBO J **23**: 3356-64.

Mangus, D. A., M. C. Evans and A. Jacobson (2003). "Poly(A)-binding proteins: multifunctional scaffolds for the post-transcriptional control of gene expression." Genome Biol **4**: 223.

Maniatis, T. and R. Reed (2002). "An extensive network of coupling among gene expression machines." Nature **416**: 499-506.

Marais, G. and L. Duret (2001). "Synonymous codon usage, accuracy of translation, and gene length in Caenorhabditis elegans." J Mol Evol **52**: 275-80.

Marguerat, S., T. S. Jensen, U. de Lichtenberg, B. T. Wilhelm, L. J. Jensen and J. Bähler (2006). "The more the merrier: comparative analysis of microarray studies on cell cycle-regulated genes in fission yeast." Yeast **23**: 261-77.

Maris, C., C. Dominguez and F. H. Allain (2005). "The RNA recognition motif, a plastic RNA-binding platform to regulate post-transcriptional gene expression." Febs J **272**: 2118-31.

Marston, A. L., W. H. Tham, H. Shah and A. Amon (2004). "A genome-wide screen identifies genes required for centromeric cohesion." Science **303**: 1367-70.

Mata, J. and J. Bähler (2003). "Correlations between gene expression and gene conservation in fission yeast." Genome Res **13**: 2686-90.

Mata, J. and J. Bähler (2006). "Global roles of Ste11p, cell type, and pheromone in the control of gene expression during early sexual differentiation in fission yeast." Proc Natl Acad Sci U S A **103**: 15517-22.

Mata, J., R. Lyne, G. Burns and J. Bähler (2002). "The transcriptional program of meiosis and sporulation in fission yeast." Nat Genet **32**: 143-7.

Mata, J., S. Marguerat and J. Bähler (2005). "Post-transcriptional control of gene expression: a genome-wide perspective." Trends Biochem Sci **30**: 506-14.

Mathews, M. B., N. Sonenberg and J. W. B. Hershey (2007). Origins and Principles of Translational Control. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 1-40.

Matlin, A. J., F. Clark and C. W. Smith (2005). "Understanding alternative splicing: towards a cellular code." Nat Rev Mol Cell Biol **6**: 386-98.

Matsuyama, A., et al. (2006). "ORFeome cloning and global analysis of protein localization in the fission yeast Schizosaccharomyces pombe." Nat Biotechnol **24**: 841-7.

Maurin, A. C., et al. (2005). "The GCN2 kinase biases feeding behavior to maintain amino acid homeostasis in omnivores." Cell Metab **1**: 273-7.

Mazan-Mamczarz, K., A. Lal, J. L. Martindale, T. Kawai and M. Gorospe (2006). "Translational repression by RNA-binding protein TIAR." Mol Cell Biol **26**: 2716-27.

McCarroll, S. A., C. T. Murphy, S. Zou, S. D. Pletcher, C. S. Chin, Y. N. Jan, C. Kenyon, C. I. Bargmann and H. Li (2004). "Comparing genomic expression patterns across species identifies shared transcriptional profile in aging." Nat Genet **36**: 197-204.

McCarthy, J. E. (1998). "Posttranscriptional control of gene expression in yeast." Microbiol Mol Biol Rev **62**: 1492-553.

McKee, A. E. and P. A. Silver (2007). "Systems perspectives on mRNA processing." Cell Res **17**: 581-90.

McLauchlan, J., D. Gaffney, J. L. Whitton and J. B. Clements (1985). "The consensus sequence YGTGTTYY located downstream from the AATAAA signal is required for efficient formation of mRNA 3' termini." Nucleic Acids Res **13**: 1347-68.

Mendell, J. T., N. A. Sharifi, J. L. Meyers, F. Martinez-Murillo and H. C. Dietz (2004). "Nonsense surveillance regulates expression of diverse classes of mammalian transcripts and mutes genomic noise." Nat Genet **36**: 1073-8.

