

Meeting report

Translation controlled

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A report of the meeting 'Translational Control', Cold Spring Harbor, USA, 3-7 September 2008.

More than 400 scientists contributed to this year's highly successful 'Translational control' meeting at the Cold Spring Harbor Laboratory. Translation is a complex and highly regulated multi-step process, fundamental to all forms of life. The meeting covered a diverse range of topics, experimental systems and approaches. Here we report a few of the highlights from the major themes of the meeting, focusing mainly on contributions not published at the time of writing.

Factors and controls in translation and mRNA decay

ATP-dependent RNA helicases are important for many aspects of RNA biology, including translation and mRNA decay. One of the key proposed roles for RNA helicases in eukaryotic translation initiation is to facilitate scanning of the mRNA 5' untranslated region (UTR) by the small ribosomal subunit and associated translation factors. Gerhard Wagner (Harvard Medical School, Boston, USA) used nuclear magnetic resonance (NMR) chemical shift mapping, as well as comparisons to the structurally similar nuclear cap-binding complex, to provide an overview of interactions involved in mRNA cap recognition and 5' UTR scanning. Wagner described how specific domains of eukaryotic initiation factor (eIF) 4G interact with the archetypal ATP-dependent RNA helicase, eIF4A, and the associated factor eIF4H. The data were combined into a model depicting how the eIF4A-eIF4G complex could associate with the ribosomal leading edge while still maintaining interaction with the cap-binding protein eIF4E.

Andrey Pisarev (State University of New York, Brooklyn, USA) presented a classical biochemical purification of an activity that restored translation of structured 5' UTR-containing mRNAs in a reconstituted *in vitro* system. This

protein, DHX29, is a DExH-domain protein with putative ATP-dependent RNA helicase activity. Although little evidence for helicase activity was found, the protein was shown to associate with the small ribosomal subunit and to possess nucleotide triphosphatase (NTPase) activity. DHX29 was also shown to promote efficient recruitment of the small ribosomal subunit to structured 5' UTR mRNAs in a manner that is synergistic with eIF4A, defining DHX29 as a novel factor required for scanning on structured 5' UTRs.

Nancy Standart (University of Cambridge, UK) presented a mutational analysis targeting the helicase domain of the protein DDX6 (also called p54 and Dhh1 in different organisms). DDX6 has been described as a translational repressor (for instance as part of the cytoplasmic polyadenylation element binding (CPEB) repressor complex) and as a component and assembling factor of processing bodies (P-bodies) involved in mRNA decay. Specific mutagenesis of the helicase domain impacted upon both of these functions, as well as leading to changes in the stoichiometry of the CPEB repressor complex.

mRNA decay and translation initiation are complex competing processes. A common perception is that mRNAs must leave the polysomal pool; that is, they must be translationally repressed to interact with enzymes responsible for mRNA decay. Evidence that severe stresses lead to translational repression and induce the accumulation of P-bodies supports this view. Wenqian Hu (Case Western Reserve University, Cleveland, USA) presented evidence that, in unstressed budding yeast, all three steps of mRNA decay (deadenylation, decapping and exonucleolytic digestion) can occur while mRNAs are still associated with the polysome. This raises a number of intriguing questions, such as what happens under stress conditions where P-bodies are induced, and what targets mRNAs for degradation?

In higher eukaryotes the 5' mRNA cap-activated deadenylase PARN enables the targeting of mRNAs for degradation. Here the deadenylase would compete with the

translational machinery for the mRNA 5' end. A molecular model for cap-dependent PARN activation was presented by Anders Virtanen (Uppsala University, Sweden) based upon crystal structures of free PARN, a poly(A)-bound form and a new crystal structure determined by Virtanen and colleagues - PARN with cap analog. In this structure PARN forms a dimer, with one molecule in an open and the other in a closed conformation, in which both cap and poly(A) sites partially overlap. These data suggest a model where one PARN monomer interacts with the cap structure to activate the nuclease domain of the partner PARN molecule, and they provide insight into the cap dependence of the nuclease.

