Getting the message in protein synthesis

Keystone Symposium on Translational Regulatory Mechanisms

Mauro Costa-Mattioli^{1†}, Michael Bidinosti¹ & Thomas E. Dever²⁺

¹McGill University, Montréal, Quebec, Canada, and ²Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA

Coeur d'Alene resort. Photograph courtesy of Tim Eberly.

The Keystone Symposium on Translational Regulatory Mechanisms was held between 28 January and 2 February 2008, in Coeur d'Alene, Idaho, USA, and was organized by N. Sonenberg, A.G. Hinnebusch and J.D. Richter.

Keywords: initiation factor; learning and memory; miRNA; mRNA stability; translational control

EMBO reports (2008) 9, 954–959. doi:10.1038/embor.2008.165

See Glossary for abbreviations used in this article.

¹Department of Biochemistry and McGill Cancer Center, 3655 Promenade Sir William Osler, McGill University, Montréal, Quebec H3G 1Y6, Canada

²Laboratory of Gene Regulation and Development, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892, USA

[†]Present address: Department of Neuroscience, Baylor College of Medicine, Houston, Texas 77030, USA

⁺Corresponding author. Tel: +301 496 4519; Fax: +301 496 8576;

E-mail: tdever@nih.gov

Submitted 8 May 2008; accepted 17 July 2008; published online 29 August 2008

Introduction

About 140 scientists gathered in snowy Coeur d'Alene, Idaho, USA, to attend the Keystone Symposium on Translational Regulatory Mechanisms. This meeting was unique in bringing together a diverse group of researchers studying the mechanism and structural biology of the translation apparatus, messenger RNA (mRNA) degradation and transport, microRNA (miRNA)-mediated control of gene expression, and the translational regulatory processes underlying development, learning and memory, and human diseases.

Translation-initiation mechanism

The initiation of protein synthesis requires the delivery of both the methionyl-initiator transfer RNA (Met-tRNA^{Met}) and the mRNA to the ribosome. These processes are coordinated by translation-initiation factors as summarized in Fig 1. Several talks provided new insights into the ordered assembly of the translating 80S ribosome. V. Ramakrishnan (Cambridge, UK) presented a structural analysis of the yeast 40S ribosomal subunit, and revealed that the binding of eIF1 and eIF1A induced the opening of the mRNA-binding channel (Passmore *et al*, 2007), perhaps providing a rationale for the ordered assembly of the initiation complex. J. Doudna (Berkeley, CA, USA) showed that the eIF3j subunit competed with eIF1A for binding to the 40S subunit, and that eIF3j prevented high-affinity mRNA binding in the absence of the ternary complex (Fraser *et al*, 2007). Therefore, eIF3j might act as a regulatory switch on the 40S complex to ensure the ordered recruitment of the ternary complex and mRNA to the 40S subunit.

After binding to the mRNA, the 43S ribosome complex scans for, and selects, an AUG start site as directed by, at least in part, codon–anticodon interactions between the tRNA^{Met} and the mRNA. In separate talks based on their mutational and biophysical studies of eIF1A, as well as examination of start-codon selection, A. Hinnebusch (Bethesda, MD, USA) and J. Lorsch (Baltimore, MD, USA) proposed that the negatively charged eIF1A carboxyl terminus promotes the open scanning-competent state of the 40S subunit and blocks initiation at non-AUG codons, whereas the positively charged amino terminus of eIF1A has the opposite function and is required for formation of the scanning-incompetent closed 43S conformation at start-codon selection (Fekete *et al*,

2007). A. Hinnebusch went on to describe a set of ribosomal RNA (rRNA) mutations that impaired reinitiation at short, upstream open reading frames on *GCN4* mRNA. These studies provide the first insights into the ribosomal determinants in eukaryotes that facilitate Met-tRNA_i^{Met} binding. Once the 43S complex locates a start site, eIF1A helps to recruit eIF5B, to promote subunit joining (Acker *et al*, 2006; Fringer *et al*, 2007).