Mendez, R. and J. D. Richter (2001). "Translational control by CPEB: a means to the end." Nat Rev Mol Cell Biol **2**: 521-9.

Mertins, P. and D. Gallwitz (1987). "A single intronless actin gene in the fission yeast Schizosaccharomyces pombe: nucleotide sequence and transcripts formed in homologous and heterologous yeast." Nucleic Acids Res **15**: 7369-79.

Meyuhas, O. (2000). "Synthesis of the translational apparatus is regulated at the translational level." Eur J Biochem **267**: 6321-30.

Minvielle-Sebastia, L., B. Winsor, N. Bonneaud and F. Lacroute (1991). "Mutations in the yeast RNA14 and RNA15 genes result in an abnormal mRNA decay rate; sequence analysis reveals an RNA-binding domain in the RNA15 protein." Mol Cell Biol **11**: 3075-87.

Miyasaka, H. (1999). "The positive relationship between codon usage bias and translation initiation AUG context in Saccharomyces cerevisiae." Yeast **15**: 633-7.

Miyasaka, H. (2002). "Translation initiation AUG context varies with codon usage bias and gene length in Drosophila melanogaster." J Mol Evol **55**: 52-64.

Moore, M. J. (2005). "From birth to death: the complex lives of eukaryotic mRNAs." Science **309**: 1514-8.

Moreno, S., A. Klar and P. Nurse (1991). "Molecular genetic analysis of fission yeast Schizosaccharomyces pombe." Methods Enzymol **194**: 795-823.

Morley, S. J. and M. J. Coldwell (2007). Matters of Life and Death: Translational Initiation during Apoptosis. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 433-458.

Murthy, K. G. and J. L. Manley (1995). "The 160-kD subunit of human cleavage-polyadenylation specificity factor coordinates pre-mRNA 3'-end formation." Genes Dev **9**: 2672-83.

Myer, V. E., X. C. Fan and J. A. Steitz (1997). "Identification of HuR as a protein implicated in AUUUA-mediated mRNA decay." EMBO J **16**: 2130-9.

Nakamura, A., K. Sato and K. Hanyu-Nakamura (2004). "Drosophila cup is an eIF4E binding protein that associates with Bruno and regulates oskar mRNA translation in oogenesis." Dev Cell **6**: 69-78.

Nelson, M. R., A. M. Leidal and C. A. Smibert (2004). "Drosophila Cup is an eIF4E-binding protein that functions in Smaug-mediated translational repression." EMBO J **23**: 150-9.

Newman, J. R., S. Ghaemmaghami, J. Ihmels, D. K. Breslow, M. Noble, J. L. DeRisi and J. S. Weissman (2006). "Single-cell proteomic analysis of S. cerevisiae reveals the architecture of biological noise." Nature **441**: 840-6.

Niessing, D., S. Blanke and H. Jackle (2002). "Bicoid associates with the 5'-cap-bound complex of caudal mRNA and represses translation." Genes Dev **16**: 2576-82.

Nott, A., S. H. Meislin and M. J. Moore (2003). "A quantitative analysis of intron effects on mammalian gene expression." RNA **9**: 607-17.

Oishi, K., R. Sugiura, H. Shuntoh and T. Kuno (1996). "Cloning and characterization of hsc1+, a heat shock cognate gene of the fission yeast Schizosaccharomyces pombe." Gene **181**: 45-9.

Olsen, P. H. and V. Ambros (1999). "The lin-4 regulatory RNA controls developmental timing in Caenorhabditis elegans by blocking LIN-14 protein synthesis after the initiation of translation." Dev Biol **216**: 671-80.

Orphanides, G. and D. Reinberg (2002). "A unified theory of gene expression." Cell **108**: 439-51.

Parker, R. and H. Song (2004). "The enzymes and control of eukaryotic mRNA turnover." Nat Struct Mol Biol **11**: 121-7.

Patel, A. A. and J. A. Steitz (2003). "Splicing double: insights from the second spliceosome." Nat Rev Mol Cell Biol **4**: 960-70.