MicroRNAs (miRNAs) have variably been shown to down-regulate both mRNA decay and translation, again highlighting the connection between these pathways. In her plenary presentation, Joan Steitz (Yale University, New Haven, USA) presented studies by Shobha Vasudevan that add further intricacy to miRNA-mediated control of gene expression. First, miRNAs can be stimulatory as well as repressive, and second, the stimulatory phase of their activity may be restricted to quiescent cells. Two presentations highlighted further complexities with regard to the source of miRNAs. Christine Ender (Max Planck Institute of Biochemistry, Munich, Germany) and Ashesh Saraiya (University of California, San Francisco, USA) both showed that, in human cells and *Giardia* parasites, respectively, RNA fragments derived from small nucleolar RNAs control translation initiation in an Argonaute- and Dicer-dependent manner, giving them many of the hallmarks of miRNAs.

Diversification of translation factor activities

Studies presented at the meeting challenge our current view of the functions of several individual factors, in that a number of factors initially defined as being involved in one process might have broader or different roles. Stefano Biffo (San Raffaele Science Institute, Milan, Italy) used heterozygous eIF6-null mouse embryonic fibroblasts to show a defect in insulin-stimulated translation with little change in ribosome biogenesis. Hence, eIF6, which was initially identified as a ribosome subunit anti-association factor and later defined as a factor involved in large ribosomal subunit biogenesis in the nucleus, can once again be tied to the regulation of translation initiation in the cytoplasm.

eIF5A is also a factor with a long history of suggested functions. This factor is particularly intriguing by virtue of a unique, essential, post-translational modification of a lysine residue to hypusine. Preeti Saini (National Institutes of Health, Bethesda, USA) presented studies that extend the list of suggested eIF5A functions by implicating the budding yeast factor in the process of translation elongation. Results were also presented linking eIF5A with eukaryotic elongation factor 2 (eEF2), thus providing mechanistic detail for this proposed function. In addition, using inhibitors of hypusine

modification in human cells, Michael Matthews (University of Medicine and Dentistry of New Jersey, Newark, USA) proposed that eIF5A plays roles in nonsense-mediated mRNA decay as well as affecting the translation of only a subset of mRNAs.

More evidence for functional divergence of individual factors came from the mitochondrial translation initiation system. Umesh Varshney (State University of New York, Albany, USA) presented mutant complementation studies in *Escherichia coli* showing that mitochondrial initiation factor 2 (IF2mt) serves the functions of both the bacterial proteins IF1 and IF2. This difference from bacterial translation was explained by an insertion of 37 amino acids in IF2mt that is necessary for IF1 functions.

In a study of *E. coli* translation Haiou Qin (University of Pennsylvania, Philadelphia, USA) used single-molecule fluorescence resonance energy transfer (FRET) studies to support the maintenance of IF2 on the 70S ribosome during recruitment of the EF-Tu elongation factor. As both factors share an overlapping binding site on the ribosome, this was a highly unexpected finding. Qin presented a model to accommodate the coexistence of both proteins, and discussed the potential for a sustained interaction between IF2 and the ribosome during the first round of elongation.

Ribosome dynamics, hybrid states and ratcheting

The translating ribosome has three tRNA-binding sites: A, P and E. Hybrid A/P and P/E intermediate states were originally proposed by Moazed and Noller to explain tRNA movement through the ribosome (translocation) catalyzed by the G protein EF-G (EF2). Understanding the mechanism of translocation is a major focus in the field. Joachim Frank (Columbia University, New York, USA) presented the results of cryo-electron microscopic reconstructions performed at low magnesium concentrations that, for the first time visualize two forms of the 70S ribosome with two tRNAs bound. Both 'pre-ratcheted' (tRNAs bound in the A and P sites) and 'ratcheted' (A/P and P/E hybrid state with tRNAs bound) complexes were seen, suggesting that ratcheting is spontaneous. In the ratcheted state tRNA anticodon stem loops reside in the A and P sites of the 30S subunit, whereas the acceptor ends are found in the P and E sites, respectively, of the 50S subunit. tRNA movement in the 50S is accompanied by a counter-clockwise rotation (ratcheting) of the small subunit in which multiple changes in the relative positioning of several ribosomal RNA and protein components occur (Figure 1). Thus, the ribosome is a dynamic rather than a rigid structure.

