A report on the 2nd EMBO Conference on Protein Synthesis and Translational Control, Heidelberg, Germany, 12-16 September 2007.

The recent EMBO conference on protein synthesis and translational control held at the European Laboratory of Molecular Biology (EMBL) in Heidelberg provided an update on developments in a field that has become a major focus of gene-expression research. A packed audience thoroughly enjoyed contributions on ribosome structure and molecular details of the initiation, elongation and termination steps of translation. Many examples of translational control were presented, showcasing its biological significance and the depth of insight into its mechanisms that is now achievable.

The ribosome and mechanisms of translation

On the structural side, two current challenges are to obtain structures of ribosomes complexed with their ligands and structures at higher resolution. Maria Selmer (Uppsala University, Uppsala, Sweden) presented a crystal structure of the 70S ribosome from \textit{Thermus thermophilus} in complex with mRNA and three tRNAs at 2.8 Å resolution. Christian Spahn (Charité Medical University, Berlin, Germany) reported a cryo-electron microscopy reconstruction of ribosomes from \textit{Escherichia coli} stalled in the decoding state with tRNA and elongation factor Tu at significantly improved resolution (less than 7 Å).

Gene expression can be regulated at the level of initiation of protein biosynthesis via structural elements present in the 5' untranslated region of mRNAs. Bruno Klaholz (Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Strasbourg, France) reported a series of cryo-electron microscopy snapshots of prokaryotic ribosomal complexes directly visualizing either the mRNA structure blocked by repressor protein S15 or the unfolded, active mRNA. A comparative structure and sequence analysis suggests the existence of a universal stand-by site on the ribosome at the 30S platform dedicated for binding regulatory 5' mRNA elements. One of us (M.R.) reported results from rapid kinetics techniques that show that the structure of the mRNA translation initiation region determines the timing of molecular events leading to formation of elongating 70S ribosomes from the 30S initiation complexes, which reveals a second control step in initiation in addition to formation of the 30S initiation complex. X-ray studies reported by Lasse Jenner (IGBMC) showed how mRNA is anchored to the 30S ribosomal subunit during initiation and moves inside the ribosome upon transition from initiation to elongation. These studies suggest that recognition of the translation initiation region of mRNA by the 30S subunit and conversion of this complex into the 70S ribosome constitute important checkpoints of translation initiation. Clearly, understanding the conformational dynamics of ribosomal initiation complexes is very important. To this end, Scott Blanchard (Cornell University New York, USA) presented a study by single-molecule techniques that revealed a dynamic exchange between three metastable configurations of tRNA bound to \textit{E. coli} ribosome, one of which is a previously unidentified hybrid state in which only deacylated tRNA adopts its hybrid (P/E) configuration.

Another frontier is to understand how the active center on the \textit{E. coli} 50S subunit catalyzes the hydrolysis of peptidyl-tRNA during termination of protein synthesis. Rachel Green (Johns Hopkins University, Baltimore, USA) showed that
release factors make two distinct contributions to catalysis of peptidyl-tRNA hydrolysis - a relatively nonspecific activation of the catalytic center and specific selection of water as a nucleophile facilitated by a glutamine in the conserved GGQ motif in release factors. Interestingly, the ribosome-recycling step differs markedly between prokaryotes and eukaryotes. In prokaryotes, recycling requires the action of ribosome-recycling factor together with elongation factor G. In contrast, Tatyana Pestova (State University of New York, New York, USA) has found that in mammals recycling is achieved by a combination of the eukaryotic initiation factors (eIF) 3, 1, and 1A, which explains why a ribosome-recycling factor is not found in eukaryotic cells.

