Protein synthesis and translational control: at eye level with the ribosome

Kathrin Leppek*, Johanna Schott* & Georg Stoecklin

At the EMBO Conference on 'Protein Synthesis and Translational Control' held in Heidelberg in September 2011, scientists shared their latest findings on the structure and function of the ribosome, mRNA-specific regulation of translation and the numerous quality control mechanisms that ensure accurate protein synthesis.

At its heart, understanding protein synthesis means understanding the ribosome. From the amazing structural work of the past decade—for which Venkatraman Ramakrishnan, Thomas Steitz and Ada Yonath were awarded the Nobel Prize in 2009—we learned that the catalytic centre of the bacterial ribosome is formed entirely by RNA, reminding us of an ancient world that relied extensively on RNA-driven catalysis. Knowing that translational control is much more complex in eukaryotes, we are finally gaining a clear view of the eukaryotic ribosome as well. Having solved the first crystal structure of the yeast ribosome (Ben-Shem *et al*, 2010), Marat Yusupov (IGBMC, Illkirch, France) now presented its structure at a greatly improved 3.0 Å resolution. Together with the crystal structure of the small ribosomal subunit (40S) of *Tetrahymena* in complex with eukaryotic initiation factor (eIF) 1, presented by Julius Rabl from the Ban laboratory (ETH Zurich, Switzerland; Rabl *et al*, 2011), these data provide, for the first time, a detailed landscape of all RNA and protein domains of the eukaryotic ribosome. While the ribosomal core responsible for mRNA decoding and peptidyl transfer is virtually identical in the bacterial and eukaryotic ribosome, the outer surface differs vastly between the two. The eukaryotic ribosome, for example, contains two proteins with long α -helices that appear to clamp the ribosomal subunits together. These structures will greatly help the community to understand the complex

mechanisms that regulate both global and specific protein synthesis in eukaryotes.

To initiate or not to initiate

Eukaryotic ribosomes decide where to initiate protein synthesis with the help of several eIFs. Once loaded with the initiator methionine tRNA in a ternary complex with GTP-bound eIF2, the ribosomal 40S subunit scans the mRNA from the 5' end for an AUG codon in a favourable sequence context. Scanning is assisted by the RNA helicase eIF4A, which unwinds secondary structures. While eIF1 stabilizes the open, scanning-competent conformation of the ribosome, its antagonist eIF5 locks the ribosome in its closed conformation and thereby promotes start codon selection. By analysing eIF1, eIF1A and eIF2b yeast mutants with increased or decreased rates of initiation at near-cognate UUG start codons, Pilar Martin-Marcos from the Hinnebusch laboratory (NIH/NICHD, Bethesda, USA) found that these factors also discriminate against start codons in an unfavourable context. The data indicate that start codon and context are recognized as a unit by the same factors. Since eIF1 has a poor AUG context in its own mRNA, this also serves as an autoregulatory mechanism by which high levels of eIF1 suppress eIF1 mRNA translation (Martin-Marcos *et al*, 2011). In human cells, Iyaylo Ivanov from the Atkins laboratory (University College Cork, Ireland) could expand the autoregulation of eIF1 to a homeostatic network of cross-regulation with its antagonist eIF5. Since the eIF5 mRNA harbours inhibitory upstream (u)ORFs with start codons in a poor context,

high levels of eIF1 promote translation of the eIF5 main ORF by impeding translation of the eIF5 uORFs. When eIF5 levels are high, the ribosome is less selective, which stimulates translation of eIF1 and inhibits translation of the eIF5 main ORF. This mechanism elegantly adjusts eIF1 and eIF5 expression levels to each other.

As start codon selection requires scanning on most mRNAs, scientists at the meeting were surprised to hear that eukaryotic ribosomes are able to recognize AUGs on mRNAs that have 5'UTRs too short for proper scanning. Rivka Dikstein (Weizmann Institute, Rehovot, Israel) identified a regulatory element known as translation initiator of short 5'UTR (TISU), present in up to 5% of all mRNAs, which allows initiation at AUGs positioned as close as 5 nucleotides to the 5' cap (Fig 1A). Nucleotides downstream from the TISU start codon compensate for the missing upstream contacts between the ribosome and the mRNA. Interestingly, at the DNA level, the TISU sequence serves as a promoter element that enhances transcription of TISU-containing mRNAs. Since TISU-mediated translation does not depend on eIF1 or eIF4A, this mechanism might ensure translation of several proteins involved in basic cellular functions when scanning is impeded due to low levels of eIF1 or eIF4A (Elfakess *et al*, 2011).

