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mRNA fate
Life and death of the mRNA in the cytoplasm

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The life of an mRNA molecule begins with transcription and ultimately ends in degradation. In the course of its life, however, mRNA is examined, modified in various ways and transported before being eventually translated into proteins. All these processes are performed by proteins and non-coding RNAs whose complex interplay in the cell contributes to determining the proteome changes and the phenotype of cells. On May 23–26, 2012, over 150 scientists from around the world convened in the sunny shores of Riva del Garda, Italy, for the workshop entitled: "mRNA fate: Life and Death of mRNA in the Cytoplasm." Sessions included mRNA trafficking, mRNA translational control, RNA metabolism and disease, RNA-protein structures and systems biology of RNA. This report highlights some of the prominent and recurring themes at the meeting and emerging arenas of future research.

mRNA Trafficking: The Importance of Being in the Right Place at the Right Time

mRNA localization is an important process to restrict certain transcripts and proteins to specific subcellular domains, thus spatially controlling gene expression. The importance of the subcellular mRNA transport for the formation and function of the nervous system is now generally accepted. Defects in mRNA localization are found to be linked to several neurological disorders.

The fragile X syndrome (FXS), the most common form of inherited mental retardation, is one notable example of such diseases. FXS is caused by mutations in the FMR1 locus encoding the fragile X mental retardation protein (FMRP), a dendritically localized RNA-binding protein (RBP) that functions as a translational repressor.

Claudia Bagni (Catholic University of Leuven) reported the identification of CYFIP1 as a new protein interactor of FMRP in neurons.[1] Interestingly, CYFIP1 also networks with eIF4E suggesting a role of CYFIP1 in translational control. CYFIP1 is found in the same messenger ribonucleoprotein (mRNP) complex with FMRP and eIF4E that is transported in dendrites together with the cargo mRNAs. The assembly of this complex ensures that the mRNA stays in a translationally silent condition during transport. Upon synaptic stimulation, the CYFIP1-FMRP complex dissociates from the eIF4E and translation can occur.

Cytoplasmic elongation of the poly(A) tail was originally identified as a mechanism to activate maternal mRNAs, stored as silent transcripts with short poly(A) tails, during meiotic progression. A family of RBPs named CPEBs, which recruit the translational repression or cytoplasmic polyadenylation machineries to their target mRNAs, directly mediates cytoplasmic polyadenylation. CPEBs have been shown to regulate the translation of hundreds of mRNAs in both somatic and germ cells and to drive events as diverse as learning and memory, cell cycle progression and tumor development.

Raul Méndez (Institute for Research in Biomedicine, Barcelona) reported a new function for CPEB1 and presented to the audience a global model for the regulation of gene expression by the CPEB family in cell cycle and cell differentiation.[2] He described that CPEB1 moonlights as a nuclear factor responsible for the pre-mRNA processing of the same mRNAs that, later, in the cytoplasm. The protein recognizes the same *cis*-acting element in the cytoplasmic mature mRNA as in the nuclear pre-mRNA, recruiting the cleavage and polyadenylation machinery that mediates both the cytoplasmic polyadenylation and the nuclear pre-mRNA cleavage and polyadenylation at specific polyadenylation sites. In turn, at least in some cases, this affects alternative splicing of the CPEB-regulated transcripts. This is a new function for CPEB, where hundreds of mRNAs are regulated by alternative processing in the nucleus in a coordinated manner and associated with cell cycle and tumor development.

One key and general feature of mRNA localization is that this event should precede translation. As a consequence, mRNAs have to be kept translationally silent during their transport toward the proper target compartment.

Jacqueline Trotter (University of Mainz) presented new data on the mechanisms regulating the translation of the myelin basic protein (MBP) in oligodendrocytes.[3] *MBP* mRNA assembles into mRNPs that are transported to the distal oligodendroglial processes. Two members of the heterogeneous nuclear ribonucleoproteins (hnRNPs), namely hnRNPA2 and hnRNPF, have been identified as components of these mRNPs where they have a role in silencing the transcripts during transport. Both proteins become phosphorylated upon (local) activation of FYN, triggered by the initial contact between the axon and the glia cell. Upon

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phosphorylation, hnRNPA2 and hnRNPF are released from the mRNPs. As a consequence, *MBP* mRNA can be locally translated. Interestingly, in several leukodystrophies, the expression of hnRNPs is altered: this dysregulation may inhibit translation of MBP mRNA causing myelination problems.