The many factors participating in eukaryotic translation initiation are well characterized in terms of their biochemical activity and their interactions with each other. Less well established are their dynamic roles as initiation progresses through its various sub-steps. Alan Hinnebusch (National Institute of Child Health and Human Development, NIH, Bethesda, USA) characterized novel mutations in eIF1 and eIF1A using genetic screens and biochemical assays in Saccharomyces cerevisiae. One screen assays the altered responses of cells to amino-acid starvation. To overcome starvation the cells depend on activation of the Gcn4 transcription factor by a translational reinitiation mechanism involving the eIF2α kinase Gcn2. Mutants displayed various phenotypes including Gcd- (general control derepressed) in which Gcn4 translation is derepressed independent of eIF2α phosphorylation, Sui- (suppressor of Sui-) and Ssu- (suppressor of Sui). These results indicate that both eIF1 and eIF1A function in the assembly of the translation preinitiation complex (PIC), ribosome scanning and start codon recognition by effecting conformational changes in the PIC. Lori Passmore (MRC Laboratory of Molecular Biology, Cambridge, UK) complemented these findings with cryo-EM based models of yeast 40S subunit complexes that suggested that eIF1 and eIF1A maintain an open, scanning-competent PIC that clamps down on mRNA upon start codon recognition and eIF1 release. Leoš Valašek (Academy of Sciences of the Czech Republic, Prague, Czech Republic) noted a Gcn- (general control non-derepressible) phenotype, which is unable to induce the starvation response, in strains carrying amino-acid starvation regulon (non-derepressible) phenotype, which is unable to induce the starvation response, in strains carrying amino-acid starvation regulon (non-derepressible) phenotype, which is unable to induce the starvation response, in strains carrying amino-acid starvation regulon (non-derepressible) phenotype. He noted that FRMP, in concert with the small neuronal noncoding RNA BC1, may direct the repressor to target mRNAs.

The Sex-lethal (SXL) protein of Drosophila melanogaster is involved in sex determination and dosage compensation. It binds to sites in the 5' and 3' untranslated regions (UTRs) of the mRNA encoding MSL-2, a component of the dosage compensation complex (DCC), and inhibits its translation in females. This well studied paradigm of translational control continues to provide valuable insight into mechanism. SXL bound to the 5' UTR of msl-2 mRNA interferes with ribosomal scanning, and Jan Medenbach (EMBL, Heidelberg, Germany) has used in vitro translation and primer extension analysis (toe-printing) to show that SXL diverts ribosomes to an upstream AUG codon, thus decreasing translation initiation at the main coding region. It is already known that SXL bound to the msl-2 RNA 3' UTR recruits the cold-shock domain protein UNR and inhibits binding of the 43S PIC at the 5' end of the mRNA. Solenn Fatalano (Centre for Genomic Regulation, Barcelona, Spain) reported that the amino-terminal half of UNR carries most of the repressor function. She also described genome-wide analyses that revealed that UNR binds to mRNAs for other DCC components in females and, furthermore, that UNR is required for DCC maintenance in males, suggesting that it performs opposing sex-specific roles in dosage compensation.

In the eggs of Xenopus laevis, the translational awakening of stored maternal mRNAs is controlled by the cytoplasmic polyadenylation element-binding protein (CPEB). During X. laevis oocyte maturation and early embryonic divisions, recruitment of the poly(A) polymerase GLD-2 to the 3' UTRs of mRNAs leads to their polyadenylation and translation. Perrine Benoit (Institute of Human Genetics, Montpellier, France) described how D. melanogaster GLD-2 also interacts with the Drosophila CPEB homolog Orb and polyadenylates oscar and nanos mRNA during oogenesis. Similarly, in Caenorhabditis elegans, the RNA-binding protein GLD-3 recruits GLD-2 to the gld-1 mRNA. Christian Eckmann (Max Planck Institute of Molecular Cell Biology, Dresden, Germany) described GLD-4, a novel pol(A) polymerase in C. elegans. GLD-4 is enriched in germ-cell-specific P granules, interacts with GLD-3 via a bridging factor GLS-1, and functions in germ-cell differentiation.