While cap-dependent translation in eukaryotes is complicated and requires a set of eIFs, certain mRNAs take shortcuts for initiation. Internal ribosomal entry sites (IRESs) are often used by viruses to 'hijack' the host's translation machinery, and there are examples of cellular mRNAs harbouring IRESs.

*Kathrin Leppek and Johanna Schott contributed equally to the Meeting Point.

Fig 1 | Novel ways to control translation. (A) A regulatory element termed translation initiator of short 5'UTR (TISU) enables translation of mRNAs with ultra-short 5'UTRs. TISU, as a promoter element, also enhances transcription of the corresponding genes in the nucleus. (B) IRES-mediated translation of FGF1 mRNA is coupled to transcription. hnRNPM and p54nrb are recruited to the FGF1 promoter, associate with the IRES in the FGF1 mRNA and promote its translation in the cytoplasm. (C) Ribosome release after stalling requires cleavage of the mRNA and the Dom34–Hbs1–Rli1 complex. For details please refer to the main text. FGF1, fibroblast growth factor 1; IRES, internal ribosomal entry site.

During myoblast differentiation, the synthesis of fibroblast growth factor 1 (FGF1) depends on an IRES in the 5'UTR of its mRNA. Anne-Catherine Prats (University of Toulouse, France) found that two proteins, hnRNPM and p54nrb, are required not only for IRES-dependent translation of FGF1, but also for transcription of the FGF1 mRNA (Fig 1B). The two proteins associate with each other on the FGF1 promoter in the nucleus, are loaded co-transcriptionally onto the pre-mRNA and remain bound to the mRNA to drive its translation in the cytoplasm. FGF1 thus provides the first example of direct coupling between transcription and IRES activity.

Despite elaborate control mechanisms that ensure accurate start codon selection, ribosomes do not always stick to the text book. Laura Ranum (University of Florida, USA) presented unexpected findings on CAG•CTG trinucleotide expansions, which are the cause of many hereditary neurological diseases. In neurons of affected patients, the aggregation of proteins with extended glutamine stretches, encoded by the CAG repeats, is thought to contribute to the pathological process. Ranum has shown that long CAG repeats cause the ribosome to go wild. Independently of AUGs, CAG repeats induce translation initiation and elongation in all three reading frames, regardless of whether the repeats are located in the coding or untranslated region. This gives rise to a mixture of protein species with poly-glutamine, poly-alanine and poly-serine

stretches (Zu *et al*, 2011). Thus, it turns out that what we thought were poly-glutamine or non-coding RNA diseases might additionally result from a toxic cocktail of mutant homopolymeric expansion proteins.

Termination is not the end

If all things must come to an end—and incarnate—why should the ribosome not do the same? Translation terminates when a stop codon enters the aminoacyl (A)-site in the ribosome, where it is recognized by the eukaryotic release factor (eRF) 1. With help from the GTPase eRF3, eRF1 then promotes cleavage of the tRNA from the polypeptide and triggers its release. Leos Valasek (Institute of Microbiology, AS CR, Prague, Czech Republic) argued that the multi-protein complex eIF3 provides a molecular bridge connecting initiation with termination. Not only is eIF3 critical for the assembly and function of pre-initiation complexes, it also remains temporarily bound to elongating 80S ribosomes to promote re-initiation at the main ORF after translation of a short uORF (Muzarová *et al*, 2011). Valasek reported that eIF3 might even play a direct role in translation termination by describing mutations in eIF3 subunits that specifically affect the rate of stop codon read-through. In support of these genetic data, eIF3 subunits were found to interact with eRFs *in vivo* and *in vitro*. It is intriguing to speculate that this new 'terminator' role of eIF3 is connected with its proposed function in the full recycling of

post-termination ribosomes to allow their 'rebirth' on the same mRNA, initiating the next translation cycle.