Sarah Walker (Ohio State University, Columbus, USA) analyzed the effects of 23S rRNA mutations predicted to inhibit P/E site formation in a steady-state kinetic study of

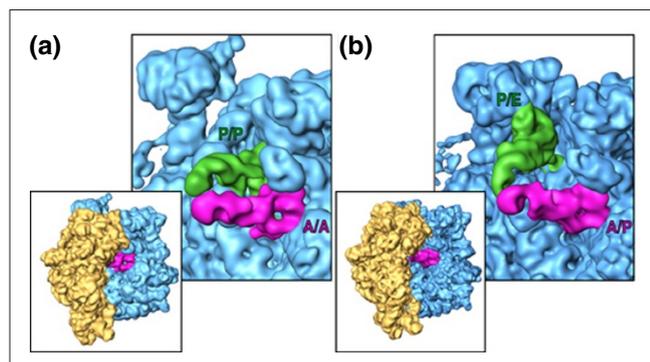


Figure 1

Transfer RNA hybrid states. The figure depicts two cryo-electron-microscopic reconstructions of the ribosome, one (a) showing two tRNAs (green and magenta) in the canonical A (A/A) and P (P/P) positions, the other (b) showing them in the hybrid A/P and P/E positions relative to the 50S subunit (blue) with the 30S subunit (yellow; see inset) computationally removed. tRNA reconfiguration accompanies a spontaneous rotation of the small subunit with respect to the large subunit (ratchet motion). The repositioning of the tRNAs accompanies a change in their immediate environment that is caused by the rotation of the small subunit. Figure courtesy of Xabier Agirrezabala and Joachim Frank, with permission from *Quarterly Reviews in Biophysics*.

translocation using a labeled mRNA. Her results implied that a P/E hybrid tRNA forms before the A/P hybrid and that EF-G•GTP hydrolysis to a bound EF-G•GDP•Pi state can precede tRNA movement. In his plenary presentation, Jody Puglisi (Stanford University School of Medicine, USA) continued the 'dynamic ribosome' theme, describing single-molecule FRET experiments with labeled tRNAs and/or rRNAs to analyze subunit joining, peptide-bond formation and translocation in real time. He noted that his data complemented the structural and kinetic experiments of others, demonstrating that A-site tRNA binding and peptide-bond formation favors ribosome ratcheting to the hybrid state and that EF-G-mediated translocation reverses the ribosome conformational change, and that together these events should drive unidirectional motion of the ribosome ready for the next elongation cycle.

Memory and brain disease

mRNA-specific translation is regulated by a family of proteins called 4E binding proteins (4E-BPs), which compete with eIF4G for binding to the 5' cap binding factor eIF4E. 4E-BP-eIF4E binding is regulated by mTOR (target of rapamycin)-dependent phosphorylation of the 4E-BPs. Knockout mice have revealed that the 4E-BPs are important for long-term memory and other physiological responses. Michael Bidinosti (McGill University, Montreal, Canada) presented the identification of a novel 4E-BP2 post-translational modification that occurs during early brain development: asparagine deamination to aspartate. The modification influences 4E-BP2 binding to the mTOR partner protein, Raptor, and might

be important for establishing the dendritic network in mammalian neurons.

Claudia Bagni (Catholic University of Leuven, Belgium) described work showing that the cytoplasmic FMRP-interacting protein (CYFIP1), a protein that interacts with the fragile X mental retardation protein (FRMP), is a neuronal 4E-BP. Interestingly, this protein lacks the classical eIF4E-binding motif and instead possesses a structurally related motif. CYFIP1 binds eIF4E and FMRP to repress neuronal translation of certain FMRP targets. Bagni showed that neuronal stimulation led to both CYFIP1 dissociation from eIF4E and activation of local protein synthesis at synapses.

