Mol Genet Genomics (2007) 277:341–355
DOI 10.1007/s00438-007-0221-5

REVIEW

Cytoplasmatic post-transcriptional regulation and intracellular signalling

Per Sunnerhagen

Received: 8 December 2006 / Accepted: 6 February 2007 / Published online: 1 March 2007

Abstract Studies of intracellular signalling have traditionally focused on regulation at the levels of initiation of transcription on one hand, and post-translational regulation on the other. More recently, it is becoming apparent that the post-transcriptional level of gene expression is also subject to regulation by signalling pathways. The emphasis in this review is on short-term regulation of mRNAs at the levels of degradation and frequency of translation. Interplay between the mRNA translation and degradation machineries and mainly the TOR, stress-induced MAP kinase (SAPK), and DNA damage checkpoint pathways is discussed. Since a large fraction of the molecular mechanisms has been dissected using molecular genetics methods in yeast, most of the examples in this review are from budding and fission yeast. Some parallels are drawn to plant and animal cells. This review is intended for those more familiar with intracellular signalling, and who realise that post-transcriptional regulation may be an underemphasised level of signalling output.

Keywords Translational control · mRNA turnover · MAPK

Introduction

The post-transcriptional level of regulation comprises many steps from synthesis of the primary transcript to the final degradation of the mRNA molecule: 5′-capping, splicing, poly(A)-site cleavage and polyadenylation, RNA editing, nonsense-mediated decay, nuclear export, localisation in cytoplasmic compartments, initiation and elongation of translation, and the different steps in mRNA decay.

In the last years, it has become clear that certain modes of post-transcriptional control may be quite widespread and can have far-reaching consequences. Thus, alternative splicing is being put forward as an important source of protein variety for multicellular organisms, thus acting as a qualitative expression control. In many higher plants and animals, the required numbers of different proteins are sometimes perceived to greatly exceed the number of recognised genes (Roberts and Smith 2002). To what extent alternative splicing is modulated by signalling pathways remains comparatively unexplored. Regulation through small RNAs (RNA interference; RNAi) can affect translation and stability of mRNA, but can also exert a more long-lasting effect on transcription through establishment of heterochromatin.

The emphasis in this review is on quantitative control mediated through signalling pathways of the later, cytoplasmic, steps in post-transcriptional control: regulation of translational initiation and elongation, through mechanisms including upstream open reading frames, and mRNA turnover. Since post-transcriptional regulation acts on pre-existing mRNAs and thus is inherently faster than regulation of transcriptional initiation, it may be more relevant in certain situations to view events on the post-transcriptional level after, e.g. hormone stimulation as earlier in the causative chain than up-regulation of transcription (Prendergast 2003).

Communicated by T. Nyström.

P. Sunnerhagen (✉)
Department of Cell and Molecular Biology,
Lundberg Laboratory, Göteborg University,
P.O. Box 462, 405 30 Göteborg, Sweden
e-mail: Per.Sunnerhagen@molbio.gu.se

There are well-documented cases of post-transcriptional control in organism development and as a consequence of particular circumstances. Thus, extensive post-transcriptional regulation is of necessity in neurons, with an extremely long distance to the nucleus (Klann et al. 2004). Those cases will not be considered here as the intent is to highlight short-term effects in response to abiotic or hormonal stimuli.

Post-transcriptional control

Most of the control exerted on translation is believed to act on the initiation step. For translation to initiate, the amino acid-charged initiator tRNA must first associate with the small subunit of the ribosome. Then, the mRNA attaches and the start codon is recognised. Finally, the large ribosomal subunit joins. The individual steps of this process are facilitated by translation initiation factors (abbreviated eIFs in eukaryotes). As initiation of translation requires interactions between the ribosome and both ends of the transcript, many eIFs bind to the 5′ or the 3′ ends of mRNAs. Much of the known regulation of translation through signalling pathways converges at eIF2α (Fig. 1a). The initiation factor eIF2 in its GTP-bound form together with charged initiator tRNA (ternary complex) is brought to the 40S subunit of the ribosome, to form a preinitiation complex. Phosphorylation of eIF2α-GDP (in mammals by either of the four kinases GCN2, PERK, HRI or PKR; in budding yeast solely by Gcn2) converts it to a high-affinity inhibitor of eIF2B, its own GDP/GTP exchange factor, preventing recharging of eIF2α with GTP. The overall effect is a down-regulation of translation initiation. Another central mechanism for regulation of the initiation step occurs through eIF4E-binding proteins (4E-BPs; see Fig. 1b). These are a family of small proteins that share a conserved binding site for eIF4E. By competing with eIF4G (which has the same binding site) for binding to eIF4E, a 4E-BP can prevent formation of the cap-binding eIF4E-eIF4G-eIF4A complex, collectively called eIF4F,

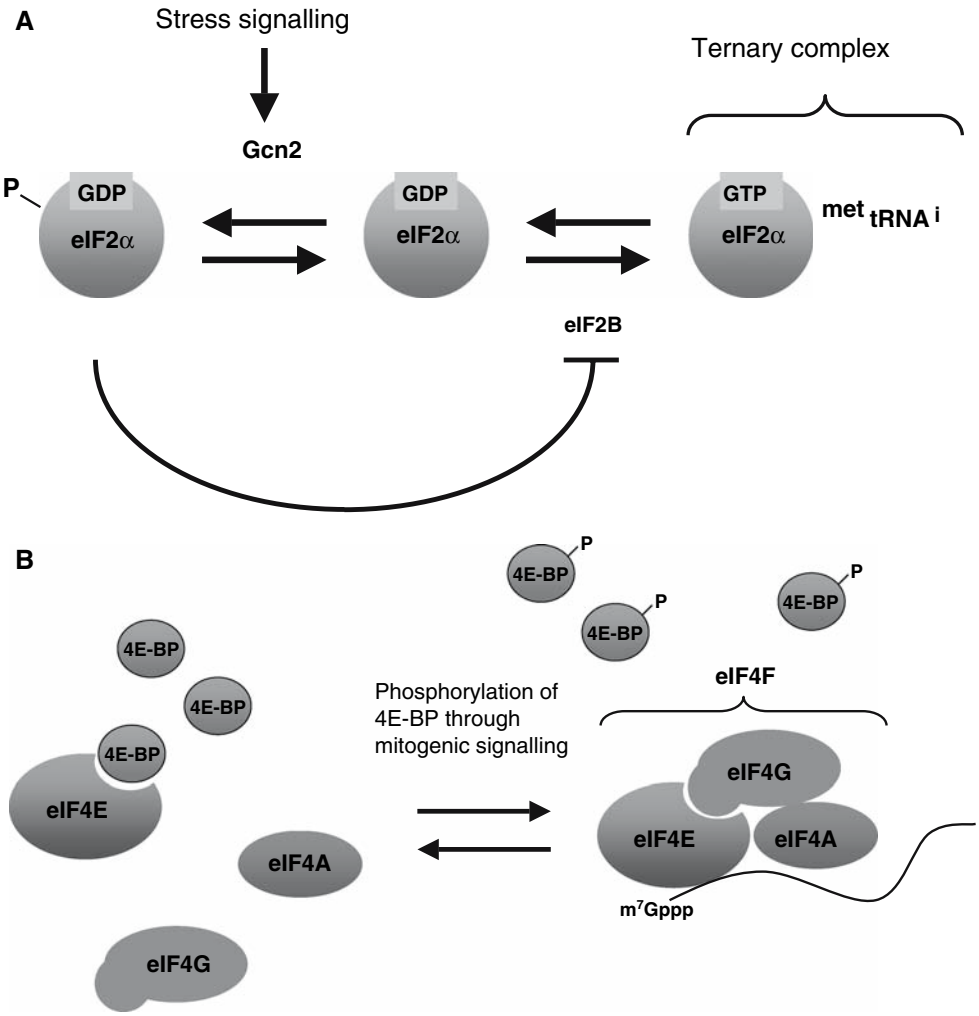

Fig. 1 Overview of mechanisms for regulation of translation initiation. **a** Inhibition of translation initiation through stress-induced phosphorylation of eIF2α by Gcn2. The phosphorylated form of eIF2α inhibits eIF2B, preventing recharging of eIF2α with GTP, which leads to decreased initiation. **b** Stimulation of translation initiation through growth stimuli-induced phosphorylation of 4E-BP. The 4E-BPs use the same binding surface on eIF4E as eIF4G. The phosphorylated forms of 4E-BP are unable to bind. Adapted from Dever 2002 and Gallie 1998

which is required for all cap-dependent translation. Binding of a 4E-BP to eIF4E is modulated by phosphorylation through a multitude of signalling pathways, after activation by, e.g. growth factors or cytokines. Hyperphosphorylation of 4E-BP prevents binding to eIF4E, ultimately leading to activation of translation. For reviews of general translation and control of translation initiation, see Dever 2002 and Preiss and Hentze 2003.

Two major pathways of mRNA degradation exist in eukaryotes. In both cases, shortening of the poly(A) tail is the first, time-limiting, step. Three distinct protein complexes (the Pan2/Pan3, or PAN complex; poly(A)-specific exonuclease, PARN; and the Ccr4/Pop2 complex) govern this deadenylation. After deadenylation, degradation can occur from the 3′ to the 5′ end by the RNase-containing exosome complex. In an independent pathway, deadenylation is followed by removal of the 7-methyl-guanosine cap of mRNAs and then proceeds in the 5′–3′ direction. The mechanisms of mRNA turnover have been reviewed recently (Meyer et al. 2004).

