Meeting report

Translational regulation of gene expression

Stephanie Kervestin and Nadia Amrani

Address: Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655-0122, USA.

Correspondence: Nadia Amrani. E-mail: nadia.amrani@umassmed.edu

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A report on the Cold Spring Harbor Laboratory meeting 'Translational Control', Cold Spring Harbor, USA, 7-12 September 2004.

There have been major breakthroughs in recent years in understanding both the mechanism of mRNA translation and its control. High-resolution structures have revealed the ribosome's role in the decoding process and the ribozyme activity of its peptidyl transferase center. The importance of post-transcriptional mechanisms in the regulation of gene expression is also much better appreciated today. The 2004 Cold Spring Harbor 'Translational Control' meeting addressed a variety of these mechanisms and provided new insights into the regulatory roles of RNA elements and RNA-binding protein complexes.

Ribosomal structure and the mechanism of translation

The crystal structures of ribosomes published in the past few years have revolutionized our understanding of the structural basis of tRNA selection and the peptide-bond-forming activity of the ribosome. The precise mechanisms of the distinct steps of protein synthesis are still unknown, however. This issue was addressed by several speakers, including Venki Ramakrishnan (MRC Laboratory of Molecular Biology, Cambridge, UK), who presented his recent work showing that the ribosome promotes accurate tRNA selection at the ribosomal A site and that recognition of cognate codon-anticodon interaction induces the 30S ribosome subunit to adopt a closed conformation. This movement most probably accelerates the rate of GTP hydrolysis and the following accommodation step, observed by other groups from kinetic analysis. Other presentations focused on structural rearrangements of the ribosome during elongation and translocation and, together, these structural data highlighted the dynamic nature of ribosome structure during the different steps of translation and prompted the audience to ponder which conformational changes are rate-limiting during translation.

Structural analysis of the eukaryotic ribosome when associated with translation factors has also brought new insights. In eukaryotes, initiation of translation is generally dependent on the presence of a 5′ cap structure on the messenger RNA. Cap-dependent translation initiation is a complex process, facilitated by a large number of initiation factors (eIFs) that form a complicated network of cooperative interactions with the 40S ribosomal subunit. John McCarthy (Manchester Interdisciplinary Biocentre, UK) reported cryo-electron microscopy (cryo-EM) reconstructions, which indicate that binding of eIF1A to the 40S ribosomal subunit induces significant conformational changes in the subunit. These movements may create a recruitment-competent state of the 40S subunit that mediates the cooperative binding of other eIFs to form the 43S initiation complex. Moreover, the structure of the 43S complex indicates that the 40S to 43S transition involves a large rotation of the head of the small subunit; this is thought to reflect the opening of the mRNA channel which, in turn, may facilitate mRNA binding and subsequent scanning.

The cap-independent pathway of translation initiation, utilized by both viral and cellular mRNAs, exploits highly structured translation-initiation regions on mRNAs dubbed internal ribosome entry sites (IRESs). The IRES from the cricket paralysis virus (CrPV) directly assembles elongation-competent ribosomes in the absence of the canonical eIFs and the initiator tRNA, methionyl-$tRNA_i$. Eric Jan, from Peter Sarnow's group (Stanford University, USA) described experiments exploiting cryo-EM to visualize the CrPV-IRES bound to human 40S subunits and the 80S ribosome. The

IRES was shown to form specific contacts with the components of the ribosomal A, P and E sites and to induce conformational changes in the ribosome. These changes were similar to those observed when the hepatitis C virus (HCV) IRES binds to the 40S subunit and when the elongation factor eEF2 binds to the 80S ribosome. This suggests that the CrPV IRES functions as an RNA-based translation factor that actively manipulates the ribosome to mediate the virus's unusual mode of translation initiation. Collectively, the structural data on the ribosome and its associated complexes presented during the meeting led the audience to an appreciation of the ribosome as a dynamic machine whose contortions are subject to the considerable influence of both regulatory proteins and RNA structures.