Pende, M., et al. (2004). "S6K1(-/-)/S6K2(-/-) mice exhibit perinatal lethality and rapamycin-sensitive 5'-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein kinase-dependent S6 kinase pathway." Mol Cell Biol **24**: 3112-24.

Pestova, T. V., S. I. Borukhov and C. U. Hellen (1998). "Eukaryotic ribosomes require initiation factors 1 and 1A to locate initiation codons." Nature **394**: 854-9.

Pestova, T. V. and V. G. Kolupaeva (2002). "The roles of individual eukaryotic translation initiation factors in ribosomal scanning and initiation codon selection." Genes Dev **16**: 2906-22.

Pestova, T. V., V. G. Kolupaeva, I. B. Lomakin, E. V. Pilipenko, I. N. Shatsky, V. I. Agol and C. U. Hellen (2001). "Molecular mechanisms of translation initiation in eukaryotes." Proc Natl Acad Sci U S A **98**: 7029-36.

Pestova, T. V., I. B. Lomakin, J. H. Lee, S. K. Choi, T. E. Dever and C. U. Hellen (2000). "The joining of ribosomal subunits in eukaryotes requires eIF5B." Nature **403**: 332-5.

Pestova, T. V., J. R. Lorsch and C. U. T. Hellen (2007). The Mechanism of Translational Initiation in Eukaryotes. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 87-128.

Petersen, C. P., M. E. Bordeleau, J. Pelletier and P. A. Sharp (2006). "Short RNAs repress translation after initiation in mammalian cells." Mol Cell **21**: 533-42.

Pillai, R. S., S. N. Bhattacharyya, C. G. Artus, T. Zoller, N. Cougot, E. Basyuk, E. Bertrand and W. Filipowicz (2005). "Inhibition of translational initiation by Let-7 MicroRNA in human cells." Science **309**: 1573-6.

Preiss, T., J. Baron-Benhamou, W. Ansorge and M. W. Hentze (2003). "Homodirectional changes in transcriptome composition and mRNA translation induced by rapamycin and heat shock." Nat Struct Biol **10**: 1039-47.

Preiss, T. and M. W. Hentze (1998). "Dual function of the messenger RNA cap structure in poly(A)-tail-promoted translation in yeast." Nature **392**: 516-20.

Preiss, T. and M. W. Hentze (2003). "Starting the protein synthesis machine: eukaryotic translation initiation." Bioessays **25**: 1201-11.

Proudfoot, N. and J. O'Sullivan (2002). "Polyadenylation: a tail of two complexes." Curr Biol **12**: R855-7.

Proudfoot, N. J., A. Furger and M. J. Dye (2002). "Integrating mRNA processing with transcription." Cell **108**: 501-12.

Qin, X., S. Ahn, T. P. Speed and G. M. Rubin (2007). "Global analyses of mRNA translational control during early Drosophila embryogenesis." Genome Biol **8**: R63.

Qin, X. and P. Sarnow (2004). "Preferential translation of internal ribosome entry site-containing mRNAs during the mitotic cycle in mammalian cells." J Biol Chem **279**: 13721-8.

Raghavan, A., R. L. Ogilvie, C. Reilly, M. L. Abelson, S. Raghavan, J. Vasdewani, M. Krathwohl and P. R. Bohjanen (2002). "Genome-wide analysis of mRNA decay in resting and activated primary human T lymphocytes." Nucleic Acids Res **30**: 5529-38.

Raineri, I., D. Wegmueller, B. Gross, U. Certa and C. Moroni (2004). "Roles of AUF1 isoforms, HuR and BRF1 in ARE-dependent mRNA turnover studied by RNA interference." Nucleic Acids Res **32**: 1279-88.

Rajasekhar, V. K., A. Viale, N. D. Socci, M. Wiedmann, X. Hu and E. C. Holland (2003). "Oncogenic Ras and Akt signaling contribute to glioblastoma formation by differential recruitment of existing mRNAs to polysomes." Mol Cell **12**: 889-901.