Several labs have contributed to dissecting the molecular mechanisms involved in subcellular mRNA localization in different cell types, and research over the last decade has shown that orthologs of key components of the mRNA localization machinery exist from yeast to humans. Key questions remain open: do mRNPs involved in the transport of different mRNAs share common molecular components? What is the complete molecular composition of transport mRNPs and which are the functions of the individual components? How does the assembly of the mRNPs occur? How specific transport RNPs are for their cargo mRNAs? What is the size of the resulting complex? Trying to address these questions in neurons might be a difficult task. As shown by Dierk Niessing (University of Munich), budding yeast *S. cerevisiae* is a model that can yield mechanistic insights to solve some of these issues.[4] Using elegant biochemical approaches, a comparably simple transport complex from yeast, the ASH1 mRNPs, has been reconstituted in order to determine how transport specificity is achieved and how the single molecular components of the transport machinery assemble. Interestingly, none of the previously implicated RBPs showed specificity in the mRNA recognition. Instead, a cytoplasmic co-complex that mediates the required specific mRNA recognition for transport has been identified. Niessing's data suggest that the nuclear assembly of pre-mRNPs is not very specific. Instead, cytoplasmic events ensure that only localization element-containing mRNAs are transported. Finally, the core *ASH1* transport complex has also been reconstituted and the stoichiometric ratios of the components and the molecular weight of the respective assemblies have been determined. Importantly, the reconstituted mRNPs are fully functional, moving along actin filaments. The reconstitution of the mRNA-transport complex provides an important step forward in our mechanistic understanding of mRNA localization and transport, establishing an extremely useful model for further studies and applications.

Transcripts can be directed either to free, cytoskeletal-associated polysomes or to ER-bound polysomes, the last for the synthesis of membrane or secreted proteins. Translating mRNAs are targeted to the ER by the interaction between the Signal Recognition Particle (SRP) and the signal peptide in the nascent protein chain. Yoav Arava (Technion, Israel Institute of Technology) that PMP1 mRNA requires its 3'UTR rather than a translated signal peptide and that the UTR is necessaru and sufficient for the transcript targeting to the ER in yeast. Chimeric transcripts containing the 3' UTR of *PMP1* show ER-association. Which RBPs are responsible for the targeting to the ER? Arava's group identified within the *PMP1* 3' UTR two UAAU repeat binding site for the RBP Puf2.[5] In addition to ER-bound ribosomes, polyribosomes enriched for mRNAs that encode for mitochondrial proteins are localized around mitochondria. Indeed, several mRNAs are targeted to these organelles. In contrast to the 3' UTR-mediated targeting to the ER, Arava's group described mitochondria-targeting mechanisms that involve translated sequences. The ribosome-associated Nascent chain-Associated Complex (NAC), the nascent-chain associated chaperone Ssa1[6] and the mitochondrial protein receptor Tom20[7] have been involved by his work. These proteins sequentially interact with the nascent chain. Interestingly, deletion of each factor causes mRNA mis-localization, supporting a translation-dependent targeting mechanism as shown for the classical ER targeting.

Translational Silencing: Making Head or Tail Out of It

It has been long-known that in eukaryotes, translating mRNA molecules can form circular structures due to an interaction between the 5' cap-binding protein eIF4E and the poly(A) binding protein (PABP), which both bind to eIF4G forming an mRNA-protein-mRNA bridge. This is the so-called "closed loop," which is thought to promote recycling of polysomes leading to time-efficient translation and may also function to ensure that only intact mRNAs are translated. Generally speaking, microRNAs (miRNAs) inhibit gene expression by base pairing to the 3' untranslated region (3' UTR) of target mRNAs and by repressing translation and/or initiating poly(A) tail deadenylation and mRNA destabilization. The circularization of the mRNA, therefore, might also explain how a miRNA bound to the 3' UTR of an mRNA could affect translation, which starts at the 5' end. However, the exact mechanism by which miRNAs inhibit translation has been a long-standing and debated question in the field.