The secondary structure near the 5' end of mRNA impairs translation by preventing binding or scanning of the 43S complex. Ribosome access to the mRNA is facilitated by the cap-binding complex eIF4F. The eIF4F complex consists of the cap-binding protein eIF4E, the RNA helicase eIF4A and the scaffolding protein eIF4G, and is thought to unwind secondary structures near the cap that prevent 43S-complex binding. The helicase activity of eIF4A is promoted by eIF4B and/or eIF4H. M. Moore (Worcester, MA, USA) reported that the binding of either eIF4B or eIF4H to eIF4A is mutually exclusive. Moreover, she drew a parallel between the interaction of eIF4B or eIF4H with eIF4A and the related exon-junction complex (EJC) in which the RNA-binding protein MLN51 interacts with the helicase eIF4AIII. Interestingly, analysis of eIF4A/eIF4AIII chimaeras led to the identification of an element in eIF4A that is crucial for the interaction with eIF4B or eIF4H. In a short talk, A. Marintchev (Boston, MA, USA) presented structural and functional studies of the eIF4A•4G•4H complex (Marintchev & Wagner, 2005; Oberer *et al*, 2005).

R. Jackson (Cambridge, UK) discussed the issue of reinitiation after translation of a short versus long ORF in mammalian cell extracts. Reinitiation after a short ORF required the primary initiation event to use eIF4F (or at least the central domain of eIF4G), indicating that constituents of the primary 43S complex might be retained through translation of the short ORF and promote re-binding of the ternary complex or other factors (Poyry *et al*, 2004). By contrast, reinitiation after a long ORF is rare. One exception is the bicistronic subgenomic region of feline calicivirus. The binding of eIF3 to the distal 87 nucleotides of the upstream pre-VP1 ORF positively correlated with the efficiency of reinitiation at the downstream ORF (Poyry *et al*, 2007). It will be interesting to determine whether this eIF3 dependence is related to the function of the factor in 40S recruitment or its role in promoting ribosome recycling (Pisarev *et al*, 2007).

Translational control and disease models

Translational control in eukaryotes is centred on the eIF2-dependent binding of Met-tRNA_i^{Met} to the 40S subunit and the eIF4F-dependent binding of the 43S complex to an mRNA. Phosphorylation of Ser 51 on eIF2 α impairs the function of the guanine-nucleotide exchange factor (GEF) and eIF2B, and blocks the conversion of inactive eIF2•GDP to functional eIF2•GTP. T. Dever (Bethesda, MD, USA) reported on the structural basis for substrate recognition by the antiviral eIF2 α kinase, PERK (Dar *et al*, 2005; Dey *et al*, 2005). Interestingly, binding of the kinase apparently induces a conformational change in eIF2 α to expose Ser51 to the kinase active site. Identification of PERK mutants that were resistant to inhibition by a poxvirus pseudosubstrate revealed an overlap with kinase residues that had been under positive selection during PERK evolution, lending support to the idea that poxviruses have had an important role in driving PERK evolution.

D. Ron (New York, NY, USA) showed that eIF2 α phosphorylation is also important in regulating intermediary metabolism in the

Glossary

ASH1	asymmetric synthesis of HO1
ATF4	activating transcription factor 4
C/EBP	CCAAT/enhancer-binding protein
DCP	decapping protein
eIF1	eukaryotic initiation factor
eRF	eukaryotic release factor
GADD34	growth arrest and DNA-damage-inducible protein 34
GCN	general control non-derepressible
GW182	glycine–tryptophan protein of 182 kDa
HCV	hepatitis C virus
HSV-1	herpes simplex virus 1
IRF7	interferon-regulatory factor 7
MLN51	metastatic lymph node 51
mTOR	mammalian target of rapamycin
PABP	poly(A)-binding protein
PERK	protein kinase R-like endoplasmic reticulum kinase
PKR	protein kinase R
PP1	phosphatase 1
S6K1	S6 kinase 1
SE2	splicing factor 2
SKAR	S6K Aly/Ref-like target
UPF	up-frameshift
VP1	major capsid protein of calicivirus
ZBP1	zipcode-binding protein 1