Raught, B. and A.-C. Gingras (2007). Signaling to Translation Initiation. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 369-400.

Read, R. L. and C. J. Norbury (2002). "Roles for cytoplasmic polyadenylation in cell cycle regulation." J Cell Biochem **87**: 258-65.

Richter, J. D. (2007). "CPEB: a life in translation." Trends Biochem Sci **32**: 279-85.

Richter, J. D. and N. Sonenberg (2005). "Regulation of cap-dependent translation by eIF4E inhibitory proteins." Nature **433**: 477-80.

Rivera-Pomar, R., D. Niessing, U. Schmidt-Ott, W. J. Gehring and H. Jackle (1996). "RNA binding and translational suppression by bicoid." Nature **379**: 746-9.

Ron, D. and H. B. Harding (2007). eIF2α Phosphorylation in Cellular Stress Response and Disease. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 345-368.

Rowlands, A. G., R. Panniers and E. C. Henshaw (1988). "The catalytic mechanism of guanine nucleotide exchange factor action and competitive inhibition by phosphorylated eukaryotic initiation factor 2." J Biol Chem **263**: 5526-33.

Rustici, G., et al. (2004). "Periodic gene expression program of the fission yeast cell cycle." Nat Genet **36**: 809-17.

Rustici, G., H. van Bakel, D. H. Lackner, F. C. Holstege, C. Wijmenga, J. Bähler and A. Brazma (2007). "Global transcriptional responses of fission and budding yeast to changes in copper and iron levels: a comparative study." Genome Biol **8**: R73.

Ruvinsky, I. and O. Meyuhas (2006). "Ribosomal protein S6 phosphorylation: from protein synthesis to cell size." Trends Biochem Sci **31**: 342-8.

Ruvinsky, I., N. Sharon, T. Lerer, H. Cohen, M. Stolovich-Rain, T. Nir, Y. Dor, P. Zisman and O. Meyuhas (2005). "Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis." Genes Dev **19**: 2199-211.

Sachs, A. (2000). Physical and Functional Interactions between the mRNA Cap Structure and the Poly(A) Tail. Translational Control of Gene Expression. N. Sonenberg, J. W. B. Hershey and M. B. Mathews, Cold Spring Harbor Laboratory Press**:** 447-466.

Saklatvala, J., J. Dean and A. Clark (2003). "Control of the expression of inflammatory response genes." Biochem Soc Symp: 95-106.

Salles, F. J. and S. Strickland (1995). "Rapid and sensitive analysis of mRNA polyadenylation states by PCR." PCR Methods Appl **4**: 317-21.

Samuelsen, C. O., V. Baraznenok, O. Khorosjutina, H. Spahr, T. Kieselbach, S. Holmberg and C. M. Gustafsson (2003). "TRAP230/ARC240 and TRAP240/ARC250 Mediator subunits are functionally conserved through evolution." Proc Natl Acad Sci U S A **100**: 6422-7.

Sandelin, A., P. Carninci, B. Lenhard, J. Ponjavic, Y. Hayashizaki and D. A. Hume (2007). "Mammalian RNA polymerase II core promoters: insights from genome-wide studies." Nat Rev Genet **8**: 424-36.

Sandoval, J., et al. (2004). "RNAPol-ChIP: a novel application of chromatin immunoprecipitation to the analysis of real-time gene transcription." Nucleic Acids Res **32**: e88.

Schäfer, B., M. Hansen and B. F. Lang (2005). "Transcription and RNA-processing in fission yeast mitochondria." RNA **11**: 785-95.

Scheuner, D., B. Song, E. McEwen, C. Liu, R. Laybutt, P. Gillespie, T. Saunders, S. Bonner-Weir and R. J. Kaufman (2001). "Translational control is required for the unfolded protein response and in vivo glucose homeostasis." Mol Cell **7**: 1165-76.

Schneider, R. J. and N. Sonenberg (2007). Translational Control in Cancer Devlopment and Rpogression. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 401-431.