Nahum Sonenberg (McGill University), a pioneer in mRNA translational control studies, gave a somewhat historical account tracing the observations that led him into the field of miRNA-mediated control of gene expression. Sonenberg reported how his group established a mouse Krebs-2 ascites extract that faithfully recapitulates miRNA action in cells. By the use of clever biochemical experiments, in collaboration with Witold Filipowicz's group, they demonstrated that the let-7 miRNA inhibits translation of a reporter mRNA at the initiation step.[8] Translation inhibition is subsequently consolidated by let-7-mediated deadenylation requiring both PABP and the CAF1 deadenylase, which interacts with the let-7 miRNA-loaded RNA-induced silencing complex (miRISC).[9] Building on his own work and several other studies, Sonenberg proposed a two-step model of miRNA-mediated gene silencing.[10] First miRISC inhibits translation initiation by interfering with eIF4F-cap recognition through its eIF4E subunit and 40S ribosomal subunit recruitment, in a deadenylation-independent manner. The interaction of the GW182 protein, a core component of the miRISC, with PABP might interfere with the closed-loop formation together with the eIF4G-PABP interaction, thus contributing to the repression of translation initiation. Importantly, Sonenberg reported that GW182 directly interacts with PABP via the DUF domain within its C terminus.[11] Second, miRISC would direct deadenylation of its target mRNA by its binding to the CNOT-CCR4 and PAN2-PAN3 deadenylase complexes.[12] Following deadenylation, both decapping and destabilization of targeted mRNAs finally occur.

Witold Filipowicz (Friedrich Miescher Institute) showed that both the N- and C-terminal domains of GW182 are required to recruit the CCR4-NOT deadenylase complex through the redundant tryptophan-containing short sequence elements, W-motifs, which are present in non-structured regions of GW182 and act additively.[13] Surprisingly, the CCR4-NOT complex functions not only as a deadenylase, but also in inhibiting translation during miRNA-mediated repression. Which mechanism comes first? Many questions about miRNA control of gene expression remain open, such as the relative contribution of translation inhibition and mRNA degradation and the relationship between deadenylation and translational repression. Part of this problem has been explored by using HeLa cell lines expressing inducible reporters bearing 3' UTRs of known miRNA targets.[14] This study has indicated that translational repression precedes mRNA deadenylation and degradation. Target mRNAs containing AU-rich regulatory elements (AREs) in the 3' UTR have been previously demonstrated to be relieved from miRNA repression upon cellular stress. De-repression happens by binding of the HuR protein to mRNA 3' UTRs. Is it possible to uncouple relief from stress? How does HuR relieve miRNA repression? The use of recombinant miRISC and purified HuR has revealed that HuR may function by oligomerizing along the mRNA leading to displacement of miRISC, even when this is positioned at a distance from the primary HuR-binding site.

Sonenberg also presented fascinating new results from his ongoing analysis of translation and introduced the concept of "translational homeostasis."[15] As translational control of gene expression plays a key role in many biological processes, the activity of the translational apparatus is under tight homeostatic control. eIF4E is a major target for translational control and is regulated by a family of repressor proteins named 4E-binding proteins (4E-BPs). Sonenberg reported the surprising finding that, despite the importance of eIF4E for translation, its drastic knockdown caused only minor reduction in translation efficiency. He could explain this conundrum by the finding that hypo-phosphorylated 4E-BP1 is targeted by the KLHL25-CUl3 ubiquitin ligase and rapidly degraded in eIF4E-knockdown cells. Thus, both ubiquitination and the proteasome pathway control the levels of translation repressors to maintain cellular homeostasis.

Stefano Biffo (DIBIT-HSR) focused on the eIF6 translation initiation factor in the context of tumorigenesis and nutrient metabolism. eIF6 is an anti-association factor that blocks the improper aggregation of the 60S to the 40S ribosome subunits and if upregulated in cancer cells, predicts malignancy. Biffo reported that eIF6 haploinsufficiency reduces tumorigenesis in Myc-induced mouse lymphoma model.[16] In eIF6 +/-, there is a reduction of the protein level in the cytoplasm, but not in the nucleus leading to a reduction in translation. Moreover, the protein is able to dissociate the 80S complex in tumor extracts. These results suggest that the anti-association activity of eIF6 is required for efficient translation. eIF6 +/- mice show a specific reduction of translation in the liver and a metabolic signature characterized by reduced fatty acid synthesis/glycolysis leading to a lean phenotype, and reduced cell cycle progression. Biffo and coworkers suggest that eIF6 acts downstream growth factors/nutrient sensing by reprogramming the metabolic status of the cell, and is activated by PKC-induced phosphorylation.