liver of obese mice. Conditional liver-specific expression of the eIF2 α -targeting GADD34 subunit of PP1 resulted in an anti-diabetic phenotype: that is, reduced glycogen and fat levels, susceptibility to hypoglycaemia, enhanced glucose tolerance and diminished hepato-steatosis in animals fed a high-fat diet. Phosphorylation of eIF2 α stimulates translation of hepatic transcription factors, such as C/EBP α and C/EBP β , which might mediate the deleterious effects of nutrient excess. D. Cavener (University Park, PA, USA) reported that tissue-specific knockout of the endoplasmic-reticulum-resident eIF2 α kinase, double-stranded RNA-dependent PERK, in the exocrine pancreas caused the cells to atrophy without affecting glucose homeostasis. By contrast, endocrine pancreas-specific knockout of PERK resulted in a diabetic phenotype due to a reduced proliferation rate of β -cells (Iida *et al*, 2007; Zhang *et al*, 2006). Therefore, PERK function and eIF2 α phosphorylation are crucial determinants of glucose control in mammals.

The eIF4F-dependent binding of mRNA to the 43S complex is regulated by eIF4E-binding proteins (4E-BPs) that disrupt the eIF4F complex by competing with eIF4G for binding to eIF4E. M. Costa-Mattioli (Montreal, QC, Canada) showed that cells and mice that are devoid of both 4E-BP1 and 4E-BP2 display a marked resistance to virus infections due to increased translation of *IRF7* mRNA and upregulation of type-I-interferon production (Colina *et al*, 2008). These findings are consistent with the idea that mRNAs burdened with secondary structure near the cap require greater amounts of eIF4F and its associated helicase activity for their translation. I. Mohr (New York, NY, USA) further demonstrated the importance of eIF4F-complex formation by describing the various strategies that viruses use to enhance eIF4F activity. Whereas HSV-1 encodes a chaperone that promotes eIF4G association with eIF4E, cytomegalovirus apparently drives eIF4F assembly by markedly increasing the levels of eIF4E, eIF4G and the poly(A)-binding protein (PABP), which

Fig 1 | Simplified model of translation initiation. In the canonical cap-dependent initiation pathway in eukaryotes (reviewed in Hershey & Merrick, 2000), the eIF2•GTP•methionyl-initiator transfer RNA ($\text{Met-tRNA}_i^{\text{Met}}$) ternary complex (TC) precedes mRNA in binding to the 40S ribosomal subunit. A latch (blue) between the head and body of the vacant 40S subunit blocks mRNA binding. Binding of eIF1 and eIF1A opens the latch and creates a new connection (denoted in grey and marked by an asterisk). Subsequent binding of the eIF2•GTP• $\text{Met-tRNA}_i^{\text{Met}}$ TC (GTP is denoted by a green circle), eIF5 and eIF3 generates the 43S pre-initiation complex. For simplicity, only the eIF3j subunit, which binds near the A-site of the 40S ribosome, is depicted. Secondary structure in the mRNA prevents ribosome association; the helicase eIF4A unwinds the structure and facilitates ribosome binding in conjunction with eIF4B/eIF4H, eIF4E, eIF4G and PABP. The 43S complex binds to the mRNA near the cap and scans in a 3' direction. Recognition of the AUG start codon through base pairing with $\text{tRNA}_i^{\text{Met}}$ triggers eIF1 displacement and release of Pi from eIF2•GDP (GDP is denoted by a red circle; Algire *et al*, 2005; Unbehaun *et al*, 2004). Subsequent release of eIF5, eIF2•GDP and eIF3, and association of eIF5B•GTP through, in part, the carboxyl terminus of eIF1A, promotes 60S subunit association. Formation of the 80S ribosome triggers GTP hydrolysis and release of eIF5B and eIF1A, and the ribosome proceeds to translation elongation (Acker *et al*, 2006; Fringer *et al*, 2007; Unbehaun *et al*, 2004). eIF2, eukaryotic initiation factor 2; n, 40S neck; mRNA, messenger RNA; PABP, poly(A)-binding protein; Pi, inorganic phosphate; pt, platform.