During translation elongation, ribosomes are thought to occasionally stall, for example when they encounter strong secondary structures within the mRNA or run into long stretches of rare codons. Stalling is recognized by an intricate surveillance mechanism that causes release of the ribosome on the one hand, and cleavage of the mRNA on the other. The recycling of stalled ribosomes requires three factors: Dom34, a homologue of eRF1; Hbs1, a GTPase with close homology to eRF3; and Rli1, an ATPase required for ribosome recycling both after regular termination and stalling (Fig 1C). Christopher Shoemaker from the Green laboratory (Johns Hopkins University, Baltimore, USA) showed that ribosomes are most effectively recycled if Hbs1 identifies ribosomes with a truncated mRNA 3' end, suggesting that mRNA cleavage has to occur before the ribosomal subunits dissociate. Structural data from Thomas Becker in the Beckmann laboratory (LMU Munich, Germany) support this idea, as Hbs1 is positioned at the site where the mRNA enters the ribosome. Based on cryoEM structures, Becker also argued that Rli1 acts, together with Dom34, as a wedge to separate the ribosomal subunits. Stalling thus causes an unusual termination event that occurs in the absence of a stop codon, yet requires Dom34 and Hbs1 as alternative RFs that are recruited by cleavage of the mRNA.

As much as ribosomes have learned to cope with mRNA-internal obstacles by clipping the RNA, tRNAs also need to undergo quality control to avoid errors in translation. Jeremy Wilusz from the Sharp laboratory (MIT, Cambridge, USA) revealed an entirely unexpected mechanism by which defective tRNAs are degraded. Normally, the CCA adding enzyme adds CCA to the 3' end of every tRNA as an essential step in its maturation. Wilusz reported that the same enzyme adds a second CCA to structurally unstable tRNAs that contain mutations or lack key nucleotide modifications. The CCACCA sequence then marks the defective tRNA for degradation by the exosome, thereby efficiently eliminating it from the pool of circulating tRNAs (Wilusz *et al*, 2011).

Ribosome profiling—a new goldmine

For decades, the translation field lacked a technique to monitor the synthesis of proteins on a genome-wide level. This changed 2 years ago when the Jonathan Weissman laboratory (UC San Francisco, USA) developed ribosome profiling, the deep sequencing of ribosome-protected mRNA fragments by which the ribosomes' footprints can be read with single-codon resolution. What we have already learned from this technique is mind-boggling: not only do ribosomes in yeast start slowly and then speed up during elongation, but they also use non-AUG start codons far more frequently than anybody thought, especially at uORFs (Ingolia *et al*, 2009). This meeting witnessed an impressive array of new applications of ribosome profiling to address fundamental questions about gene expression.

Gloria Brar from the Weissman laboratory used ribosome profiling to follow genome-wide translation during yeast meiosis, where gene expression underlies tight temporal control. Brar reported that nearly half of all mRNAs show a change in translation efficiency of more than threefold during the yeast meiotic programme, while dramatic changes of more than tenfold are observed with some 200 mRNAs. There is also an increase in non-canonical translation, with 30% of all meiotic ribosome footprints mapping to novel short ORFs and uORFs. Many uORFs initiate at non-AUG start codons and their ribosome occupancy is associated with enhanced translation of their downstream ORF. Extended 5'UTRs were also identified on many mRNAs, often including novel AUG uORFs that show a competitive relationship with their downstream ORF. It

will be interesting to probe further the precise mechanistic role of AUG and non-AUG uORFs in translational control.