Regulation of mRNA utilization by *cis* elements and *trans*-acting factors

The expression of many proteins is influenced by the structure and cellular localization of their mRNAs, features often dictated by specific RNA-binding proteins. Several talks and posters addressed the functions of these RNA-binding translational activators and repressors, as well as the intricacies of their RNA targets. One of the best characterized models of translational control involving *cis*-acting RNA elements is the regulation of translation of maternally inherited mRNA by cytoplasmic polyadenylation in *Xenopus* oocytes. Polyadenylation is required before the mRNA can be translated, and regulation of this step is therefore necessary for oocyte maturation and embryonic development. Cytoplasmic polyadenylation depends on phosphorylation of CPEB, a protein that is bound to specific cytoplasmic polyadenylation elements (CPEs) in the 3′ ends of transcripts regulated in this manner. In *Xenopus*, phosphorylation is mediated by the protein kinases Eg2 (known as Aurora A in the mouse) and Cdc2. The role of the CPEs in the timing of polyadenylation and translational activation was addressed by Raul Mendez (Center for Genomic Regulation, Barcelona, Spain). He showed that the CPEs in the 3′ untranslated regions (UTRs) of the mRNAs of cyclins B1 through B5 regulate translation of these mRNAs in a sequential manner during *Xenopus* oocyte maturation. The presence of one CPE close to, but not overlapping with, the hexanucleotide sequence AAUAAA was shown to promote early polyadenylation, and thus to stimulate translation, while the presence of a cluster of CPEs in which one overlapped the AAUAAA sequence (or was separated from it by only one to three nucleotides) promoted repression of translation and late polyadenylation.

Sequence elements in UTRs can also enhance translation. Eva Harris (University of California, Berkeley, USA) showed that the 5′ and 3′ UTRs in dengue virus RNA can act together to promote translation. Dengue RNA has a 5′ m^7G cap and a non-polyadenylated 3′ UTR, and undergoes cap-dependent translation. But Harris showed that, under conditions in which cap-dependent translation is inhibited, cap-independent translation occurs, and that this mechanism is distinct from IRES-driven activity and requires both the 5′ and 3′ UTRs. This shows for the first time that translation initiation in a viral system can switch from being cap-dependent to cap-independent, a property previously described only for cellular mRNAs.

RNA-binding proteins have key roles in the regulation of nearly every aspect of gene expression. They often display a modular architecture, exert multiple functions and participate in more than one step of the gene-expression pathway. Stefan Hüttelmaier, from the laboratory of Robert Singer (Albert Einstein College of Medicine, New York, USA), described how the *trans*-acting factor zipcode-binding protein 1 (ZBP1), which binds to the 3′ UTR of β-actin mRNA, regulates not only the localization of this mRNA in fibroblasts and neurons, but also its translation. He showed that binding of ZBP1 probably inhibits translation by interfering with the formation of the 80S ribosome during initiation. Two different pathways for phosphorylating ZBP1 are involved in this regulation. Serine phosphorylation of ZBP1 by extracellular-regulated kinases (ERKs) induces repression of translation. In contrast, tyrosine phosphorylation of ZBP1 by Src kinases inhibits its binding to RNA, thus antagonizing the repression.

In addition to being part of the translation machinery, some ribosomal proteins have roles in translational control. Paul Fox (Lerner Research Institute, Cleveland, USA) reported the identification of ribosomal protein L13a as a component of the GAIT complex. This complex binds to a previously identified stem-loop structure in the 3′ UTR of ceruloplasmin mRNA, and is responsible for silencing its translation in macrophages associated with inflammation. Fox showed that, when L13a is phosphorylated, it is released from its site in the 60S ribosomal subunit to form, with three other proteins, the functional GAIT complex that binds to ceruloplasmin mRNA and blocks its translation.