As a key metabolic factor, insulin level is tightly regulated by different transcriptional and post-transcriptional mechanisms. In response to acute elevations in circulating glucose, the timely rise in insulin production is primarily controlled by rapid increases in insulin mRNA translation in β cells, but the mediators of this translational elevation were so far unknown.

Myriam Gorospe (National Institute of Health, NIH) reported that the RBP HuD is a pivotal regulator of insulin translation in pancreatic β cells.[17] Gorospe's group discovered that HuD, previously believed to be present only in neurons and gonads, is expressed in pancreatic β cells under control of the insulin receptor signaling pathway. They were able to show that HuD associates in β cells with a 22-nucleotide segment of the 5' UTR of preproinsulin (*Ins2*) mRNA. Modulating HuD abundance did not alter *Ins2* mRNA levels, but HuD overexpression decreased Ins2 mRNA translation and insulin production, and conversely HuD silencing enhanced *Ins2* mRNA translation and insulin production. Following treatment with glucose, HuD rapidly dissociated from the *Ins2* mRNA and enabled insulin biosynthesis. Importantly, HuD-knockout mice displayed higher insulin levels in pancreatic islets, while HuD-overexpressing mice exhibited lower insulin levels in islets and in plasma. In light of this result, it will be important to test if the functions of HuD are aberrant in patients with type 2 diabetes.

RNA Metabolism and Disease: Life Hang by a Thread ... of mRNA

The processes promoting or preventing translation in a certain cell location and at a certain time are responsible for controlling major cellular events and for orchestrating development. Several examples were provided concerning the derangement of these processes and their involvement in pathogenesis.

Primary miRNAs (pri-miRNAs) are processed to precursor miRNAs (pre-miRNAs) and then to mature miRNAs through endonucleolytic cleavages operated by distinct multiprotein complexes including the enzymes Drosha and Dicer. Different proteins, participating as co-regulators of these enzymes in the control of specific miRNAs maturation, have been identified. Thus, an altered control of miRNA precursor maturation can impact on the mature miRNA deregulated expression observed in cancer and many other diseases.

Roberto Gherzi (Istituto Nazionale per la Ricerca sul Cancro) discussed the role of KH-type splicing regulatory protein (KSRP) in miRNA maturation. He reported that the dynamic exchange of three co-regulators (KSRP, DDX5 and SMADs) orients the differentiative potential of the pluripotent mesenchymal cell line C2C12 by controlling miR-206 and miR-133 "myo-miRs" maturation.[18] In rapidly proliferating undifferentiated C2C12 cells, KSRP is complexed with DDX5 and its myo-miRs precursors processing activity is impaired. In cells committed to differentiate into myotubes, KSRP phosphorylation by AKT favors its association to pri-miR206 and pri-miR-133b, leading to accumulation

of the mature miRNAs. Conversely, when C2C12 cells are induced to differentiate into osteoblasts by activation of the BMP signaling pathway, KSRP, although phosphorylated, associates with SMAD proteins and its ability to induce pri-miR206 and pri-miR-133b maturation is blocked. Low levels of miR-206 and miR-133b are essential for osteoblastic differentiation to occur and KSRP knockdown in undifferentiated C2C12 cells induces their phenotypic conversion into osteoblasts.

Regulation of telomere length by telomerase plays an essential role in premature senescence and maintaining genetic stability. In mice, lack of adequate telomerase expression results in progressive telomere shortening over several generations.

Robert Schneider (NYU School of Medicine) showed that AUF1, a major attenuator of the inflammatory response, is also an essential regulator of telomere length and telomerase activity in mice.[19] AUF1, which is also known as hnRNP D, destabilizes mRNAs containing AREs in their 3' UTRs. AREs are largely present in pro-inflammatory cytokines' mRNAs. AUF1 consists of four protein isoforms generated by alternative RNA splicing: the two smallest isoforms associate with targeted ARE-mRNA degradation. Late generation AUF1-deficient mice exhibit premature senescence due to increased stabilization of AUF1 target and cell cycle-inhibiting mRNAs, as well as decreased lifespan, shorter telomeres, chromosomes without detectable telomere signals and a significant increase in DNA damage foci at telomeres. Backcross of late-generation mice to wild-type mice rescues the decrease in the number and survival of AUF1-/- progeny. AUF1-/- mice also exhibit an increased rate of tumorigenesis. What is the mechanism linking AUF1 to telomerase maintenance? Schneider reported that in AUF1-deficient cells, there is a reduction in the expression of the two core telomerase components, the catalytic subunit, TERT and the RNA subunit, TERC. The two largest AUF1 isoforms act in part by promoting the transcription of TERC and TERT RNAs and can be footprinted to their promoters. In synthesis, Schneider's results demonstrate that cessation of the inflammatory response by AUF1 is mechanistically linked to maintenance of telomere length, normal aging and reduced carcinoma and is likely involved in human disease.