There is a competition between ribosomes and ribonucleases for binding to mRNA, so actively translated mRNAs are generally protected from translation. Insertion of elements in the 5′-UTR that interfere with efficient translation will destabilise the mRNA (Muhlrad et al. 1995). PolyA-tail shortening proteins generally compete with translation factors and the ribosome for access to mRNA, and so highly translated mRNAs are degraded more slowly than weakly translated ones (Prieto et al. 2000). Stalling of ribosomes at internal sites in an mRNA will also induce endonucleolytic cleavage and degradation (Doma and Parker 2006). In general, therefore, for a given mRNA species we should expect a negative correlation between translational activity and degradation.

Regulation through upstream open reading frames

An additional mechanism for translational control operates through short open reading frames (upstream open reading frames; uORFs) present in the 5′-UTR of certain genes. These can interfere with translation of the main ORF by preventing reinitiation after translation has been terminated at the end of the uORF. The paradigm case for uORF regulation is *Saccharomyces cerevisiae GCN4*. There, translation of uORF4 located close to the start codon of the main ORF precludes translation of the main ORF. Translation of uORF1, located further upstream, can result in reinitiation downstream of it. The different probabilities of reinitiation downstream of uORF1 and uORF4 stem from the shortness of uORF1 and A/U-rich sequences immediately downstream of it, and uORF4 being longer and having G/C-rich sequences downstream. If reinitiation occurs at uORF4, translation of the main ORF will be prevented. If scanning ribosomes can ignore the uORF4 start codon and continue scanning downstream of uORF4, however, the *GCN4* main ORF will be translated and Gcn4, a transcription factor regulating many genes required in amino acid metabolism, will be produced (Abastado et al. 1991; Hinnebusch 1996). The nutrition status of the cell modulates *GCN4* translation through the level of ternary complex. Under conditions of high nutrient availability, the concentration of ternary complex is high. This leads to a high frequency of reinitiation, and so uORF4 will be translated, but not *GCN4* itself. Starvation activates Gcn2 to phosphorylate eIF2α, reducing ternary complex levels, and so the reinitiation frequency decreases. This means scanning will resume downstream of uORF4, and so translation of Gcn4 increases (Dever et al. 1992).

Regulation through uORFs may be more widespread than believed previously. Genome comparisons between man and mouse (Crowe et al. 2006), *Aspergillus* species (Galagan et al. 2005), and *Saccharomyces* species (Zhang and Dietrich 2005) indicate that many uORFs are conserved in evolution with respect to sequence and position.

Localised degradation and storage of mRNA

There is evidence that in undisturbed cells, mRNA decay occurs in discrete cytoplasmic foci containing many components involved in mRNA degradation such as decapping enzymes, exonucleases, and mRNA degradation intermediates. Such structures have alternately been called processing bodies (P bodies), cytoplasmic foci, or GW bodies for their content of the glycine–tryptophan repeat-rich protein GW182 (Cougot et al. 2004; Sheth and Parker 2003; Yang et al. 2004). Under conditions of severe stress (oxidative, metal, and heat), eukaryotic cells in addition accumulate cytoplasmic particles called “stress granules”, which have been shown to contain mRNA as well as ribosomal components, translation initiation factors and specialised proteins required for their structural integrity. Stress granules were first described in plant cells (Nover et al. 1983) and later in mammalian cells (Kedersha et al. 1999) as well as in the fission yeast (Dunand-Sauthier et al. 2002). So far they have not been unequivocally demonstrated to exist in the budding yeast, however. Formation of stress granules depends on interaction domains of the proteins TIA

and TIA-R in a process that has been compared to prion formation (Gilks et al. 2004; Kedersha et al. 2000). mRNAs contained in stress granules or P bodies are translationally silent.

A direct connection between polysomes on one hand, and stress granules and cytoplasmic foci on the other, becomes obvious through the interesting observations that drugs such as emetine or cycloheximide, translation inhibitors that also block disassembly of polysomes, simultaneously inhibit formation of stress granules under arsenite stress, or accumulation of cytoplasmic foci. By contrast, application of puromycin, which induces premature termination of translation and dissociation of polysomes, causes stress granule formation even without external stress factors (Cougot et al. 2004; Kedersha et al. 2000). This suggests a dynamic relationship between mRNA at actively translating ribosomes and stress granules. When the cell encounters strongly adverse conditions, one of its first actions is to strongly down-regulate the translational machinery. This serves the dual purpose of conserving energy—protein synthesis represents a major part of the cell's energy expenditure—and of facilitating rapid redirection of synthesis resources from gene products required for rapid growth under near-optimal conditions to products protecting the cell from stress. The amount of polysomes in such cells rapidly decreases, and in cases where stress conditions are severe, stress granules appear in the cytoplasm. It is natural to presume that mRNA and ribosomal components from disassembled polysomes appear in stress granules, which are characterised by a rapid exchange of RNA and proteins with the environment.

A model for the relationship between stress granules and P bodies has been proposed (Kedersha et al. 2005): mRNAs are first sorted from polysomes to stress granules under conditions of severe stress. They can then be recruited back to polysomes, or alternatively, be transferred to P bodies for degradation. P bodies can also form in the absence of stress granules, however (Kedersha et al. 2005). Both stress granules and P bodies contain components of the 5′–3′ mRNA degradation pathway (Kedersha et al. 2005; Sheth and Parker 2003; Yang et al. 2004); a composition difference between them is that translation initiation factors are mainly found in stress granules but not in P bodies (Kedersha et al. 2005). It should be noted that evidence also exists for recruitment of mRNA from P bodies to polysomes in budding yeast when translation resumes (Brengues et al. 2005).

Signalling through phosphorylation of eIF2α fundamentally affects formation of stress granules. Expression of a mutated allele mimicking the phosphorylated form of eIF2α causes stress granules to form even in the absence of external stimuli (Kedersha et al. 1999; McEwen et al. 2005), whereas an allele expressing a non-phosphorylatable form of eIF2α blocks formation of stress granules (Kedersha et al. 1999).

Techniques to study post-transcriptional regulation

Translational activity

For an individual protein, it is possible to determine the formation rate through short term labelling in vivo with radioactive amino acid followed by immunoprecipitation. The workhorses of proteomics, 2D gel electrophoresis (2D-PAGE) and mass spectrometry, determine steady-state levels of proteins, which in turn are a function of the individual production and degradation rates. Pulse-labelling techniques can be used to estimate individual protein synthesis rates in 2D-PAGE, but are limited to the more abundant proteins. To measure degradation rates on a global scale, Belle et al. 2006 employed an exhaustive collection of *S. cerevisiae* strains expressing TAP-tagged proteins, where individual decay rates were measured after inhibition of protein synthesis with cycloheximide.

Polysomal association

When in the elongation phase of translation, mRNAs are associated with typically several ribosomes, an arrangement called a polysome. Because of their high molecular weight and abundance, polysomes can be easily separated from other fractions. This can be done both analytically and preparatively, by sedimentation ultracentrifugation of crude lysates, treated with cycloheximide to prevent ribosomes from dissociating from mRNAs (Foyt et al. 1991; Marcus et al. 1967). After separation, the contents of the centrifugation tube can be collected from one end of the gradient. By online UV monitoring, one obtains a polysomal profile (Fig. 2), which itself can be used to diagnose many aspects of the overall translational state of the cell. The amount of polysomes reflects the overall level of translational activity in the cell. Further, the gradient can be fractionated and the fractions analysed for their content of individual mRNAs by hybridisation. Northern blotting can be used to monitor the precise distribution between the different polysomal, monosomal, and submonosomal peaks for a particular mRNA. It is also feasible to use the fractions from a polysome gradient (individual peaks or, e.g. pools of polysomes) for hybridisation to DNA arrays. By comparing the intensity

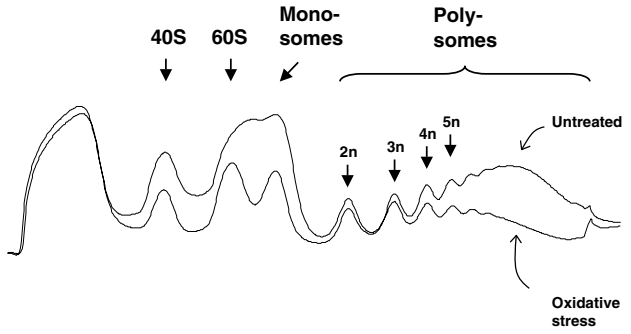

Fig. 2 Polysomal profiles from *S. cerevisiae* cells with or without exposure to oxidative stress. Total cell lysates were prepared in the presence of cycloheximide to preserve ribosome/mRNA complexes, and separated on sucrose gradients by ultracentrifugation. Absorbance at 254 nm was recorded online while collecting the gradients; higher mass complexes are located to the right in the diagram. 40S, 60S, and monosomes represent the small, the large ribosomal subunit, and the 80S "monosomal" peak, respectively. The numbers above peaks in the polysomal range represents the number of mRNA-associated ribosomes. Adapted from Swaminathan et al. 2006

patterns obtained from polysomal RNA with those from total or subpolysomal RNA, we can deduce the translational efficiency (as measured by the specific polysomal association) for any mRNA.