Schuman, E. M., J. L. Dynes and O. Steward (2006). "Synaptic regulation of translation of dendritic mRNAs." J Neurosci **26**: 7143-6.

Sen, G. L. and H. M. Blau (2005). "Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies." Nat Cell Biol **7**: 633-6.

Serikawa, K. A., X. L. Xu, V. L. MacKay, G. L. Law, Q. Zong, L. P. Zhao, R. Bumgarner and D. R. Morris (2003). "The Transcriptome and Its Translation during Recovery from Cell Cycle Arrest in Saccharomyces cerevisiae." Mol Cell Proteomics **2**: 191-204.

Sharma, N., S. Marguerat, S. Mehta, S. Watt and J. Bähler (2006). "The fission yeast Rpb4 subunit of RNA polymerase II plays a specialized role in cell separation." Mol Genet Genomics.

Shatkin, A. J. and J. L. Manley (2000). "The ends of the affair: capping and polyadenylation." Nat Struct Biol **7**: 838-42.

Shenton, D., J. B. Smirnova, J. N. Selley, K. Carroll, S. J. Hubbard, G. D. Pavitt, M. P. Ashe and C. M. Grant (2006). "Global Translational Responses to Oxidative Stress Impact upon Multiple Levels of Protein Synthesis." J Biol Chem **281**: 29011-21.

Sheth, U. and R. Parker (2003). "Decapping and decay of messenger RNA occur in cytoplasmic processing bodies." Science **300**: 805-8.

Shimanuki, M., et al. (2007). "Two-step, extensive alterations in the transcriptome from G0 arrest to cell division in Schizosaccharomyces pombe." Genes Cells **12**: 677-92.

Shin, B. S., D. Maag, A. Roll-Mecak, M. S. Arefin, S. K. Burley, J. R. Lorsch and T. E. Dever (2002). "Uncoupling of initiation factor eIF5B/IF2 GTPase and translational activities by mutations that lower ribosome affinity." Cell **111**: 1015-25.

Shock, J. L., K. F. Fischer and J. L. Derisi (2007). "Whole-genome analysis of mRNA decay in Plasmodium falciparum reveals a global lengthening of mRNA half-life during the intra-erythrocytic development cycle." Genome Biol **8**: R134.

Smirnova, J. B., et al. (2005). "Global gene expression profiling reveals widespread yet distinctive translational responses to different eukaryotic translation initiation factor 2B-targeting stress pathways." Mol Cell Biol **25**: 9340-9.

Spahr, H., et al. (2001). "Analysis of Schizosaccharomyces pombe mediator reveals a set of essential subunits conserved between yeast and metazoan cells." Proc Natl Acad Sci U S A **98**: 11985-90.

Spence, J., B. M. Duggan, C. Eckhardt, M. McClelland and D. Mercola (2006). "Messenger RNAs under differential translational control in Ki-ras-transformed cells." Mol Cancer Res **4**: 47-60.

Spence, J., R. R. Gali, G. Dittmar, F. Sherman, M. Karin and D. Finley (2000). "Cell cycle-regulated modification of the ribosome by a variant multiubiquitin chain." Cell **102**: 67-76.

Spriggs, K. A., M. Bushell, S. A. Mitchell and A. E. Willis (2005). "Internal ribosome entry segment-mediated translation during apoptosis: the role of IRES-trans-acting factors." Cell Death Differ **12**: 585-91.

St Johnston, D. (2005). "Moving messages: the intracellular localization of mRNAs." Nat Rev Mol Cell Biol **6**: 363-75.

Stevenson, A. L. and C. J. Norbury (2006). "The Cid1 family of non-canonical poly(A) polymerases." Yeast **23**: 991-1000.

Stewart, M. (2007). "Ratcheting mRNA out of the nucleus." Mol Cell **25**: 327-30.

Stoneley, M. and A. E. Willis (2004). "Cellular internal ribosome entry segments: structures, trans-acting factors and regulation of gene expression." Oncogene **23**: 3200-7.