directly bind to eIF4G. By contrast, poxvirus infection causes the destruction of 4E-BP, and the sequestration and concentration of eIF4F components near discrete cytosolic centres of viral replication (Walsh *et al*, 2008). R. Schneider (New York, NY, USA) reported that eIF4G is overexpressed in inflammatory breast cancer, which led him to assess the impact of depleting eIF4G in cells. Surprisingly, small-interfering RNA (siRNA) targeting the eIF4GI isoform had little impact on general protein synthesis; however, importantly, subsets of mRNAs containing many upstream ORFs or of low abundance were excluded from polysomes (Ramirez-Valle *et al*, 2008). These findings further support the idea that, based on their structural features, mRNAs require different levels of eIF4F for their translation. G. Wagner (Boston, MA, USA) described the identification of small molecules that inhibit formation of either the eIF4E•eIF4G or the eIF4A•eIF4G complexes. Interestingly, the eIF4E•eIF4G complex inhibitor, 4EGI-1 (Moerke *et al*, 2007), binds to eIF4E but does not

disrupt the binding of 4E-BP1; instead, it stabilizes the eIF4E•4E-BP complex. As 4EGI-1 inhibits the proliferation of some cancer cells, these findings raise the possibility that eIF4F assembly might be a promising target for cancer and antiviral drugs.

mRNA localization and translational control in development

The localization of mRNAs to achieve concentrations of specific proteins in different regions of a cell seems to be a common regulatory mechanism in development and neuronal signalling. For this localization to be effective, translation should be suppressed until after the mRNA is transported to its final destination. In his keynote address, R. Singer (Bronx, NY, USA) presented two cases in which 60S-subunit joining is inhibited until the mRNA is transported. In mammalian fibroblasts and neurons, binding of the protein ZBP1 to elements in the 3' untranslated region (UTR) of the β -actin mRNA inhibits translation during sorting of the mRNA. Localized

phosphorylation of ZBP1 by SRC kinase relieved this translational repression (Huttelmaier *et al*, 2005). In an analogous fashion, binding of Puf6 to the 3' UTR of the yeast *ASH1* mRNA represses translation and promotes localization of the mRNA to the daughter bud. Interestingly, the association of Puf6 with the subunit-joining factor eIF5B was required for proper regulation of *ASH1* mRNA *in vivo*, and phosphorylation of Puf6 by casein kinase II in the daughter cell relieved the translational inhibition (Deng *et al*, 2008).

Several talks reported on the identification of mRNA targets of transport factors. J. Casolari (Stanford, CA, USA) reported on his systematic screen of mRNAs associated with the cytoskeletal motor proteins in yeast. Interestingly, only a subset of the actin-based motors, and none of the microtubule motor proteins, was associated with mRNAs. J. Darnell (New York, NY, USA) described a crosslinking and immunoprecipitation methodology (CLIP) that she has been using to identify polysome-associated *in vivo* mRNA targets of the fragile X mental retardation protein (FMRP). J. Carson (Farmington, CT, USA) reported that, in neurons, several mRNAs are combined into granules for transport along microtubules to the synapse where individual mRNAs are then translated (Gao *et al*, 2008).

The cytoplasmic polyadenylation element-binding protein (CPEB) was initially found to govern cytoplasmic polyadenylation of mRNAs and to have an essential role in mitotic cycling in *Xenopus* egg extracts. So, it was a surprise when CPEB-knockout mice were found to be viable. J. Richter (Worcester, MA, USA) reported that mouse embryonic fibroblasts (MEFs) lacking CPEB bypass senescence, at least in part, through derepression of c-Myc expression (Groisman *et al*, 2006). It will be interesting to learn whether the c-Myc mRNA is a direct target of CPEB, and how loss of CPEB increases c-Myc expression.

Translational control in learning and memory

A crucial requirement for the conversion of labile short-term memory into consolidated long-term memory is new protein synthesis. Similar to memory, activity-dependent modulation of synaptic strength (long-term potentiation; LTP)—a key cellular mechanism by which information is stored—has two temporally distinct phases: early LTP, which depends on the modification of pre-existing proteins; and late LTP (L-LTP), which requires new protein synthesis. If new protein synthesis is the rate-limiting step that is necessary to strengthen existing synaptic connections between neurons, what are the signalling pathways involved in these processes?