It is logical to assume that mRNAs associated with two or more ribosomes are actually being actively translated, although it is conceivable that under conditions of cellular stress or nutrient deprivation a fraction of them could represent cases where translation has stopped, but dissociation of the complexes has not yet taken place. The interpretation of the monosome peak is more ambiguous. Beside mRNAs where initiation has taken place but elongation not yet started, this fraction also contains ribosomes in the elongation phase attached to mRNAs that are too short to house more than one ribosome at a time, and mRNAs where the translation rate is so low that they are on average occupied by only one ribosome. To bypass this uncertainty, the majority of analyses exclude the monosome peak from the fraction of actively translated mRNAs and consider only the polysomal peaks.

mRNA decay

In vivo labelling methods

It is possible to estimate the half-life of individual mRNAs in intact cells without interfering with either general transcription or the transcription rate of the gene of interest. In "approach to steady-state" labelling (Kim and Warner 1983), radioactive RNA precursors are added to living cells for various time lengths, followed by extraction of total RNA and hybridisation to immobilised probes. The rate by which the specific radioactivity comes closer to that obtained at extended labelling times (steady-state) is an indirect measure of the turnover time of the pool of the mRNA species in question. The advantage of this method is that the cells are left undisturbed, including the transcription rate of the gene under study. Drawbacks include the relatively low sensitivity, limiting this approach to highly expressed genes, and a low temporal resolution.

Promoter switch-off

Putting a gene of interest under control of a switchable promoter allows one to follow the fate of an individual mRNA species. If transcription can be rapidly turned off, then the decay of the mRNA species in question can be monitored. Popular promoters with this property that are used in yeast include *Schizosaccharomyces pombe nmt1*, *S. cerevisiae GAL1*, and variations of the heterologous *Tet* system, where transcription is quickly terminated by addition of thiamine, glucose, or a tetracycline-type drug, respectively. Obvious disadvantages of this approach are the workload involved in creation of the regulatable constructs, and that only one mRNA species can be measured in an individual strain. In the case of glucose, genes under regulation of carbon source will be more difficult to study using this regime.

Arrest of RNA polymerase II

Another avenue is to abolish all transcription by RNA polymerase II (Pol II). This allows simultaneous measuring of individual half-lives of all mRNAs in the cell under the same conditions. Clearly, a cell where mRNAs are no longer being made is a dying cell. Interpretation of this type of experiments generally relies on the assumption that the integrity of the cell is maintained, including the state of mRNA degradation machineries, and so one always has to keep reservations in mind about possible artefacts arising because of this major disturbance of the state of the cell.

The first way to halt transcription is the use of conditional mutants defective in Pol II components. Temperature-sensitive Pol II mutants such as *rpb1-1* (Nonet et al. 1987) are readily available in yeast. Temperature upshift causes Pol II transcription to arrest within a few minutes in such a mutant. Studies using temperature-sensitive Pol II mutants have provided a large body of valuable insights into the mechanisms of mRNA decay. They suffer, obviously, from the limitation that controls have to be provided, as far as possible, to account for the effects of the temperature shift itself.

The other option is to arrest Pol II by chemical inhibitors, side-stepping the requirement for a temperature shift (Caponigro and Parker 1996 and references therein). The most commonly used drug is 1-10-phenantroline, a zinc chelator. Although zinc is a co-factor of many enzymes, the effect of intracellular zinc exhaustion on RNA polymerases, particularly Pol II, is immediate and extensive. As a result, cellular mRNA production virtually ceases shortly after addition of 1-10-phenantroline. Side effects of the chemical inhibitor are an issue to be considered. Beside inhibitory effects on other enzymes than the targeted one (such as loss of activity of zinc-requiring proteins in the example of 1-10-phenantroline), we have to consider general stress responses (Causton et al. 2001; Gasch et al. 2000) as well as compensatory mechanisms elicited by the drug. This can be seen, e.g. in 1-10-phenantroline treatment as up-regulation of several genes required for zinc import and metabolism. A systematic comparison of the readout in the form of mRNA decay rates has been performed of different Pol II inhibitors (1-10-phenantroline, thiolutin, 6-azauracil, ethidium bromide, and cordycepin) side by side with *rbs1-1* mutants (Grigull et al. 2004). It was found that thiolutin and 1-10-phenantroline produced patterns that were most similar to that of *rbs1-1* mutants. Overall, there was a high degree of overlap between all five different inhibitors and *rbs1-1* mutants, as expected. However, certain subgroups of genes display aberrant, inhibitor-specific, effects.

High-throughput methods

Two of the techniques described above, Pol II inactivation and polysomal association, lend themselves well to global studies using DNA arrays. Polysomal analysis measures physical association with ribosomes, and is thus only an indirect estimation of translational activity. Because of its appropriateness for large-scale studies, however, it can be used to connect proteomic and transcript array data. Global technologies for study of translational activity have recently been surveyed (Beilharz and Preiss 2004). As already pointed out, it is also possible to measure mRNA decay rates on a global scale using DNA arrays (Fig. 3). A technical issue in such experiments is the need for an external or internal standard that is assumed not to change its abundance with time. An avenue sometimes taken is to use groups of RNAs known to be stable, and measure mRNA decay rates relative to those.

To analyse post-transcriptional regulation requires more detailed information about the fine structure of RNA species expressed from a gene. Identification of genes with a potential for post-transcriptional regulation should be greatly enhanced by the novel high-density arrays that have been used to fine-map the

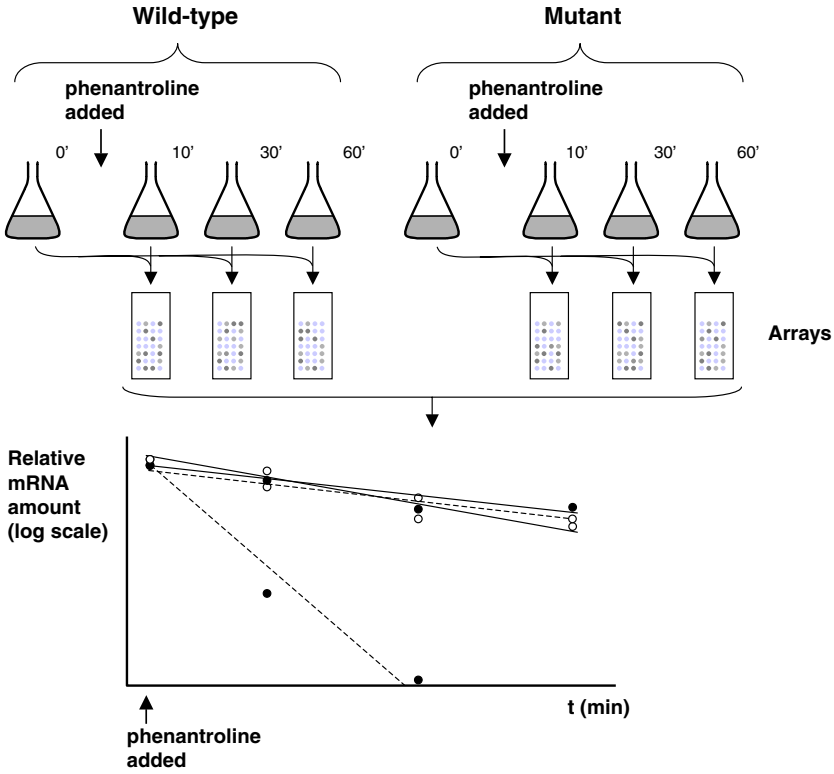

Fig. 3 Determination of mRNA decay rates using microarray analysis. Synthesis of mRNA is arrested using an inhibitor of RNA polymerase II such as 1-10-phenantroline. RNA samples taken from wild-type or mutant cells before phenantroline addition are hybridised to arrays together with samples taken at various times after addition. Decay rates of individual mRNAs are calculated from differences in relative hybridisation intensity. In the bottom diagram, filled circles represent an mRNA under stability control of the pathway affected in the mutant

yeast transcriptome (David et al. 2006), yielding the exact ends of each transcript, as well as the presence of anti-sense transcripts.

A biochemical global technique complementing estimates of transcript stability and polysome association is isolation of the mRNA bound to an affinity-tag labelled protein, followed by DNA array analysis of the mRNA population compositions. This has allowed functional profiling of RNA-stabilising proteins and translation initiation factors (Duttagupta et al. 2005; Gerber et al. 2004, 2006; Zhou et al. 2005). For example, the homologue of TIA/TIA-R in yeast, Pub1, has been shown by array analysis of the associated mRNA population to selectively bind about 5% of all mRNA species. At the same time, about 10% of all transcripts display a reduced half-life in a *pub1* mutant. The mRNA set which was found to be both physically associated with Pub1 and to require this protein for stability encoded mainly proteins for ribosome biogenesis and intermediary metabolism (Duttagupta et al. 2005).