Swaminathan, S., T. Masek, C. Molin, M. Pospisek and P. Sunnerhagen (2006). "Rck2 is required for reprogramming of ribosomes during oxidative stress." Mol Biol Cell **17**: 1472-82.

Swiercz, R., M. D. Person and M. T. Bedford (2005). "Ribosomal protein S2 is a substrate for mammalian PRMT3 (protein arginine methyltransferase 3)." Biochem J **386**: 85-91.

Takagaki, Y., L. C. Ryner and J. L. Manley (1989). "Four factors are required for 3'-end cleavage of pre-mRNAs." Genes Dev **3**: 1711-24.

Taylor, D. J., J. Frank and T. G. Kinzy (2007). Structure and Function of the Eukaryotic Ribosome and Elongation Factors. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 59-85.

Teixeira, D., U. Sheth, M. A. Valencia-Sanchez, M. Brengues and R. Parker (2005). "Processing bodies require RNA for assembly and contain nontranslating mRNAs." RNA **11**: 371-82.

Temple, M. D., G. G. Perrone and I. W. Dawes (2005). "Complex cellular responses to reactive oxygen species." Trends Cell Biol **15**: 319-26.

Tenenbaum, S. A., C. C. Carson, P. J. Lager and J. D. Keene (2000). "Identifying mRNA subsets in messenger ribonucleoprotein complexes by using cDNA arrays." Proc Natl Acad Sci U S A **97**: 14085-90.

Thermann, R. and M. W. Hentze (2007). "Drosophila miR2 induces pseudo-polysomes and inhibits translation initiation." Nature **447**: 875-8.

Thomas, J. D. and G. J. Johannes (2007). "Identification of mRNAs that continue to associate with polysomes during hypoxia." RNA **13**: 1116-31.

Thompson, B., M. Wickens and J. Kimble (2007). Translational Control in Development. Translational Control in Biology and Medicine. M. B. Mathews, N. Sonenberg and J. W. B. Hershey, Cold Spring Harbor Laboratory Press**:** 507-544.

Tomari, Y. and P. D. Zamore (2005). "Perspective: machines for RNAi." Genes Dev **19**: 517-29.

Tusher, V. G., R. Tibshirani and G. Chu (2001). "Significance analysis of microarrays applied to the ionizing radiation response." Proc Natl Acad Sci U S A **98**: 5116-21.

Unterholzner, L. and E. Izaurralde (2004). "SMG7 acts as a molecular link between mRNA surveillance and mRNA decay." Mol Cell **16**: 587-96.

Valencia-Sanchez, M. A., J. Liu, G. J. Hannon and R. Parker (2006). "Control of translation and mRNA degradation by miRNAs and siRNAs." Genes Dev **20**: 515-24.

van Dijk, E., N. Cougot, S. Meyer, S. Babajko, E. Wahle and B. Seraphin (2002). "Human Dcp2: a catalytically active mRNA decapping enzyme located in specific cytoplasmic structures." EMBO J **21**: 6915-24.

Vasudevan, S., S. W. Peltz and C. J. Wilusz (2002). "Non-stop decay--a new mRNA surveillance pathway." Bioessays **24**: 785-8.

Walden, W. E., T. Godefroy-Colburn and R. E. Thach (1981). "The role of mRNA competition in regulating translation. I. Demonstration of competition in vivo." J Biol Chem **256**: 11739-46.

Walhout, A. J. (2006). "Unraveling transcription regulatory networks by protein-DNA and protein-protein interaction mapping." Genome Res **16**: 1445-54.

Wang, W., J. L. Martindale, X. Yang, F. J. Chrest and M. Gorospe (2005). "Increased stability of the p16 mRNA with replicative senescence." EMBO Rep **6**: 158-64.

Wang, Y., C. L. Liu, J. D. Storey, R. J. Tibshirani, D. Herschlag and P. O. Brown (2002). "Precision and functional specificity in mRNA decay." Proc Natl Acad Sci U S A **99**: 5860-5.

Watson, A., J. Mata, J. Bähler, A. Carr and T. Humphrey (2004). "Global gene expression responses of fission yeast to ionizing radiation." Mol Biol Cell **15**: 851-60.