The eIF2 α protein regulates two fundamental processes that are essential for the generation of long-lasting memories. Phosphorylation of eIF2 α inhibits *de novo* protein synthesis but it also stimulates the synthesis of a specific protein, ATF4 (also known as CREB2), which is a well-known memory repressor that blocks CREB-mediated gene expression. N. Sonenberg (Montreal, QC, Canada) reported that deletion of the eIF2 α kinase GCN2 or genetic reduction of eIF2 α phosphorylation—both of which are conditions that lead to impaired expression of ATF4—reduced the threshold for induction of LTP, and led to enhanced learning and memory (Costa-Mattioli *et al*, 2005, 2007). By contrast, induction of eIF2 α phosphorylation with a small molecule not only leads to an inhibition of protein synthesis and increased expression of ATF4, but also has the opposite depressant effects on lasting synaptic changes and memory. These data indicate that eIF2 α phosphorylation could represent a type of molecular switch that contributes to enduring long-lasting memories.

E. Klann (New York, NY, USA) underscored the role of the mTOR signalling pathway, which is a known modulator of translation, in long-lasting synaptic plasticity and memory. Mice with genetic deletion of either of the mTOR substrates 4E-BP2 or S6K1—the phosphorylation of which is involved in translation initiation—displayed alterations in L-LTP and impaired long-term contextual fear memory. Moreover, genetic deletions of negative modulators of mTOR resulted in mice with enhanced L-LTP and long-term contextual fear memory, and behaviours reminiscent of autism spectrum disorders. J.-C. Lacaille (Montreal, QC, Canada) reported that knockdown of Staufen1—an RNA-binding protein that functions in mRNA transport, localization, translational control and decay—in hippocampal pyramidal cells blocked L-LTP, decreased the frequency and amplitude of spontaneous excitatory synaptic activity, and reverted dendritic spines to phenotypically immature shapes (Lebeau *et al*, 2008).

Coupling of translation to mRNA stability

The surveillance mechanism by which eukaryotic cells remove potentially deleterious mRNAs harbouring a premature termination codon (PTC) is known as nonsense-mediated decay (NMD). Two models of NMD have been proposed. In the first model, which has been best studied in yeast, aberrant termination—defined as the failure of ribosomes to terminate near the normal 3' UTR and poly(A) tail—triggers NMD. Consistently, recruitment of PABP to the PTC blocks NMD (Amrani *et al*, 2006). In the second model, which has been best studied in mammals, the recognition of proteins (such as an EJC) deposited downstream from the PTC targets an mRNA for NMD. In normal mRNAs, these factors are displaced by the scanning ribosome during a pioneer round of translation, whereas their retention in aberrant mRNAs precipitates degradation (Isken & Maquat, 2007). At least three core factors, UPF1, UPF2 and UPF3, are required to execute NMD. UPF1 interacts with translation-release factors eRF1 and eRF3; in addition, UPF1 triggers decapping and subsequent mRNA decay through recruitment of DCP1 and/or DCP2.

Studying mammalian NMD, L. Maquat (Rochester, NY, USA) showed that UPF1 phosphorylation suppresses translation initiation through binding to eIF3. This interaction prevents the eIF3-dependent formation of 80S•mRNA initiation complexes, thereby promoting the degradation of PTC-containing mRNAs (Isken *et al*, 2008). She also reported that increased cellular levels of the splicing factor SF2/ASF shifted NMD of the exported mRNA from the cytoplasm to a nuclear-associated form, and proposed that the pioneer round of translation governs the efficiency of subsequent rounds (Sato *et al*, 2008). In a similar vein, J. Blenis (Boston, MA, USA) described a new function of the mTOR pathway in regulating the translation of spliced mRNAs by way of the EJC. Activated mTOR substrate p70-S6K is recruited to the EJC of newly synthesized mRNAs by SKAR during the pioneer round of translation. This leads to the enhanced translation of intron-containing mRNAs (Ma *et al*, 2008). A. Jacobson (Worcester, MA, USA) reported that ~50% of the Upf1-associated mRNAs in yeast might not be NMD substrates (as defined by twofold increases in NMD-deficient cells); he proposed that these mRNAs might rely on the translation-termination function of Upf1 (Johansson *et al*, 2007). Consistently, depletion of Upf1 in yeast led to defective reuse of ribosomes after termination. O. Muhlemann (Bern, Switzerland) reported that extending the 3' UTR in human cells destabilized an mRNA in a manner that was dependent on UPF1 and could be rescued by recruitment of PABP close to the termination codon (Eberle