Stefan Huttelmaier (Martin-Luther University) reported the role of the RBP IGF2BP1 in controlling the metabolism of specific mRNAs in cancer cells.[20] In developing neurons, IGF2BP1 promotes neurite outgrowth and the migration of neuronal crest cells. Like neurons, metastatic tumor cells form extensive lamellipodia and filopodia that are important for both migration and invasiveness. In tumor cells, IGF2BP1 enhances the direct migration by regulating post-transcriptionally the expression of two proteins: the mitogen-activated protein kinase 4 (MAPK4) and the phosphatase and tensin homolog (PTEN). This occurs in two ways: first, the IGF2BP1-dependent inhibition of MAPK4 interferes with the phosphorylation of the heat-shock protein 27 (HSP27) induced by the MAPK-activated protein kinase-5 (MK-5). This leads to a reduced recruitment of G-actin that enhances cell adhesion and increases the speed of tumor cell migration. Second, IGF2BP1 increases the stability of PTEN by interfering with the turnover of its mRNA. Increased expression of PTEN enhances RAC1-dependent cell polarization, which promotes the directionality of tumor cell migration. Taken together, Huttelmaier's results identify IGF2BP1 as a potent oncogenic factor that regulates several aspects of tumor cell properties such as adhesion, migration and invasiveness.

Anne Willis (MRC Toxicology Unit) showed how regulatory elements in 5' and 3' UTRs can modify sensitivity of cancer cells to platinum-based chemotherapy.[21] Platinum chemotherapeutic agents, such as cisplatin and carboplatin, have a broad range of activity in malignant diseases and are used to treat many types of cancer. Unfortunately, acquired resistance to platinum-based chemotherapy limits the efficacy of these agents. The cellular events that seem to mainly contribute to this response are alterations of DNA repair processes. Willis and coworkers started by measuring the relative amount of cyclo-pyrimidine dimers and global protein synthesis rates in cells exposed to a nonlethal dose of UVB light showing a significant increase in DNA damage and a general reduction in protein synthesis after treatment. By investigating the role of a post-transcriptional control in the process, they observed that a subset of mRNAs is effectively subject to differential translational regulation: despite the general decrease of protein synthesis, activation of the DNA damage response (DDR) resulted in the translational upregulation of nucleotide excision repair (a specific program of DNA repair) proteins such as ERCC1, ERCC5, DDB1, XPA, XPD and OGG1. This was demonstrated by evaluating the polysome/subpolysome distribution in untreated and UVB-irradiated cells using two-color cDNA microarray and northern analysis. Of interest, it was noted that the group of mRNAs that remained associated to polysomes had enrichment in upstream open reading frames (uORFs). Using report vectors, which contained the 5' UTRs of some of the mRNAs required for the DDR, they showed that these mRNA elements were sufficient to permit translational reprogramming of reporter mRNAs following exposure of compounds that caused bulky adduct damage such as ciplatin, mitomycin C and UV.

Fabrizio Loreni (University "Tor Vergata") reported about the mechanism underlying "ribosomopathies." These diseases, whose best known example is Diamond Blackfan Anemia, are caused by a defect in ribosome biogenesis that brings to a cell response called "ribosomal stress." The ultimate result is the inhibition of cell proliferation and apoptosis. Loreni's group observed that the cell cycle regulator serine/threonine kinase PIM1 interacts with the ribosomal protein S19 and co-sediments with ribosomes.[22] S19 deficiency causes a strong destabilization of PIM1 that results in an increase in the cell cycle inhibitor p27Kip1 and blocks cell proliferation. Loreni also presented data supporting a model by which ribosomal stress causes an increase of the phosphorylation of eEF2 translation elongation factor possibly through the eEF2 kinase, with a consequent decrease of the translational elongation and an increase of the recruitment of ribosomal proteins' mRNAs to polysomes.