Ariel Bazzini from the Giraldez laboratory (Yale University, USA) applied ribosome profiling to address a hotly debated question: What is the order of events once a microRNA (miRNA) has bound to its target mRNA? Previous steady-state analysis indicated that miRNAs predominantly act by degrading rather than translationally repressing mRNAs (Guo *et al*, 2010). Bazzini took up the issue in the zebrafish embryo, where only a single miRNA, miR-430, is expressed 3–24 h post-fertilization. Comparing the wild type with Dicer mutants, Bazzini showed that target mRNAs of miR-430 are repressed translationally at 4 h post-fertilization, whereas the levels of these mRNAs declined only by 6 h. In zebrafish, it thus appears that the same miRNA first induces translational repression before causing mRNA degradation. In support of this hypothesis, Sergej Djuranovic from the Green laboratory provided evidence for temporal separation of miRNA activity in somatic *Drosophila* cells—observing repression of translation initiation prior to mRNA decay. Future work will have to establish the molecular mechanism that underlies the timing of these two sequential events.

Sick from unbalanced translation

Given that important transitions in life, such as meiosis and early vertebrate development, rely on extensive translational control, it is not surprising that deregulated translation can cause severe diseases. Robert Schneider (New York University, USA) showed that elevated levels of eIF4G1 play a key role in aggressive forms of breast cancer. Importantly, eIF4G1 enhances translation of a specific group of mRNAs, many of which are required for the DNA damage response. By silencing eIF4G1 in breast cancer xenografts, Schneider could show that eIF4G1 overexpression renders these tumours resistant to chemotherapy or ionizing irradiation. Hence, eIF4G1 is not only a general translation factor, it also proves to be of particular importance for the translation of a specific subset of mRNAs. A similar situation occurs in Diamond–Blackfan anaemia, an inherited disease caused by mutations in different ribosomal proteins. Marieke von Lindern (Sanquin Research, Amsterdam, The Netherlands) presented evidence for two different groups of mRNAs whose translation is either elevated or repressed in Blackfan anaemia cells (Horos *et al*, 2011). Reduced

translation is observed, for example, with Bag1 and Csde1, both of which von Lindern could show are essential for the differentiation of erythroid precursor cells into mature erythrocytes. Inflammatory breast cancer and Diamond–Blackfan anaemia thus provide two compelling examples where the alteration of general translation factors causes aberrant expression of specific proteins.

Summary

This meeting challenged some of the traditional ideas about the initiation and termination phase of translation, as exemplified by ribosomes that initiate at non-AUG start codons to an unexpected extent. We are changing our views on canonical translation factors and ribosomal proteins, many of which control specific subgroups of mRNAs. Yet the field is far away from understanding all the hidden dimensions of translational control including target and tissue specificity. Structural insights into the eukaryotic ribosome and its interactions with translation factors, together with advanced techniques addressing the translation of individual mRNAs, will help to get ever closer to the heart of protein synthesis.

ACKNOWLEDGEMENTS

We thank the organizers, Anne Ephrussi, Rachel Green, Matthias Hentze and Jon Lorsch, for putting together this wonderful meeting, and the colleagues who gave us permission to write about their findings. We apologize to all participants whose work we could not mention owing to space limitations.

REFERENCES

- Ben-Shem A *et al* (2010) *Science* **330**: 1203–1209
- Elfakess R *et al* (2011) *Nucleic Acids Res* **39**: 7598–7609
- Guo H *et al* (2010) *Nature* **466**: 835–840
- Horos R *et al* (2011) *Blood* (in the press)
- Ingolia NT *et al* (2009) *Science* **324**: 218–223
- Martin-Marcos P *et al* (2011) *Mol Cell Biol* 19 Sep 2011 [Epub ahead of print] doi:10.1128/MCB.05819-11
- Munzarová V *et al* (2011) *PLoS Genet* **7**: e1002137
- Rabl J *et al* (2011) *Science* **331**: 730–736
- Wilusz JE *et al* (2011) *Science* (in the press)
- Zu T *et al* (2011) *Proc Natl Acad Sci USA* **108**: 260–265

Kathrin Leppek, Johanna Schott and Georg Stoecklin are at the Helmholtz Junior Research Group Posttranscriptional Control of Gene Expression, German Cancer Research Center, DKFZ-ZMBH Alliance, Heidelberg 69120, Germany.
E-mail: g.stoecklin@dkfz.de

Published online 11 November 2011

EMBO reports (2011) **12**, 1214–1216.
doi:10.1038/embor.2011.224