In a related vein, Jayati Sengupta from Joachim Frank's lab (The Howard Hughes Medical Institute at the Wadsworth Center, Albany, USA), in collaboration with Poul Nissen's group (University of Aarhus, Denmark), described the identification and visualization of the protein RACK1 as a component of the small ribosomal subunit in the fungus *Thermomyces lanuginosus*. RACK1 (also called Asc1p in *Saccharomyces cerevisiae*) acts as a scaffold for recruiting proteins involved in signaling pathways and has been linked to the control of translation initiation of specific mRNAs. Cryo-EM density maps of purified wild-type 80S ribosomes were compared with those of ribosomes from mutant cells lacking RACK1. This showed that RACK1 is located on the head region of the 40S subunit, in the immediate vicinity of the mRNA exit channel. RACK1 appears to expose a platform surface to the solvent, possibly providing a scaffold for interacting factors. The location and shape of RACK1 are also

conserved in yeast ribosomes, indicating its general role in linking signal transduction pathways to the ribosome.

Translational control by cytoplasmic polyadenylation is crucial not only for development but also for regulation of 'synaptic memory' in the mammalian central nervous system. Long-term synaptic plasticity and long-term memory require the synthesis of new proteins for their consolidation. The signaling pathways that are responsible for initiating new protein synthesis are poorly understood, but most regulation is thought to take place at the level of translation initiation. An example of regulation of cap-dependent translation in synaptic plasticity and long-term memory was presented by Jessica Banko from Eric Klann's laboratory (Baylor College of Medicine, Houston, USA). Mice with a genetic knockout of eIF4E-BP2, a factor that inhibits translation, showed altered long-term synaptic plasticity and deficits in long-term memory.

Quality-control mechanisms and mRNA decay

Several post-transcriptional mechanisms are used by eukaryotic cells to control the quality of mRNA. One of them, nonsense-mediated mRNA decay (NMD), recognizes and degrades mRNAs containing a premature termination codon. Allan Jacobson (University of Massachusetts Medical School, Worcester, USA) showed that termination events are different at premature and normal termination codons. At the premature stop signal, termination is aberrant and, in the presence of the Upf1p protein (the major factor in NMD), ribosomes are able to reinitiate translation upstream or downstream. These results indicate that aberrant termination is linked to NMD. Jacobson also showed that tethering the poly(A)-binding protein Pab1p or its interacting factor Sup35p/eRF3 downstream of the premature termination site led to stabilization of an otherwise unstable mRNA. These findings support the '*faux* UTR' model, which postulates that sequences downstream of a premature termination codon fail to bind a set of regulatory factors (for example, Pab1p and/or Sup35p) required for efficient termination, thereby triggering NMD.

In both yeast and human cells, the decapping and 5′-to-3′ degradation of mRNA appear to occur in discrete foci known as processing bodies (P-bodies). Ujwal Sheth from Roy Parker's group (University of Arizona, Tucson, USA) addressed the question of whether NMD also takes place in P-bodies in yeast. Under normal growth conditions, NMD factors are randomly distributed in the cytoplasm. In a *dcp1Δ* strain, which carries a deletion of the gene encoding the decapping enzyme Dcp1p, however, the Upf factors and decay intermediates containing premature termination codons are localized in P-bodies. P-bodies are highly dynamic and their size is dependent on the type and severity of stress conditions. The relationship between P-bodies and stress granules in mammalian cells was addressed by Nancy Kedersha from Paul Anderson's laboratory (Brigham and Women's Hospital, Boston, USA). Stress granules are dynamic foci initiated by the phosphorylation of eIF2α and are thought to be sites for mRNA triage and messenger ribonucleoprotein (mRNP) remodeling. Under stress conditions, P-bodies are physically clustered around stress granules, and some proteins and mRNAs are detected in both the P-bodies and stress granules. These, and other observations, led to a model whereby, in response to a stress, mRNAs go from the stress granules, where translation initiation is inhibited and the mRNP modified, to the P-bodies for subsequent degradation.

The examples of regulation of protein expression by signal-dependent translation (or inhibition of translation) presented at the meeting are consistent with the general prediction that specialized translational mechanisms frequently control the synthesis of biologically active proteins. At the meeting, the current state of knowledge of different aspects of translation and translation regulation was addressed with particular reference to the role of the dynamics of the ribosome as well as the importance of the RNA structure and the discovery of new regulators. We look forward to seeing advances in these and other areas at the next 'Translational Control' meeting in 2006.