RNA-Protein Structure: A Complex Point of View

During its whole lifespan, the mRNA molecule in the cell is physically associated to RNA-protein machineries such as the

RNA polymerase, the spliceosome, the ribosome and the exosome, which ultimately influence gene expression and, in turn, affect cell growth, proliferation, differentiation and response to environmental changes. The use of structural approaches to gain information on mRNA-protein machineries is of great interest to build models of functional states of biologically active complexes, as well as to get a better comprehension of how mRNA processing has changed during evolution. Structural techniques, such as NMR, cryo-electron microscopy, X-ray crystallography, combined with molecular biology or biochemistry, have proven to be highly informative and successful approaches to investigate the structure and function of macromolecular complexes and their interactions with mRNAs, significantly changing our understanding of post-transcriptional events in the control of gene expression.

Gabriele Varani (University of Washington) described the structure of regulatory enzymes, proteins and RNAs involved in 3'-end processing performed by complexes bound to the RNA polymerase II C-terminal domain (CTD).[23] Varani and collegues took advantage of new NMR methods to identify the protein-protein interfaces in the complete complex of 300 kDa of Rna15-Hrp1 and Rna14. The structure of the complex between Rna15, Hrp1 and 3'-end processing signals was described portraying a cooperative mechanism aimed at allowing rapid changes in the recruitment of processing factors as the 3'-end of mRNA approaches.

Kiyoshi Nagai (MRC Laboratory of Molecular Biology) presented a wide number of structural studies of spliceosomal snRNPs, comprising the five large RNA-protein complexes U1, U2, U4, U5 and U6. The crystal structure of the U4 snRNP core domain at 3.6 Å resolution was shown,[24] revealing the mechanism of the Sm site (AUUUUUG) binding to the central hole of the heptameric ring of Sm proteins. This interaction occurs one-to-one with SmE-SmG-SmD3-SmB-SmD1-SmD2-SmF. The resolved structures reveal that each nucleotide interacts with four key residues at equivalent positions in the L3 and L5 loops of the Sm fold. The crystal structure of the functional core of the U1 snRNP at 5.5 Å resolution shows a hierarchical network of elaborate interactions among RNA and protein subunits.[25] In particular, the N-terminal polypeptide of U1-70K was found to wrap around the seven Sm proteins' core domain, representing a crucial position for the 5'-splice-site recognition.

Robert Gilbert (University of Oxford) focused on the RNA turnover describing the structure of a 3' miRNA-targeted uridylltransferase.[26] The crystal structure of the cytoplasmic 3' uridylyltransferase Cid1 from *S. pombe* revealed a specific mechanism of uridine selection with respect to the mitochondrial terminal uridyl transferases (TUTs) and displayed a close relationship to both other TUTs and the DNA polymerase β family proteins.

Elena Conti (Max Planck Institute of Biochemistry) reviewed the structural insights on RNA degradation by the 3'-5' exosome complex, underling that the RNA exosome channeling mechanism seems to be conserved in exosome-like complexes from all domains of life, and might have been present in the most recent common ancestor.[27,28] The structural data, coupled with biochemical and molecular biology approaches, demonstrate that RNA, representing the substrate of the exosome machinery, reaches the active site by threading through the central channel of the barrel. The eukaryotic exosome has been shown to contain a minimum of 10 different proteins, among which the 9-subunit core (Exo-9) shares a similar architecture with prokaryotic complexes, even though it was found to be catalytically inactive, being the RNase activity provided by Rrp44. Nevertheless, the structural data demonstrate that the recruitment and binding of RNA have been widely conserved from prokaryotes to eukaryotes.

The Big Picture: Cell-wide Portraits of mRNA Fate

An increasingly important perspective on the cytoplasmic behavior of mRNAs is that offered by the unbiased study of thier dynamics at the whole-cell level. As well as for transcriptional networks, the recent technical advances in parallel detection and sequencing of nucleic acids allow us to identify, for example, virtually all mRNAs bound by a certain RBP in a specific condition of the cell.