Coupling of intracellular signalling pathways and post-transcriptional control

Nitrogen starvation and nutrient sensing

The TOR pathway is distinctive for having global effects on the translational machinery. Amino acid starvation, or addition of the TOR inhibitor rapamycin rapidly shuts down synthesis of ribosomal components. The TOR pathway acts on the translational machinery at different levels (transcriptional, post-transcriptional and post-translational) and through multiple mechanisms, including regulation of RNA polymerases I and II, activation of eIF4E through hyperphosphorylation of 4E-BPs, and the ribosomal protein S6 kinase. In yeast, two eIF4E-binding proteins have been identified, Caf20 and Eap1. As *eap1* mutants are rapamycin-resistant, this opens the possibility that the modulation of translational activity by the TOR pathway are in part channelled through phosphorylation of Eap1, in analogy with 4E-BP control in mammalian cells (Cosentino et al. 2000). Signalling through the TOR pathway also promotes translation through the phosphatase Sit4, which promotes conversion of eIF2α to its unphosphorylated, active, form (Cherkasova and Hinnebusch 2003; Rohde et al. 2004). Beside these general effects on translation of mRNA, the TOR pathway exerts post-transcriptional control on specific mRNAs. In mammalian cells, translation of mRNAs containing a pyrimidine-rich stretch in the 5′-UTR (Table 1), is promoted by TOR activity (Jefferies et al. 1994). This sequence feature is present in many mRNAs encoding proteins needed for translation, including ribosomal proteins, again linking the TOR pathway to protein synthesis in general.

In budding yeast, passage from G_1 to S-phase is critically dependent on the protein level of the cyclin Cln3. Translation of *CLN3* mRNA is reduced under conditions of low protein synthesis rate and slow growth, and this regulation is dependent on a uORF in the 5′-UTR (Polymenis and Schmidt 1997). The G_1 arrest induced by rapamycin can be overcome by replacing

Table 1 Some elements in mRNA sequences important for regulation of stability and translation

Element	Consensus sequence[a]	Reference
AREs class I	Several scattered copies of [AUUUA] followed by U-rich sequence	Chen and Shyu 1995
AREs class II	[UUAUUUA(U/A)(U/A]$_2$ flanked by U-rich sequence	Chen and Shyu 1995
mRNA instability upon iron deprivation in yeast	UUAUUUAUU (UAUUUAUU, UUAUUUAU)	Puig et al. 2005
Mammalian iron-responsive element	[*inv. repeat*]-CAGWGH-[*inv. repeat*]	Hentze et al. 2004
Mammalian glucose transporter 1 (GLUT1) stabilising/destabilising element	CCAACCACTC	Boado and Pardridge 1998
C-rich stabilising element of mammalian long-lived mRNAs	3 C-rich regions forming secondary structure	Waggoner and Liebhaber 2003
G-rich stage-specific stabilising element in *Trypanosoma*	[CGGGG]$_2$	D'Orso and Frasch 2001
Terminal oligo-pyrimidine tract (vertebrate mRNAs for ribosomal proteins)	[Y]$_{5–15}$	Levy et al. 1991

A compilation of sequence element in the 5′ and 3′-UTR's of eukaryotic mRNAs is found at http://www.bighost.area.ba.cnr.it/BIG/UTRHome/ (Mignone et al. 2005), and a specialised database for ARE sequences from human genes at http://www.rc.kfshrc.edu.sa/ared/ (Bakheet et al. 2003)

[a] Consensus sequences are shown as simplified versions of core elements; the actual definitions of patterns are more complex

the 5′-UTR of *CLN3* with a heterologous sequence lacking this uORF (Barbet et al. 1996). The cell-cycle gene *CDC33* encodes the cap-binding protein eIF4E. *cdc33-1* mutants arrest in G_1 with low Cln3 levels. Substitution of the 5′-UTR with the same heterologous sequence as above in the *CLN3* mRNA abolishes the G_1-specific arrest in *cdc33-1* mutants (Danaie et al. 1999), implying that translation of *CLN3* is the limiting factor for cell cycle progression in such mutants, and that eliminating the uORF in *CLN3* enhances translation sufficiently to compensate for the eIF4E deficiency of *cdc33-1* cells.

A case that is parallel in many aspects to budding yeast Cln3 has been found in fission yeast. There, the mRNAs encoding two key cell cycle regulators, the Cdk1 phosphatase Cdc25 and the mitotic cyclin Cdc13, both have extensive stem-loop structures in their 5′-UTRs (Daga and Jimenez 1999). The translation of both the *cdc25*$^+$ and *cdc13*$^+$ mRNAs are highly sensitive to the expression levels of *tif1*$^+$, which interestingly encodes eIF4A, an RNA helicase and a component of eIF4F which targets the 5′ end of mRNAs and unwinds secondary structures in the 5′-UTR.

One study investigated the global effects in yeast on translation by fusel alcohols (e.g. butanol), which accumulate under nitrogen starvation as a result of amino acid breakdown (Smirnova et al. 2005). One might then expect that amino acid starvation and butanol stress would cause similar post-transcriptional effects. Instead, they cause persistent translation, refractory to the general translational down-regulation, of distinct and mostly non-overlapping sets of mRNAs. Intriguingly, eIF2B is targeted in both cases, which underscores that additional mechanisms for control of translation under these stress conditions are likely to exist.

Glucose leads to destabilisation of mRNAs required for gluconeogenesis (the critical enzymes fructose-1, 6-*bis*phosphatase, encoded by *FBP1*, and phosphoenolpyruvate carboxykinase, encoded by *PCK1*, are specific for gluconeogenesis and are specifically targeted in this way). This is dependent on the low glucose sensor Snf3 and the phosphatase Reg1 (Yin et al. 2000). Interestingly, different pathways seem to regulate this effect under different glucose concentrations. In conditions of high glucose, the high glucose sensor Rgt2 and the Ras/cAMP pathway are required, whereas for signalling in low glucose concentrations, the low glucose sensor Snf3 is needed, but the Ras pathway is dispensable.

Stress-activated MAPK pathways

Eukaryotic cells possess multiple three-layered MAP kinase cascades, which act in parallel in response to different stimuli. The stress-activated MAP kinases (SAPK's) are stimulated by a variety of conditions, including oxidative and hyperosmotic stress, UV irradiation, and temperature changes. In mammalian cells, there are two types of SAPKs: the c-Jun NH_2-terminal protein kinase (JNK) and the p38 subfamilies. Plants possess a large number of MAP kinases, but their possible role in post-transcriptional regulation has not been established. The SAPK in *S. cerevisiae* is called Hog1, and its homologue in *Sz. pombe* is Sty1. A MAP kinase can bind to and activate a downstream kinase (MAPK-activated protein kinase; MAPKAPK). Mammalian MAPKAPK's have been directly implicated in several instances of post-transcriptional control. In yeast, there is a MAPK-activated protein kinase, which shares some homology to mammalian MAPKAPK's. The primordial gene encoding this kinase has been independently duplicated in *S. cerevisiae* and *Sz. pombe* (Hughes and Friedman 2003) to produce the paralogues Rck1 and Rck2 in the budding yeast (Dahlkvist and Sunnerhagen 1994) and Srk1/Mkp1 and Cmk2/Mkp2 in the fission yeast (Alemany et al. 2002; Asp and Sunnerhagen 2003; Smith et al. 2002).

Regulation through A/U-rich elements

The best characterised sequence elements in mRNAs involved in regulation of stability and translation are the A/U-rich elements (AREs), first defined in mammalian cells (for review, see Bevilacqua et al. 2003; Zhang et al. 2002). These elements (see Table 1), most often found in the 3′-UTR, recruit proteins with an influence on mRNA translation and stability. These proteins in turn can bind to components of the exosome to initiate 3′–5′ degradation of selected mRNAs (Anderson and Parker 1998; Chen et al. 2001).

In yeast, translation of the *MFA2* and the *TIF51A* mRNAs is down-regulated in glucose medium (Vasudevan et al. 2005). By contrast, the stability of *TIF51A* mRNA is increased in glucose whereas *MFA2* mRNA is unaffected by carbon source (Vasudevan and Peltz 2001). Both the effects on mRNA stability and translation rate are dependent on an intact *HOG* pathway and on ARE's in the 3′-UTRs of the transcripts. In mammals, expression of several interleukins is under control at the post-transcriptional level of SAPK pathways. Interleukin-2 (IL-2) mRNA is stabilised after activation of the JNK pathway, and this stabilisation is dependent on a JNK-responsive element (JRE) in the 5′-UTR of the *IL-2* mRNA (Chen et al. 1998, 2000). The mRNAs for other interleukins, IL-6 and IL-8, become stabilised by cytokine signalling mediated by another SAPK, p38. In this case, also expression of the

kinase downstream of p38, MAPKAPK-2 or MK2, is able to mediate this effect, whereas a mutated, kinase-dead, version of MK2 is unable to do so (Winzen et al. 1999). This role of SAPK's is not restricted to cytokines, as inhibition of the p38 pathway destabilises cyclooxygenase-2 mRNA in several different cell types (Lasa et al. 2001). Evidence for regulation of mRNA stability involving ARE's through the related SAPK JNK also exists. However, although there are numerous cases where similar effects are mediated from the SAPK p38 through the downstream kinase MAPK-activated protein kinase 2 (MAPKAPK-2 or MK2) (Kotlyarov et al. 1999; Lasa et al. 2000; Neininger et al. 2002; Winzen et al. 1999), this is not the case for JNK. An MK2-dependent phosphorylation of the ARE-binding protein tristetraprolin releases it from stress granules and inhibits degradation of mRNAs containing ARE's (Stoecklin et al. 2004).