Translational control of gene expression

In her keynote lecture, Erin Schuman (California Institute of Technology, Pasadena, USA) outlined methods that combine fluorescent tagging of newly synthesized proteins with photobleaching to visualize translational changes in live neurons, and also described high-throughput technologies to identify locally translated proteins in dendrites. She described how these methods were combined with the local inhibition of elongation factor eEF2 to show control of translation in dendrites by eEF2 phosphorylation. Ilaria Napoli (Università di Roma Tor Vergata, Rome, Italy) has found that the fragile X mental retardation protein (FRMP) interacts with a component of the WAVE actin-polymerization regulatory complex. This WAVE component acts as an eIF4E-binding protein to repress translation, and Napoli suggested that FRMP, in concert with the small neuronal noncoding RNA BC1, may direct the repressor to target mRNAs.
These findings highlight the intricate nature and importance of mRNA polyadenylation control as a means of regulating gene expression in development and elsewhere. The latter point was underscored by Joel Richter (University of Massachusetts, Amherst, USA) who presented new data indicating that CPEB controls mammalian somatic cell senescence. One of us (T.P.) presented genome-wide data on poly(A) tail length and other mRNA characteristics in budding yeast and in fission yeast which indicate that 3' UTR-mediated control of mRNA deadenylation influences cell-cycle progression and other processes.

**MicroRNAs and microarrays**

Despite intense interest in translational control by microRNA (miRNA), there is at present no consensus model for a molecular mechanism(s). In his keynote lecture, Withold Filipowicz (Friedrich Miescher Institute, Basel, Switzerland) championed a general model involving two-steps: inhibition of translation initiation of the mRNA followed by localization to P-bodies for storage or degradation. Together with Nahum Sonenberg’s group, he and his colleagues have recreated translational inhibition by endogenous let-7 miRNA in mouse Krebs-2 ascites extracts, confirming that it affects the cap-recognition step of translation initiation. Rolf Thermann (EMBL) has characterized translational repression by endogenous miR-2 via the reaper mRNA 3' UTR in *D. melanogaster* embryo extracts. His results indicated that miR-2 causes inhibition of cap-dependent initiation and formation of dense, puromycin-insensitive particles. One of us (T.P.) reported that in mammalian cells full repression of translation initiation by synthetic or endogenous let-7 miRNA required both a cap and a poly(A) tail on the target mRNA and featured mRNA deadenylation as the earliest detectable change.

Martin Bushell (University of Nottingham, UK) has found that the promoter on transfected plasmid reporters affects miRNA-mediated translational repression. SV40-driven reporter mRNA bearing target sites for let-7 in its 3' UTR is repressed at the step of translation initiation, whereas virtually identical mRNA derived from the thymidine kinase promoter is repressed by an alternative post-initiation mechanism. Xavier Ding (Friedrich Miescher Institute, Basel, Switzerland) reported an RNA interference (RNAi) screen in *C. elegans* that identified several eIF3 subunits as suppressors of a let-7 mutation, implicating eIF3 as a novel target of miRNA action. Thus, notwithstanding the continuing diversity of experimental results and opinions, a common thread may be that miRNA can repress translation initiation by affecting the function of the cap and that of the cap-binding protein eIF4E, as well as perhaps functions of other initiation factors. Repression is aided by mRNA deadenylation, which might attenuate translation as well as expediting mRNA decay. Viewed this way, the miRNA mechanism appears not too dissimilar to other examples of translational control: despite the considerable diversity of cis-acting RNA elements and trans-acting factors involved in controlling initiation, cap and/or poly(A) tail function are commonly the targets.