Wayne Sossin (McGill University, Montreal, Canada) presented experiments examining the mechanism of serotonin-mediated TOR activation of translation in neurons in the sea slug *Aplysia californica*, a model organism with a simple nervous system used to study synaptic plasticity in response to stress. Sossin reported that by deleting the TOR-binding site in ribosomal protein S6 kinase (S6K) to create a dominant-negative mutation, it was found that the S6K was an important mediator of active translation in the neuron cell body and for long term-synaptic plasticity, suggesting that S6K is an important TOR target for long-term memory formation.

Inherited mutations in genes encoding eIF2B subunits cause fatal human 'eIF2B-related disorders' of the brain. Orna Elroy-Stein (Tel-Aviv University, Israel) described the generation of a knock-in mouse model for the disease, which reproduced important aspects of the human condition, including delayed development of white matter and a decrease in the layer of myelin wrapping axons. Isolated homozygous knock-in primary astrocytes exhibited marked defects in cell culture. This mouse model will clearly be helpful in understanding the related human pathology.

Infection and immunity

Like other viruses, human cytomegalovirus (HCMV) must inveigle the host's translation machinery into synthesizing viral proteins. Many viruses modify translation factors to shut off host gene expression, thereby reducing competition between host and viral genes. Cesar Perez (New York University School of Medicine, USA) described experiments showing that rather than shutting off host translation, HCMV takes a different approach and elevates expression of both the eIF4F complex (consisting of eIF4E, eIF4G and eIF4A) and poly(A)-binding protein (PABP) to promote viral protein synthesis and replication. PABP expression is controlled translationally, whereas transcriptional changes mediate the eIF4F response.

One common host innate immune response to viral infection is activation of protein kinase R (PKR), which phosphorylates

eIF2 α ; this in turn helps to shut down protein synthesis in infected cells. Poxviruses have evolved a variety of strategies to counter PKR. Stefan Rothenburg (National Institutes of Health, Bethesda, USA) presented evidence that the rate of PKR evolution is more rapid than for other eIF2 α kinases throughout vertebrates, while Adam Geballe (Fred Hutchinson Cancer Research Center, Seattle, USA) presented evidence that this is so even across different ape species. Both explained this by co-evolutionary pressures on both the host PKR and poxvirus-encoded K3L and E3L, proteins that are known to target and inactivate PKR, and included results of mutation analyses in yeast to support this hypothesis.

Bradley Joyce (Indiana University School of Medicine, Indianapolis, USA) presented evidence that the pathogenic protist *Toxoplasma gondii* expresses novel members of the eIF2 α kinase family - called TgIF2K-A and TgIF2K-B - that have a novel gene architecture and mediate translational control in response to cellular stresses. Joyce also showed that eIF2 α phosphorylation accompanies the developmental transition from the invasive tachyzoite form to the quiescent bradyzoite that is required for persistence in the host. Whereas TgIF2K-A was regulated by conditions that evoke an endoplasmic reticulum stress (conditions that interfere with the functions of this organelle) in a manner similar to the mammalian eIF2 kinase PEK/PERK, TgIF2K-B is a novel member of the kinase family whose activation mechanism remains unknown. The study clearly implicates a role for eIF2 α kinase-mediated translational control in developmental switches during the parasite life cycle.

The field of translational control has burgeoned over the years and now represents a very large community of scientists with connections to almost every aspect of biology. It is clear the future is bright for translational control. There are many key areas for future studies, including further structure-function analyses to increase our understanding of the molecular details underlying translational control and extending the already wide-ranging connections with development and disease. The ribosome remains a major antimicrobial antibiotic target and we are sure that new biotechnological and therapeutic applications will emerge, strengthening links between 'translational control' and 'translational medicine'.

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