et al, 2008). These studies imply that the aberrant termination, or 'faux UTR', model of NMD is relevant in human cells.

Mechanisms of microRNA-mediated gene silencing

MicroRNAs are a family of small RNAs (~21 nucleotides in length) that regulate gene expression post-transcriptionally in different phyla. The molecular mechanism by which miRNAs silence the expression of their target mRNAs remains controversial. Several mechanisms have been reported, namely inhibition of translation initiation, inhibition of translation elongation, ribosome drop-off, co-translational protein degradation and mRNA degradation.

To investigate how miRNAs repress gene expression, M. Hentze (Heidelberg, Germany) used a cell-free translation system from *Drosophila melanogaster* embryos. In his keynote address, he demonstrated that miR2 represses the translation, but does not affect the mRNA stability, of a reporter mRNA containing six miR2-binding sites. Sucrose-gradient analysis showed that miR2 not only inhibits formation of the 80S ribosome complex, but also induces the formation of dense micro-ribonucleoproteins, which have been called 'pseudopolysomes', despite the apparent absence of ribosomes. Consistent with the idea that miRNAs inhibit translation initiation through the 5' cap (m⁷G) structure, an uncapped mRNA bearing an ApppG 5'-end (in place of m⁷GpppG) was resistant to miR2-mediated translational repression (Thermann & Hentze, 2007). E. Izaurralde (Tuebingen, Germany) revisited the miRNA-mediated translational silencing mechanism and challenged current models. Interestingly, she reported that in *Drosophila* S2 cells, it is not the interaction between the Argonaute 1 (Ago1) protein and the mRNA cap that is essential for miRNA-mediated translational repression or mRNA degradation, but rather the interaction between Ago1 and the P-body component, GW182. She further showed that miRNA-mediated repression of translation is eIF6-independent (Eulalio *et al*, 2008). J. Belasco (New York, NY, USA) described the differences among the four mammalian Ago proteins in terms of their influence on translation and on the specificity of RNA interference. Additionally, P. Sarnow (Stanford, CA, USA) reported the liver-specific positive regulation of HCV replication by miR-122. Interestingly, depletion of certain P-body markers resulted in a loss of HCV proteins. Furthermore, infection of liver cells with infectious HCV resulted in the loss of P bodies. It will be interesting to determine whether HCV gene expression is modulated by the release of general miRNA-mediated translational repression, the absence of P bodies, or the lack of certain P-body marker proteins.

Concluding remarks

By bringing together a broad range of topics, such as ribosome structure, mRNA localization and stability, and miRNA function, as well as the roles of translation in learning and memory, diabetes, obesity and virus infections, this symposium provided a comprehensive and up-to-date compendium of the translational control field. The meeting highlighted the considerable progress that has been made in deciphering the translational regulatory mechanisms governing a range of biological processes, and it is clear that new and exciting findings will continue to emerge as we delve deeper into the mechanism and control of protein synthesis.

ACKNOWLEDGEMENTS

We are grateful to J. Richter, A. Hinnebusch and N. Sonenberg for organizing this highly stimulating meeting, and we apologize to those whose data could not be discussed here owing to space limitations.