In mammalian cells exposed to certain but not all environmental stresses or mitogens, the MAPKAP kinase Mnk1 phosphorylates the cap-binding protein eIF4E. Mnk1 in turn can be activated by the p38 or ERK MAP kinases (Banko et al. 2004; Wang et al. 1998; Waskiewicz et al. 1999). SAPK-directed modulation of mRNA stability can occur also under a normal developmental process. In myoblasts, activated p38 phosphorylates and inactivates the mRNA-destabilising protein KSRP, leading to stabilisation of mRNAs critical for differentiation to myocytes (Briata et al. 2005).

It is not far-fetched to think of coordinated post-transcriptional control of functional groups of genes. In humans, expression is regulated by iron levels through iron response elements (IRE's) in the UTR's of mRNAs encoding proteins in iron metabolism. During iron starvation, the RNA-binding protein IRP1 becomes activated and attaches to the IRE in the 5′-UTR of selected mRNAs, preventing translation of proteins such as iron exporters and storage proteins. At the same time, IRP1 binds to the 3′-UTR and stabilises the mRNA for proteins required at iron scarcity such as transferrin receptor, facilitating uptake of iron from the exterior (Hentze et al. 2004). It has recently been established that a large class of genes involved in iron metabolism is under coordinated post-transcriptional control by the Cth2 protein in yeast (Puig et al. 2005). Cth2 displays sequence homology to the human ARE-binding protein tristetraproline (TTP) in the TZF region. It was also shown that a majority of the mRNAs that are dependent on Cth2 for post-transcriptional down-regulation under iron limitation carry ARE elements in their 3′-UTR. Thus, the IREs in this organism appear to be identical to, or a subset of, AREs (see Table 1). Although it is clear that a coordinated control of mRNA stability occurs in response to changes in iron levels in yeast, the responsible signalling pathway(s) have not been identified.

Other instances of MAPK-mediated post-transcriptional control

Oxidative stress in fission yeast causes phosphorylation of eIF2α, with concomitant reduction of protein synthesis. This stress-induced phosphorylation has been reported to be substantially increased in *sty1* mutants, suggesting that the SAPK pathway is involved in modulating the global translational response to stress (Dunand-Sauthier et al. 2005).

In budding yeast, an Rck2-dependent phosphorylation of EF-2 upon hyperosmotic shock has been demonstrated to occur, which in turn is dependent on phosphorylation of Rck2 by the MAPK Hog1 (Teige et al. 2001). In *hog1* and *rck2* mutants, the global adaptation (initial down-regulation and later recovery) of translation upon hyperosmotic and oxidative stress is perturbed (Swaminathan et al. 2006; Teige et al. 2001; Uesono and Toh 2002). In *rck2* mutants, a large number of genes encoding ribosomal proteins and proteins required for ribosomal assembly and modification are deregulated both on the transcriptional level and with respect to polysomal association (Swaminathan et al. 2006).

The *Sz. pombe* RNA-binding protein Csx1 is phosphorylated upon oxidative stress in a Sty1-dependent way (Rodriguez-Gabriel et al. 2003). Interestingly, this phosphorylation is specific for the type of external stress, and does not occur in, e.g. hyperosmotic shock. This leads to stabilisation of mRNA encoding Atf1, a transcription factor responsible for stimulation of transcription a large number of stress-induced genes. Two other RNA-binding proteins, Cip1 and Cip2, have subsequently been identified which act in opposition to Csx1 (Martin et al. 2006); the sensitivity of a *csx1* deletion to oxidative stress is rescued by a *cip1* or *cip2* deletion. There is as yet no evidence for Sty1-dependent phosphorylation of Cip1 and Cip2, however. As *atf1*$^{+}$ mRNA is the only identified direct target of Csx1, Cip1, and Cip2, it is not clear if the mechanism involves ARE's.

So far, we have considered regulation at the post-transcriptional level as one of the outputs of signalling chains in the cell, including MAP kinase cascades. There is one interesting example of post-transcriptional regulation acting within this signalling pathway itself. In fission yeast, the RNA-binding protein Rnc1 participates in a negative feedback mechanism leading to down-regulation of the Pmk1 MAPK pathway after

activation. This occurs through phosphorylation of Rnc1 by active Pmk1, inducing the binding of phosphorylated Rnc1 to *pmp1*$^+$ mRNA, encoding a phosphatase which deactivates Pmk1. This leads to mRNA stabilisation with ensuing dephosphorylation and deactivation of Pmk1 (Sugiura et al. 2003).

DNA damage checkpoints

Proteins in the DNA-dependent cell cycle checkpoint pathway were primarily recognised for their post-translational action on cell cycle proteins, and for their crucial role in the transcriptional induction program following DNA damage and stalled replication. This pathway may also act at the post-transcriptional level, however. Signalling from replicatively damaged DNA through the checkpoint protein Dun1 destabilises *RAD5* mRNA through interaction with the polyA nuclease (PAN) complex (Hammet et al. 2002). This leads to decreased Rad5 levels upon checkpoint activation, favouring error-free DNA repair. This action is mediated through the FHA domain of Dun1 in its interaction with the Pan3 subunit of the polyA nuclease complex. The up-regulation of UV damage endonuclease (UVDE) activity in *Sz. pombe rad9* checkpoint mutants has been ascribed to post-transcriptional effects, on mRNA stability, translation, or both (Davey et al. 1998).

There are also examples of post-transcriptional effects following DNA damage where the signalling pathway remains unidentified. UV irradiation of mammalian cells causes down-regulation of translation through Gcn2-dependent phosphorylation of eIF2α (Deng et al. 2002; Jiang and Wek 2005). Even though UV radiation activates the JNK and p38 stress-activated MAP kinases, eIF2α phosphorylation does not require either of these pathways. Conversely, UV-induced activation of JNK or p38 does not require Gcn2 (Deng et al. 2002), and so there is presently no evidence for a link between these phenomena. In yeast, however, treatment with the DNA damaging agent methyl methane sulfonate (MMS) increases Gcn4 translation in a Gcn2-dependent way (Natarajan et al. 2001). It should be emphasised that it has not been shown that DNA damage as such is the initiating event for Gcn2 activation in these experiments.

Protein kinase C

A direct coupling of protein kinase C (PKC) signalling and translation in mammalian cells is strongly suggested by the physical association of both the PKC receptor protein RACK and PKCβII to eIF6, a protein implicated in dissociation of the 40S and 60S subunits. Stimulation of PKC signalling promotes joining of the ribosomal subunits (Ceci et al. 2003). The significance of RACK/PKC signalling is perceived to lie in cell-cell adhesion and localised translation at points of cellular growth.

Global studies of translational efficiency

Global investigation of cognate protein and mRNA levels in *S. cerevisiae* have pointed out that the degree of correlation is so low that major post-transcriptional regulation has to be invoked (Futcher et al. 1999; Ghaemmaghami et al. 2003; Gygi et al. 1999; Washburn et al. 2003). This discrepancy is most pronounced for weakly expressed genes (Pradet-Balade et al. 2001). Different cellular compartments have been reported to have different characteristics with respect to translational regulation (Beyer et al. 2004). An investigation of pre-existing datasets describing mRNA abundance, protein abundance and translational efficiencies indicate that there is a positive correlation between mRNA abundance and ribosome density. Thus, mRNAs encoding proteins in energy-yielding processes tend to be translated more efficiently; those where the levels of the protein product varies rapidly (e.g. ribosomal proteins) are of relatively high abundance but their translation can be strongly up-regulated when needed ("translation on demand"; Beyer et al. 2004; Washburn et al. 2003).

Several array studies indicate that changes in the translational state and stability may affect a quite broad range of mRNAs. Blocking of the Ras and Akt pathways in mammalian brain cells leads to rapid regulation of polysomal association of selected mRNAs, arguably more so than seen in the abundance in total mRNA. A significantly higher number of transcripts relevant for Ras and Akt signalling were found to be altered in polysomal mRNA than in the total mRNA fraction (Rajasekhar et al. 2003). An early study identified 1% of transcripts in fibroblasts as being translationally regulated upon mitogenic stimulation (Zong et al. 1999). During transition from an epithelial to a fibroblastoid phenotype, 15% of all induced or repressed transcripts were exclusively translationally regulated, while for 7%, both a transcriptional and a translational component was found (Jechlinger et al. 2003). A recent study of changes on the mRNA level after exposure of tumour cells to ionising radiation found as much as 90% of all induced and 80% of all repressed mRNAs to be regulated primarily on the translational level (Lü et al. 2006). In yeast, equal numbers of mRNAs were found to be regulated on the

translational versus the transcriptional level upon amino acid starvation and butanol stress (Smirnova et al. 2005).