Wells, S. E., P. E. Hillner, R. D. Vale and A. B. Sachs (1998). "Circularization of mRNA by eukaryotic translation initiation factors." Mol Cell **2**: 135-40.

Wickens, M., E. B. Goodwin, J. Kimble, S. Strickland and M. W. Hentze (2000). Translational Control of Developmental Decisions. Translational Control of Gene Expression. N. Sonenberg, J. W. B. Hershey and M. B. Mathews, Cold Spring Harbor Laboratory Press**:** 295-370.

Wightman, B., T. R. Burglin, J. Gatto, P. Arasu and G. Ruvkun (1991). "Negative regulatory sequences in the lin-14 3'-untranslated region are necessary to generate a temporal switch during Caenorhabditis elegans development." Genes Dev **5**: 1813-24.

Wilczynska, A., C. Aigueperse, M. Kress, F. Dautry and D. Weil (2005). "The translational regulator CPEB1 provides a link between dcp1 bodies and stress granules." J Cell Sci **118**: 981-92.

Wilhelm, J. E., M. Hilton, Q. Amos and W. J. Henzel (2003). "Cup is an eIF4E binding protein required for both the translational repression of oskar and the recruitment of Barentsz." J Cell Biol **163**: 1197-204.

Wilson, G. M., Y. Sun, J. Sellers, H. Lu, N. Penkar, G. Dillard and G. Brewer (1999). "Regulation of AUF1 expression via conserved alternatively spliced elements in the 3' untranslated region." Mol Cell Biol **19**: 4056-64.

Wilusz, C. J., M. Wormington and S. W. Peltz (2001). "The cap-to-tail guide to mRNA turnover." Nat Rev Mol Cell Biol **2**: 237-46.

Wolin, S. L. and P. Walter (1988). "Ribosome pausing and stacking during translation of a eukaryotic mRNA." EMBO J **7**: 3559-69.

Wood, V., et al. (2002). "The genome sequence of Schizosaccharomyces pombe." Nature **415**: 871-80.

Wu, L., J. Fan and J. G. Belasco (2006). "MicroRNAs direct rapid deadenylation of mRNA." Proc Natl Acad Sci U S A **103**: 4034-9.

Yang, E., E. van Nimwegen, M. Zavolan, N. Rajewsky, M. Schroeder, M. Magnasco and J. E. Darnell, Jr. (2003). "Decay rates of human mRNAs: correlation with functional characteristics and sequence attributes." Genome Res **13**: 1863-72.

Yekta, S., I. H. Shih and D. P. Bartel (2004). "MicroRNA-directed cleavage of HOXB8 mRNA." Science **304**: 594-6.

Yoshioka, S., K. Kato, K. Nakai, H. Okayama and H. Nojima (1997). "Identification of open reading frames in Schizosaccharomyces pombe cDNAs." DNA Res **4**: 363-9.

Zanetti, M. E., I. F. Chang, F. Gong, D. W. Galbraith and J. Bailey-Serres (2005). "Immunopurification of polyribosomal complexes of Arabidopsis for global analysis of gene expression." Plant Physiol **138**: 624-35.

Zhan, K., K. M. Vattem, B. N. Bauer, T. E. Dever, J. J. Chen and R. C. Wek (2002). "Phosphorylation of eukaryotic initiation factor 2 by heme-regulated inhibitor kinase-related protein kinases in Schizosaccharomyces pombe is important for fesistance to environmental stresses." Mol Cell Biol **22**: 7134-46.

Zhao, J., L. Hyman and C. Moore (1999). "Formation of mRNA 3' ends in eukaryotes: mechanism, regulation, and interrelationships with other steps in mRNA synthesis." Microbiol Mol Biol Rev **63**: 405-45.

Zofall, M. and S. I. Grewal (2006). "RNAi-mediated heterochromatin assembly in fission yeast." Cold Spring Harb Symp Quant Biol **71**: 487-96.