REFERENCES

- Acker MG, Shin BS, Dever TE, Lorsch JR (2006) Interaction between eukaryotic initiation factors 1A and 5B is required for efficient ribosomal subunit joining. *J Biol Chem* **281**: 8469–8475
- Algire MA, Maag D, Lorsch JR (2005) Pi release from eIF2, not GTP hydrolysis, is the step controlled by start-site selection during eukaryotic translation initiation. *Mol Cell* **20**: 251–262
- Amrani N, Sachs MS, Jacobson A (2006) Early nonsense: mRNA decay solves a translational problem. *Nat Rev Mol Cell Biol* **7**: 415–425
- Colina R *et al* (2008) Translational control of the innate immune response through IRF-7. *Nature* **452**: 323–328
- Costa-Mattioli M *et al* (2005) Translational control of hippocampal synaptic plasticity and memory by the eIF2 α kinase GCN2. *Nature* **436**: 1166–1173
- Costa-Mattioli M *et al* (2007) eIF2 α phosphorylation bidirectionally regulates the switch from short- to long-term synaptic plasticity and memory. *Cell* **129**: 195–206
- Dar AC, Dever TE, Sicheri F (2005) Higher-order substrate recognition of eIF2 α by the RNA-dependent protein kinase PKR. *Cell* **122**: 887–900
- Deng Y, Singer RH, Gu W (2008) Translation of ASH1 mRNA is repressed by Puf6p-Fun12p/eIF5B interaction and released by CK2 phosphorylation. *Genes Dev* **22**: 1037–1050
- Dey M, Cao C, Dar AC, Tamura T, Ozato K, Sicheri F, Dever TE (2005) Mechanistic link between PKR dimerization, autophosphorylation, and eIF2 α substrate recognition. *Cell* **122**: 901–913
- Eberle AB, Stalder L, Mathys H, Zamudio Orozco R, Muhlemann O (2008) Posttranscriptional gene regulation by spatial rearrangement of the 3' untranslated region. *PLoS Biol* **6**: e92
- Eulalio A, Huntzinger E, Izaurralde E (2008) GW182 interaction with Argonaute is essential for miRNA-mediated translational repression and mRNA decay. *Nat Struct Mol Biol* **15**: 346–353
- Fekete CA, Mitchell SF, Cherkasova VA, Applefield D, Algire MA, Maag D, Saini AK, Lorsch JR, Hinnebusch AG (2007) N- and C-terminal residues of eIF1A have opposing effects on the fidelity of start codon selection. *EMBO J* **26**: 1602–1614
- Fraser CS, Berry KE, Hershey JW, Doudna JA (2007) eIF3j is located in the decoding center of the human 40S ribosomal subunit. *Mol Cell* **26**: 811–819
- Fringer JM, Acker MG, Fekete CA, Lorsch JR, Dever TE (2007) Coupled release of eukaryotic translation initiation factors 5B and 1A from 80S ribosomes following subunit joining. *Mol Cell Biol* **27**: 2384–2397
- Gao Y, Tataarty V, Korza G, Levin MK, Carson JH (2008) Multiplexed dendritic targeting of α CaMKII, NG and ARC RNAs by the A2 pathway. *Mol Biol Cell* **19**: 2311–2327
- Groisman I, Ivshina M, Marin V, Kennedy NJ, Davis RJ, Richter JD (2006) Control of cellular senescence by CPEB. *Genes Dev* **20**: 2701–2712
- Hershey JWB, Merrick WC (2000) Pathway and mechanism of initiation of protein synthesis. In *Translational Control of Gene Expression*, Sonenberg N, Hershey JWB, Mathews MB (eds), pp 33–88. Cold Spring Harbor, USA: Cold Spring Harbor Laboratory Press
- Huttemaier S, Zenklusen D, Lederer M, Dictenberg J, Lorenz M, Meng X, Bassell GJ, Condeelis J, Singer RH (2005) Spatial regulation of β -actin translation by Src-dependent phosphorylation of ZBP1. *Nature* **438**: 512–515
- Iida K, Li Y, McGrath BC, Frank A, Cavener DR (2007) PERK eIF2 α kinase is required to regulate the viability of the exocrine pancreas in mice. *BMC Cell Biol* **8**: 38
- Isken O, Maquat LE (2007) Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function. *Genes Dev* **21**: 1833–1856
- Isken O, Kim YK, Hosoda N, Mayeur GL, Hershey JW, Maquat LE (2008) Upf1 phosphorylation triggers translational repression during nonsense-mediated mRNA decay. *Cell* **133**: 314–327
- Johansson MJ, He F, Spatrick P, Li C, Jacobson A (2007) Association of yeast Upf1p with direct substrates of the NMD pathway. *Proc Natl Acad Sci USA* **104**: 20872–20877
- Lebeau G, Maher-Laporte M, Topolnik L, Laurent CE, Sossin W, Desgroseillers L, Lacaille JC (2008) Staufen1 regulation of protein synthesis-dependent long-term potentiation and synaptic function in hippocampal pyramidal cells. *Mol Cell Biol* **28**: 2896–2907
- Ma XM, Yoon SO, Richardson CJ, Julich K, Blenis J (2008) SKAR links pre-mRNA splicing to mTOR/S6K1-mediated enhanced translation efficiency of spliced mRNAs. *Cell* **133**: 303–313
- Marintchev A, Wagner G (2005) eIF4G and CBP80 share a common origin and similar domain organization: implications for the structure and function of eIF4G. *Biochemistry* **44**: 12265–12272

