Chapter 6

General discussion

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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.

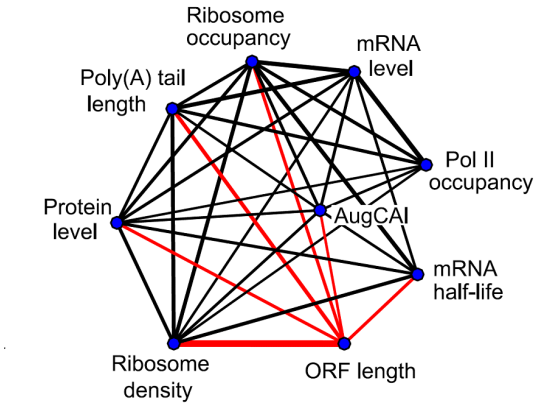

Figure 6.1 Summary of relationships between all aspects of gene expression
Weighted association map summarizing the relationships between the various aspects of gene expression analyzed here. The blue nodes represent the different data sets as labelled, black lines show significant positive correlations 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.

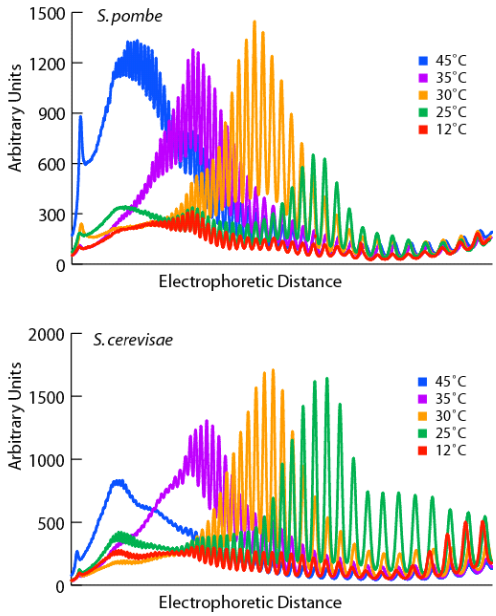

Figure 6.2 Comparison of poly(A) tail lengths between fission and budding yeast
Graph of poly(A) tail length tracts for *S. pombe* and *S. cerevisiae* showing relative intensity on the gel as a function of electrophoretic distance for all fractions, which have been eluted at different temperatures (as 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.