Comparing steady-state levels of mRNA species in the total RNA pool and their levels in polysomal RNA under one particular set of conditions gives a static view on translational control. There is a positive correlation between high expression level of an mRNA and its tendency to be highly translated (Beyer et al. 2004). Several genome-wide studies of mRNA translation profiles have been performed in yeast, aiming to identify global changes that occur upon changes in growth conditions or external stress, e.g. (Arava et al. 2003; Kuhn et al. 2001; Preiss et al. 2003; Smirnova et al. 2005; Swaminathan et al. 2006). This gives a dynamic perspective on translational regulation. The strongest overall trend is to have transcriptional and translational control reinforce each other for a particular gene, a phenomenon dubbed "homodirectional changes" (Preiss et al. 2003). For a small number of genes, there are instead "counterdirectional" changes. The budding yeast genes *CPA1*, *GCN4*, *HAC1*, and *ICY2* are more efficiently translated under conditions of stress (Beilharz and Preiss 2004; Hinnebusch and Natarajan 2002; Kuhn et al. 2001; Messenguy et al. 1983). For *GCN4*, this control is exerted through phosphorylation of eIF2α and different translation of uORFs as already described. In mammalian cells, translation of the *ATF4* mRNA (also encoding a transcription factor) is likewise positively controlled by eIF2α phosphorylation and also involves translation of uORFs. Interestingly, the mechanism is similar to that of yeast *GCN4* in that it involves a more 5′ uORF which directs scanning and downstream reinitiation, and a second, inhibitory, uORF closer to the main ORF (Vattem and Wek 2004). Another mechanism for translational control is through phosphorylation of eIF4E binding proteins, leading to release of active eIF4E. An active TOR pathway and many mitogens increase this phosphorylation. This regulation can have gene-specific effects, as translation of the *PGC1* mRNA is selectively increased in mice lacking eIF4E binding protein 1 (Tsukiyama-Kohara et al. 2001).

The global studies indicate that a considerable fraction of mRNA species change their translational efficiency in response to stress, hormones or nutrient availability. It is thus likely that beside these few well-characterised cases, other as yet distinguished mechanisms regulate potentially large classes of mRNAs on the translational level. This regulation may be surprisingly specific, as even seemingly related stress conditions will enhance polysomal association of different mRNA subsets (Smirnova et al. 2005). Further, their data suggest co-regulation of translation and transcription factors by the same signalling pathways.

Concluding remarks

The extent of posttranscriptional regulation could be different between yeast and more complex organisms. It is well recognised that the scarcity of introns provides fewer opportunities for alternative splicing in yeast. Of note is also the fact that although mammalian mRNAs encoding ribosomal proteins contain polypyrimidine tracts in their 5′-UTRs which dictate their translational regulation (Table 1), the corresponding yeast mRNAs lack these sequences and are instead regulated on the transcriptional level (Warner 1999). RNAi presents a wealth of previously unrecognised possibilities for short-term and long-term regulation of expression. This mechanism is almost ubiquitous in eukaryotes with the notable exception of hemiascomycetes (budding yeasts), among which the investigated species invariably have lost the genes encoding proteins required for the RNA processing steps in the RNAi pathway and several genes required for heterochromatin formation (Aravind et al. 2000; Axelsson-Fisk and Sunnerhagen 2006; Cerutti and Casas-Mollano 2006). Based on the distribution among eukaryotic lineages of components of the RNAi machinery, it has been suggested that its primordial functions included targeted transcript degradation, and that other functions such as guided DNA methylation could be more recent specialisations (Cerutti and Casas-Mollano 2006). There are recent hints of previously unsuspected links between the RNAi machinery and regulation of mRNA turnover. Thus, microRNA regulation of mRNA stability can be mediated through ARE elements in mammalian cells (Jing et al. 2005). It has also been found that mammalian P bodies contain protein and RNA elements of the RNAi machinery (Liu et al. 2005; Sen and Blau 2005). Knowing that formation of stress granules is downstream of phosphorylation of eIF2α, which is also a focal point of intracellular signalling through TOR and other pathways, it appears likely that temporary storage of mRNAs in response to stress is controlled through one or more of these intracellular signalling pathways. Great efforts are currently made to define the molecular mechanisms of such interactions between pathways.

So far, only few verified examples of uORF control of translation exist, and the extent of this control mechanism is unknown. The number of conserved uORFs emerging from comparative genomics studies suggests that uORF control could be quite widespread. Monitoring movement of mRNAs of interest between the

polysomal fractions and those containing free mRNAs across several array experiments, representing many different types of environmental and hormonal inputs, should be an efficient experimental way to identify mRNAs regulated by uORFs.

A global investigation of protein half-lives in budding yeast indicates that the protein products of genes that are co-regulated on the transcriptional level tend to be co-regulated also on the level of protein turnover (Belle et al. 2006). It would be interesting to see if a similar, or even stronger, correlation could be found between post-translational and post-transcriptional regulation. In more general terms, a fruitful approach to identification of mRNA classes subject to post-transcriptional regulation should be to measure translational activity (as polysomal association) and mRNA stability (after Pol II arrest) in the same experiment. Cross-species comparisons will add to the discrimination power of this approach, and may lead to the identification of sequence determinants, conserved in evolution, that are responsible for mRNA stability and translational efficiency.

Acknowledgments Thanks are due to Alan Hinnebusch for constructive criticism. Support of work in the author's laboratory from the Swedish Research Council, the Swedish Cancer Fund, the European Commission, and the National Graduate School of Genomics and Bioinformatics is acknowledged.

References

Abastado JP, Miller PF, Jackson BM, Hinnebusch AG (1991) Suppression of ribosomal reinitiation at upstream open reading frames in amino acid-starved cells forms the basis for *GCN4* translational control. Mol Cell Biol 11:486–596

Alemany V, Sanchez-Piris M, Bachs O, Aligue R (2002) Cmk2, a novel serine/threonine kinase in fission yeast. FEBS Lett 524:79–86

Anderson JS, Parker RP (1998) The 3′ to 5′ degradation of yeast mRNAs is a general mechanism for mRNA turnover that requires the SKI2 DEVH box protein and 3′ to 5′ exonucleases of the exosome complex. EMBO J 17:1497–1506

Arava Y, Wang Y, Storey JD, Liu CL, Brown PO, Herschlag D (2003) Genome-wide analysis of mRNA translation profiles in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 100:3889–3894

Aravind L, Watanabe H, Lipman DJ, Koonin EV (2000) Lineage-specific loss and divergence of functionally linked genes in eukaryotes. Proc Natl Acad Sci USA 97:11319–11324

Asp E, Sunnerhagen P (2003) Mkp1 and Mkp2, two MAPKAP-kinase homologues in *Schizosaccharomyces pombe*, interact with the MAP kinase Sty1. Mol Genet Genomics 268:585–597

Axelsson-Fisk M, Sunnerhagen P (2006) Comparative genomics and gene finding in fungi. In: Sunnerhagen P, Piškur J (eds) Comparative genomics using fungi as models. Springer, Heidelberg, pp 1–28

Bakheet T, Williams BR, Khabar KS (2003) ARED 2.0: an update of AU-rich element mRNA database. Nucleic Acids Res 31:421–423

Banko JL, Hou L, Klann E (2004) NMDA receptor activation results in PKA- and ERK-dependent Mnk1 activation and increased eIF4E phosphorylation in hippocampal area CA1. J Neurochem 91:462–470

Barbet NC, Schneider U, Helliwell SB, Stansfield I, Tuite MF, Hall MN (1996) TOR controls translation initiation and early G1 progression in yeast. Mol Biol Cell 7:25–42

Beilharz TH, Preiss T (2004) Translational profiling: the genome-wide measure of the nascent proteome. Brief Funct Genomic Proteomic 3:103–111

Belle A, Tanay A, Bitincka L, Shamir R, O'Shea EK (2006) Quantification of protein half-lives in the budding yeast proteome. Proc Natl Acad Sci USA 103:13004–13009

Bevilacqua A, Ceriani MC, Capaccioli S, Nicolin A (2003) Post-transcriptional regulation of gene expression by degradation of messenger RNAs. J Cell Physiol 195:356–372

Beyer A, Hollunder J, Nasheuer HP, Wilhelm T (2004) Post-transcriptional expression regulation in the yeast *Saccharomyces cerevisiae* on a genomic scale. Mol Cell Proteomics 3:1083–1092

Boado RJ, Pardridge WM (1998) Ten nucleotide cis element in the 3′-untranslated region of the GLUT1 glucose transporter mRNA increases gene expression via mRNA stabilization. Brain Res Mol Brain Res 59:109–113

Brengues M, Teixeira D, Parker R (2005) Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies. Science 310:486–489

Briata P, Forcales SV, Ponassi M, Corte G, Chen CY et al (2005) p38-dependent phosphorylation of the mRNA decay-promoting factor KSRP controls the stability of select myogenic transcripts. Mol Cell 20:891–903

Caponigro G, Parker R (1996) Mechanisms and control of mRNA turnover in *Saccharomyces cerevisiae*. Microbiol Rev 60:233–249

Causton HC, Ren B, Koh SS, Harbison CT, Kanin E et al (2001) Remodeling of yeast genome expression in response to environmental changes. Mol Biol Cell 12:323–337

Ceci M, Gaviraghi C, Gorrini C, Sala LA, Offenhauser N et al (2003) Release of eIF6 (p27BBP) from the 60S subunit allows 80S ribosome assembly. Nature 426:579–584

Cerutti H, Casas-Mollano JA (2006) On the origin and functions of RNA-mediated silencing: from protists to man. Curr Genet 50:81–99

Chen CY, Shyu AB (1995) AU-rich elements: characterization and importance in mRNA degradation. Trends Biochem Sci 20:465–470