Microarrays can be used as tools to study global aspects of posttranscriptional gene regulation and to discover new targets and mediators of such regulation. Gerhard Schratt (University of Heidelberg, Germany) reported on miRNAs involved in dendritic spine morphogenesis. A functional screen identified miR-138, which he showed regulated the mRNA encoding APT1, an enzyme involved in protein palmitoylation. Perturbing this regulation altered the membrane localization of APT1 target proteins and dendritic spine size. Anne Willis (University of Nottingham, UK) and Wendy Gilbert (University of California, Berkeley, USA) both described translation state array (TSA) approaches, combining isolation of polysomal RNA with microarray analyses. Willis identified mRNAs that were selectively polysome-associated in UV-irradiated mammalian cells. These miRNAs commonly encoded proteins required for the DNA-damage response and contained regulatory upstream ORFs in their 5' UTR. Gilbert studied miRNAs required for invasive growth in glucose-starved haploid yeast cells and found they contain internal ribosome entry sites. The miRNAs studied included those coding for P-body components, which is consistent with an increase in P-body size and number in starved cells.

**Translational cross-talk**

There is an increasing appreciation that different steps in eukaryotic gene expression are physically or functionally linked. Susanne Rüther (Gene Center, University of Munich, Germany), reported that the kinase Ctk1 (CDK9), which is involved in transcription elongation, also functions in translation elongation. She suggested that Ctk1 may be loaded onto nascent mRNAs and accompany them to the ribosome where it phosphorylates the 40S subunit protein Rsp2. Similarly, Heike Krebber (Institute of Molecular Biology and Tumor Research, Marburg, Germany) showed that yeast Dhp5, a DEAD box helicase and mRNA export factor, functions in translation termination. Dhp5 interacts with the eukaryotic release factor (eRF) 1, and its helicase activity is important for stop codon recognition and eRF3 recruitment. Discoveries of this kind show that despite their physical separation in eukaryotic cells, translation can receive multiple inputs from earlier steps of mRNA biogenesis.

A paradigm of such cross-talk is the nonsense-mediated decay (NMD) pathway, as pointed out by Elena Conti (Max Planck Institute of Biochemistry, Martinsried, Germany). The centerpiece of her keynote lecture was the crystal structure of the human exon junction complex (consisting of eIF4AIII, Barentsz, Mago, Y14, RNA and ATP) determined by her laboratory. The NMD pathway surveys mRNA during
translation for premature stop codons (PTCs) and in human cells triggers mRNA degradation when an exon-exon junction is present downstream of a PTC. The exon junction complex serves as a critical NMD mark, situated on the mRNA just upstream of the splice junction. Toshifumi Inada (Nagoya University, Nagoya, Japan) described investigations of NMD in yeast, where he discovered that rapid protein degradation by the proteasome contributes significantly to the repression of PTC-containing mRNA. Ana Luisa Silva (National Institute of Health, Lisbon, Portugal) observed that in human cells, mRNAs with PTCs proximal to the start codon escape NMD. She has found that tethering of poly(A)-binding protein (PABP) to a PTC-proximal position on NMD-sensitive mRNA partially blocked NMD, reminiscent of the situation in yeast. Silva suggested that PABP might travel along with the ribosome during early elongation, hindering the recruitment of the NMD factor Upf1 at AUG-proximal PTC. Thus, while NMD is universally conserved in eukaryotes, it continues to surprise with its diverse and intricate implementation strategies in different organisms.

In conclusion, the conference provided ample evidence for the central importance of translation and its regulation within gene-expression research. The traditionally strong appreciation of translational control in developmental biology and neurobiology was reflected in excellent sessions on these topics. MicroRNAs define a new frontier in gene expression and their mechanisms of action as well as their biological roles were focal points of the conference, drawing the attention of veteran translation enthusiasts as well as converts from other research areas. Last but not least, presentations on the interfaces between translational control and mRNA localization or decay highlighted functional links between different steps in the gene-expression pathway. Given the exciting prospects for translational control and RNA research, one can hope that this European conference, held in September of odd-numbered years since 2005 and partnered with the Translational Control Meetings at Cold Spring Harbor, will become a fixture in the international conference calendar.