- Moerke NJ *et al* (2007) Small-molecule inhibition of the interaction between the translation initiation factors eIF4E and eIF4G. *Cell* **128**: 257–267
- Oberer M, Marintchev A, Wagner G (2005) Structural basis for the enhancement of eIF4A helicase activity by eIF4G. *Genes Dev* **19**: 2212–2223
- Passmore LA, Schmeing TM, Maag D, Applefield DJ, Acker MG, Algire MA, Lorsch JR, Ramakrishnan V (2007) The eukaryotic translation initiation factors eIF1 and eIF1A induce an open conformation of the 40S ribosome. *Mol Cell* **26**: 41–50
- Pisarev AV, Hellen CU, Pestova TV (2007) Recycling of eukaryotic posttermination ribosomal complexes. *Cell* **131**: 286–299
- Poyry TA, Kaminski A, Jackson RJ (2004) What determines whether mammalian ribosomes resume scanning after translation of a short upstream open reading frame? *Genes Dev* **18**: 62–75
- Poyry TA, Kaminski A, Connell EJ, Fraser CS, Jackson RJ (2007) The mechanism of an exceptional case of reinitiation after translation of a long ORF reveals why such events do not generally occur in mammalian mRNA translation. *Genes Dev* **21**: 3149–3162
- Ramirez-Valle F, Braunstein S, Zavadi J, Formenti SC, Schneider RJ (2008) eIF4GI links nutrient sensing by mTOR to cell proliferation and inhibition of autophagy. *J Cell Biol* **181**: 293–307
- Sato H, Hosoda N, Maquat LE (2008) Efficiency of the pioneer round of translation affects the cellular site of nonsense-mediated mRNA decay. *Mol Cell* **29**: 255–262
- Thermann R, Hentze MW (2007) *Drosophila* miR2 induces pseudo-polysomes and inhibits translation initiation. *Nature* **447**: 875–878
- Unbehauen A, Borukhov SI, Hellen CU, Pestova TV (2004) Release of initiation factors from 48S complexes during ribosomal subunit joining and the link between establishment of codon–anticodon base-pairing and hydrolysis of eIF2-bound GTP. *Genes Dev* **18**: 3078–3093
- Walsh D, Arias C, Perez C, Halladin D, Escandon M, Ueda T, Watanabe-Fukunaga R, Fukunaga R, Mohr I (2008) Eukaryotic translation initiation factor 4F architectural alterations accompany translation initiation factor redistribution in poxvirus-infected cells. *Mol Cell Biol* **28**: 2648–2658
- Zhang W, Feng D, Li Y, Iida K, McGrath B, Cavener DR (2006) PERK EIF2AK3 control of pancreatic beta cell differentiation and proliferation is required for postnatal glucose homeostasis. *Cell Metab* **4**: 491–497

Mauro Costa-Mattioli

Michael Bidinosti

Thomas E. Dever