Chen CY, Del Gatto-Konczak F, Wu Z, Karin M (1998) Stabilization of interleukin-2 mRNA by the c-Jun NH_2-terminal kinase pathway. Science 280:1945–1949

Chen CY, Gherzi R, Andersen JS, Gaietta G, Jurchott K et al (2000) Nucleolin and YB-1 are required for JNK-mediated interleukin-2 mRNA stabilization during T-cell activation. Genes Dev 14:1236–1248

Chen CY, Gherzi R, Ong SE, Chan EL, Raijmakers R et al (2001) AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. Cell 107:451–464

Cherkasova VA, Hinnebusch AG (2003) Translational control by TOR and TAP42 through dephosphorylation of eIF2α kinase GCN2. Genes Dev 17:859–872

Cosentino GP, Schmelzle T, Haghighat A, Helliwell SB, Hall MN, Sonenberg N (2000) Eap1p, a novel eukaryotic translation initiation factor 4E-associated protein in *Saccharomyces cerevisiae*. Mol Cell Biol 20:4604–4613

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

Crowe ML, Wang XQ, Rothnagel JA (2006) Evidence for conservation and selection of upstream open reading frames

suggests probable encoding of bioactive peptides. BMC Genomics 7:16
Daga RR, Jimenez J (1999) Translational control of the cdc25 cell cycle phosphatase: a molecular mechanism coupling mitosis to cell growth. J Cell Sci 112:3137–3146
Dahlkvist A, Sunnerhagen P (1994) Two novel deduced serine/threonine protein kinases from *Saccharomyces cerevisiae*. Gene 139:27–33
Danaie P, Altmann M, Hall MN, Trachsel H, Helliwell SB (1999) *CLN3* expression is sufficient to restore G1-to-S-phase progression in *Saccharomyces cerevisiae* mutants defective in translation initiation factor eIF4E. Biochem J 340:135–141
Davey S, Han CS, Ramer SA, Klassen JC, Jacobson A et al (1998) Fission yeast *rad12*$^{+}$ regulates cell cycle checkpoint control and is homologous to the Bloom's syndrome disease gene. Mol Cell Biol 18:2721–2728
David L, Huber W, Granovskaia M, Toedling J, Palm CJ et al (2006) A high-resolution map of transcription in the yeast genome. Proc Natl Acad Sci USA 103:5320–5325
Deng J, Harding HP, Raught B, Gingras AC, Berlanga JJ et al (2002) Activation of GCN2 in UV-irradiated cells inhibits translation. Curr Biol 12:1279–1286
Dever TE (2002) Gene-specific regulation by general translation factors. Cell 108:545–556
Dever TE, Feng L, Wek RC, Cigan AM, Donahue TF, Hinnebusch AG (1992) Phosphorylation of initiation factor 2 alpha by protein kinase GCN2 mediates gene-specific translational control of GCN4 in yeast. Cell 68:585–596
Doma MK, Parker R (2006) Endonucleolytic cleavage of eukaryotic mRNAs with stalls in translation elongation. Nature 440:561–564
D'Orso I, Frasch AC (2001) Functionally different AU- and G-rich cis-elements confer developmentally regulated mRNA stability in *Trypanosoma cruzi* by interaction with specific RNA-binding proteins. J Biol Chem 276:15783–15793
Dunand-Sauthier I, Walker C, Wilkinson C, Gordon C, Crane R et al (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 13:1626–1640
Dunand-Sauthier I, Walker CA, Narasimhan J, Pearce AK, Wek RC, Humphrey TC (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
Duttagupta R, Tian B, Wilusz CJ, Khounh DT, Soteropoulos P et al (2005) Global analysis of Pub1p targets reveals a coordinate control of gene expression through modulation of binding and stability. Mol Cell Biol 25:5499–5513
Foyt HL, LeRoith D, Roberts CT Jr (1991) Differential association of insulin-like growth factor I mRNA variants with polysomes in vivo. J Biol Chem 266:7300–7305
Futcher B, Latter GI, Monardo P, McLaughlin CS, Garrels JI (1999) A sampling of the yeast proteome. Mol Cell Biol 19:7357–7368
Galagan JE, Calvo SE, Cuomo C, Ma LJ, Wortman JR et al (2005) Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. Nature 438:1105–1115
Gallie DR (1998) A tale of two termini: a functional interaction between the termini of an mRNA is a prerequisite for efficient translation initiation. Gene 216:1–11
Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB et al (2000) Genomic expression programs in the response of yeast cells to environmental changes. Mol Biol Cell 11:4241–4257
Gerber AP, Herschlag D, Brown PO (2004) Extensive association of functionally and cytotopically related mRNAs with Puf family RNA-binding proteins in yeast. PLoS Biol 2:E79
Gerber AP, Luschnig S, Krasnow MA, Brown PO, Herschlag D (2006) Genome-wide identification of mRNAs associated with the translational regulator PUMILIO in *Drosophila melanogaster*. Proc Natl Acad Sci USA 103:4487–4492
Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A et al (2003) Global analysis of protein expression in yeast. Nature 425:737–741
Gilks N, Kedersha N, Ayodele M, Shen L, Stoecklin G et al (2004) Stress granule assembly is mediated by prion-like aggregation of TIA-1. Mol Biol Cell 15:5383–5398
Grigull J, Mnaimneh S, Pootoolal J, Robinson MD, Hughes TR (2004) Genome-wide analysis of mRNA stability using transcription inhibitors and microarrays reveals posttranscriptional control of ribosome biogenesis factors. Mol Cell Biol 24:5534–5547
Gygi SP, Rochon Y, Franza BR, Aebersold R (1999) Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19:1720–1730
Hammet A, Pike BL, Heierhorst J (2002) Posttranscriptional regulation of the *RAD5* DNA repair gene by the Dun1 kinase and the Pan2-Pan3 poly(A)-nuclease complex contributes to survival of replication blocks. J Biol Chem 277:22469–22474
Hentze MW, Muckenthaler MU, Andrews NC (2004) Balancing acts: molecular control of mammalian iron metabolism. Cell 117:285–297
Hinnebusch AG (1996) Translational control of *GCN4*: gene-specific regulation by phosphorylation of eIF2. In: Hershey JWB, Mathews MB, Sonenberg N (eds) Translational control. Cold Spring Harbor Press, Cold Spring Harbor, pp 199–244
Hinnebusch AG, Natarajan K (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
Hughes AL, Friedman R (2003) Parallel evolution by gene duplication in the genomes of two unicellular fungi. Genome Res 13:1259–1264
Jechlinger M, Grunert S, Tamir IH, Janda E, Ludemann S et al (2003) Expression profiling of epithelial plasticity in tumor progression. Oncogene 22:7155–7169
Jefferies HB, Reinhard C, Kozma SC, Thomas G (1994) Rapamycin selectively represses translation of the "polypyrimidine tract" mRNA family. Proc Natl Acad Sci USA 91:4441–4445
Jiang HY, Wek RC (2005) GCN2 phosphorylation of eIF2α activates NF-κB in response to UV irradiation. Biochem J 385:371–380
Jing Q, Huang S, Guth S, Zarubin T, Motoyama A et al (2005) Involvement of microRNA in AU-rich element-mediated mRNA instability. Cell 120:623–634
Kedersha NL, Gupta M, Li W, Miller I, Anderson P (1999) RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 alpha to the assembly of mammalian stress granules. J Cell Biol 147:1431–1442
Kedersha N, Cho MR, Li W, Yacono PW, Chen S et al (2000) Dynamic shuttling of TIA-1 accompanies the recruitment of mRNA to mammalian stress granules. J Cell Biol 151:1257–1268
Kedersha N, Stoecklin G, Ayodele M, Yacono P, Lykke-Andersen J et al (2005) Stress granules and processing bodies are dynamically linked sites of mRNP remodeling. J Cell Biol 169:871–884
Kim CH, Warner JR (1983) Messenger RNA for ribosomal proteins in yeast. J Mol Biol 165:79–89
Klann E, Antion MD, Banko JL, Hou L (2004) Synaptic plasticity and translation initiation. Learn Mem 11:365–372

Kotlyarov A, Neininger A, Schubert C, Eckert R, Birchmeier C et al (1999) MAPKAP kinase 2 is essential for LPS-induced TNF-α biosynthesis. Nat Cell Biol 1:94–97

Kuhn KM, DeRisi JL, Brown PO, Sarnow P (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 21:916–927

Lasa M, Mahtani KR, Finch A, Brewer G, Saklatvala J, Clark AR (2000) Regulation of cyclooxygenase 2 mRNA stability by the mitogen-activated protein kinase p38 signaling cascade. Mol Cell Biol 20:4265–4274

Lasa M, Brook M, Saklatvala J, Clark AR (2001) Dexamethasone destabilizes cyclooxygenase 2 mRNA by inhibiting mitogen-activated protein kinase p38. Mol Cell Biol 21:771–780

Levy S, Avni D, Hariharan N, Perry RP, Meyuhas O (1991) Oligopyrimidine tract at the 5′ end of mammalian ribosomal protein mRNAs is required for their translational control. Proc Natl Acad Sci USA 88:3319–3323

Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R (2005) MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. Nat Cell Biol 7:719–723

Lü X, de la Peña L, Barker C, Camphausen K, Tofilon PJ (2006) Radiation-induced changes in gene expression involve recruitment of existing messenger RNAs to and away from polysomes. Cancer Res 66:1052–1061

Marcus L, Ris H, Halvorson HO, Bretthauer RK, Bock RM (1967) Occurrence, isolation, and characterization of polyribosomes in yeast. J Cell Biol 34:505–512

Martin V, Rodriguez-Gabriel MA, McDonald WH, Watt S, Yates JR 3rd et al (2006) Cip1 and Cip2 are novel RNA-recognition-motif proteins that counteract Csx1 function during oxidative stress. Mol Biol Cell 17:1176–1183

McEwen E, Kedersha N, Song B, Scheuner D, Gilks N et al (2005) Heme-regulated inhibitor kinase-mediated phosphorylation of eukaryotic translation initiation factor 2 inhibits translation, induces stress granule formation, and mediates survival upon arsenite exposure. J Biol Chem 280:16925–16933

Messenguy F, Feller A, Crabeel M, Pierard A (1983) Control-mechanisms acting at the transcriptional and post-transcriptional levels are involved in the synthesis of the arginine pathway carbamoylphosphate synthase of yeast. EMBO J 2:1249–1254

Meyer S, Temme C, Wahle E (2004) Messenger RNA turnover in eukaryotes: pathways and enzymes. Crit Rev Biochem Mol Biol 39:197–216

Mignone F, Grillo G, Licciulli F, Iacono M, Liuni S et al (2005) UTRdb and UTRsite: a collection of sequences and regulatory motifs of the untranslated regions of eukaryotic mRNAs. Nucleic Acids Res 33:D141–D146

Muhlrad D, Decker CJ, Parker R (1995) Turnover mechanisms of the stable yeast *PGK1* mRNA. Mol Cell Biol 15:2145–2156

Natarajan K, Meyer MR, Jackson BM, Slade D, Roberts C et al (2001) Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. Mol Cell Biol 21:4347–4368

Neininger A, Kontoyiannis D, Kotlyarov A, Winzen R, Eckert R et al (2002) MK2 targets AU-rich elements and regulates biosynthesis of tumor necrosis factor and interleukin-6 independently at different post-transcriptional levels. J Biol Chem 277:3065–3068

Nonet M, Scafe C, Sexton J, Young R (1987) Eucaryotic RNA polymerase conditional mutant that rapidly ceases mRNA synthesis. Mol Cell Biol 7:1602–16011

Nover L, Scharf KD, Neumann D (1983) Formation of cytoplasmic heat shock granules in tomato cell cultures and leaves. Mol Cell Biol 3:1648–1655

Polymenis M, Schmidt EV (1997) Coupling of cell division to cell growth by translational control of the G1 cyclin *CLN3* in yeast. Genes Dev 11:2522–2531

Pradet-Balade B, Boulme F, Beug H, Mullner EW, Garcia-Sanz JA (2001) Translation control: bridging the gap between genomics and proteomics? Trends Biochem Sci 26:225–229

Preiss T, Hentze MW (2003) Starting the protein synthesis machine: eukaryotic translation initiation. Bioessays 25:1201–1211

Preiss T, Baron-Benhamou J, Ansorge W, Hentze MW (2003) Homodirectional changes in transcriptome composition and mRNA translation induced by rapamycin and heat shock. Nat Struct Biol 10:1039–1047

Prendergast GC (2003) Signal transduction: putting translation before transcription. Cancer Cell 4:244–245

Prieto S, de la Cruz BJ, Scheffler IE (2000) Glucose-regulated turnover of mRNA and the influence of poly(A) tail length on half-life. J Biol Chem 275:14155–14166

Puig S, Askeland E, Thiele DJ (2005) Coordinated remodeling of cellular metabolism during iron deficiency through targeted mRNA degradation. Cell 120:99–110

Rajasekhar VK, Viale A, Socci ND, Wiedmann M, Hu X, Holland EC (2003) Oncogenic Ras and Akt signaling contribute to glioblastoma formation by differential recruitment of existing mRNAs to polysomes. Mol Cell 12:889–901

Roberts GC, Smith CW (2002) Alternative splicing: combinatorial output from the genome. Curr Opin Chem Biol 6:375–383

Rodriguez-Gabriel MA, Burns G, McDonald WH, Martin V, Yates JR 3rd et al (2003) RNA-binding protein Csx1 mediates global control of gene expression in response to oxidative stress. EMBO J 22:6256–6266

Rohde JR, Campbell S, Zurita-Martinez SA, Cutler NS, Ashe M, Cardenas ME (2004) TOR controls transcriptional and translational programs via Sap-Sit4 protein phosphatase signaling effectors. Mol Cell Biol 24:8332–8341

Sen GL, Blau HM (2005) Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. Nat Cell Biol 7:633–636

Sheth U, Parker R (2003) Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. Science 300:805–808

Smirnova JB, Selley JN, Sanchez-Cabo F, Carroll K, Eddy AA 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 25:9340–9349

Smith DA, Toone WM, Chen D, Bähler J, Jones N et al (2002) The Srk1 protein kinase is a target for the Sty1 stress-activated MAPK in fission yeast. J Biol Chem 277:33411–33421

Stoecklin G, Stubbs T, Kedersha N, Wax S, Rigby WF et al (2004) MK2-induced tristetraprolin:14-3-3 complexes prevent stress granule association and ARE-mRNA decay. EMBO J 23:1313–1324

Sugiura R, Kita A, Shimizu Y, Shuntoh H, Sio SO, Kuno T (2003) Feedback regulation of MAPK signalling by an RNA-binding protein. Nature 424:961–965

Swaminathan S, Masek T, Molin C, Pospisek M, Sunnerhagen P (2006) Rck2 is required for reprogramming of ribosomes during oxidative stress. Mol Biol Cell 17:1472–1482

Teige M, Scheikl E, Reiser V, Ruis H, Ammerer G (2001) Rck2, a member of the calmodulin-protein kinase family, links protein synthesis to high osmolarity MAP kinase signaling in budding yeast. Proc Natl Acad Sci USA 98:5625–5630

Tsukiyama-Kohara K, Poulin F, Kohara M, DeMaria CT, Cheng A et al (2001) Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1. Nat Med 7:1128–1132

Uesono Y, Toh EA (2002) Transient inhibition of translation initiation by osmotic stress. J Biol Chem 277:13848–13855

Vasudevan S, Peltz SW (2001) Regulated ARE-mediated mRNA decay in *Saccharomyces cerevisiae*. Mol Cell 7:1191–1200

Vasudevan S, Garneau N, Tu Khounh D, Peltz SW (2005) p38 mitogen-activated protein kinase/Hog1p regulates translation of the AU-rich-element-bearing *MFA2* transcript. Mol Cell Biol 25:9753–9763

Vattem KM, Wek RC (2004) Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. Proc Natl Acad Sci USA 101:11269–11274

Waggoner SA, Liebhaber SA (2003) Identification of mRNAs associated with alphaCP2-containing RNP complexes. Mol Cell Biol 23:7055–7067

Wang X, Flynn A, Waskiewicz AJ, Webb BL, Vries RG et al (1998) The phosphorylation of eukaryotic initiation factor eIF4E in response to phorbol esters, cell stresses, and cytokines is mediated by distinct MAP kinase pathways. J Biol Chem 273:9373–9377

Warner JR (1999) The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 24:437–440

Washburn MP, Koller A, Oshiro G, Ulaszek RR, Plouffe D et al (2003) Protein pathway and complex clustering of correlated mRNA and protein expression analyses in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 100:3107–3112

Waskiewicz AJ, Johnson JC, Penn B, Mahalingam M, Kimball SR, Cooper JA (1999) Phosphorylation of the cap-binding protein eukaryotic translation initiation factor 4E by protein kinase Mnk1 in vivo. Mol Cell Biol 19:1871–1880

Winzen R, Kracht M, Ritter B, Wilhelm A, Chen CY et al (1999) The p38 MAP kinase pathway signals for cytokine-induced mRNA stabilization via MAP kinase-activated protein kinase 2 and an AU-rich region-targeted mechanism. EMBO J 18:4969–4980

Yang Z, Jakymiw A, Wood MR, Eystathioy T, Rubin RL et al (2004) GW182 is critical for the stability of GW bodies expressed during the cell cycle and cell proliferation. J Cell Sci 117:5567–5678

Yin Z, Hatton L, Brown AJ (2000) Differential post-transcriptional regulation of yeast mRNAs in response to high and low glucose concentrations. Mol Microbiol 35:553–565

Zhang Z, Dietrich FS (2005) Identification and characterization of upstream open reading frames (uORF) in the 5′ untranslated regions (UTR) of genes in *Saccharomyces cerevisiae*. Curr Genet 48:77–87

Zhang T, Kruys V, Huez G, Gueydan C (2002) AU-rich element-mediated translational control: complexity and multiple activities of trans-activating factors. Biochem Soc Trans 30:952–958

Zhou C, Arslan F, Wee S, Krishnan S, Ivanov AR et al (2005) PCI proteins eIF3e and eIF3m define distinct translation initiation factor 3 complexes. BMC Biol 3:14

Zong Q, Schummer M, Hood L, Morris DR (1999) Messenger RNA translation state: the second dimension of high-throughput expression screening. Proc Natl Acad Sci USA 96:10632–10636