Undoubtedly a pioneer of this field is Jack Keene (Duke University), whose long standing interest on RBP biology brought him to develop 10 y ago the first adaptation of antibody-mediated pull-down to the parallel isolation of RBP targets, the ribonucleoprotein immunoprecipitation on chip (RIP-chip) technique.[29] From his first results with this method, he built a conceptual framework for the description of post-transcriptional networks, indicating as the "ribonome" the space in which single mRNPs with associated RBPs and ncRNAs functionally interact through the sharing of common regulators. He called these network entities post-transcriptional RNA operons or regulons. A predicted feature of post-transcriptional RNA regulons, subsequently validated in a number of studies, was that they tend to aggregate mRNAs coding for complementary protein functions in the same regulatory unit, in analogy to what happens with bacterial operons. After having reviewed his initial studies on the ELAV family of RBPs, Keene reported about the first application of RIP-chip and of a new deep sequencing-based technique, PAR-CLIP,[30] to the description of the network generated by an ELAV member, HuR. While RIP-chip selects for stable RBP-mRNA interactions, PAR-CLIP identifies with high resolution more transient RBP binding sites on mRNAs. The results of this study demonstrate two important high-order features of HuR, the integration of splicing with mRNA stability and the tendency to bind preferentially on the 3' UTR of target mRNAs around miRNA-binding sites. The power of the post-transcriptional RNA regulon model in producing meaningful interpretations of transcriptome data are demonstrated by the study of mRNA dynamics during T-cell activation, immune cells in which again the HuR protein has a fundamental role. The model also brings, by the use of a system of correspondence between transcriptome profiles and activity of small molecules as the Connectivity Map, to the identification of compounds able to compensate or reproduce the effects on mRNAs typical of the RBPs action. This approach could lead to the repositioning of drugs able to induce phenotypic changes by coordinately affecting post-transcriptional events in the target cell.

The work of André Gerber (University of Surrey) is perfectly framed in the context of the RNA regulon theory, besides his

early participation to the first identification of functional regulons in yeast. Gerber showed convincingly not only that the yeast is a robust model to dissect mRNP networks, but also that it can be exploited to obtain important insights about the biological function of RBPs involved in human diseases. On the first line of his activity, he showed the results of a screening conducted with high-density protein microarrays, which allowed him to identify new RBPs on the basis of their consistent binding to mRNAs.[31] Surprisingly, the larger fraction of these proteins resulted to be represented by metabolic enzymes, a finding recently confirmed by other genome-wide approaches[32,33] after having been suggested by single mechanistic studies in the past. Why molecular evolution repetitively wired in moonlighting proteins such two divergent functions, catalysis of essential small molecules and mRNA binding, awaits further investigation. Another interesting feature of RNA regulons comes from the network representation of 69 yeast RBPs and of their mRNAs, by which Gerber showed a high degree of connectivity (more than 10) and a much higher level of autoregulation than for transcription factors, being about 40% of these RBPs able to bind their own mRNA.[34]

But the study of post-transcriptional networks could also be enlightened by the ability to look quantitatively, and on a large scale, to proteomes, in order to match transcriptome dynamics to corresponding proteome dynamics and, therefore, derive ultimately the degree of impact of these networks on the cell phenotype. This is the direction followed by Matthias Selbach (Max Delbruck Center) on his landmark work correlating steady-state transcriptome and proteome profiles in mammalian cells. A double parallel metabolic RNA/protein pulse-labeling approach, based respectively on 4sU incorporation and SILAC quantitative proteomics, allowed him and his colleagues to measure simultaneously the absolute quantities and the turnover of more than 5,000 genes in murine exponentially growing cells.[35] This simple experimental setup provided a wealth of new information about the architecture of gene expression in mammalian cells. First, from this study, we had confirmation that the dynamic range of protein quantities is much higher than that of mRNAs (five and two orders of magnitude, respectively), as well as the average relative abundance (almost three orders of magnitude higher in proteins than mRNAs). The steady-state correlation between mRNAs and proteins is, in agreement with other studies, around 0.4, but the surprising finding is that this value reaches 0.95 in the proposed quantitative model when considering translation rate constants. This means that translational control is by far the main determinant of gene expression in mammalian cells, more than mRNA synthesis and degradation, which together determine mRNA steady-state levels, and more than protein degradation. This rather unexpected finding becomes obviously of paramount interest in the context of the workshop. Moreover, the average translation rate is 20 times faster than the average transcription rate, while using 90%, again following the proposed model, of the estimated cellular energy involved in gene expression. A picture confirmed by looking at a human cell line which, in conclusion, stresses the chief role of mRNA fate in determining protein abundance.[36]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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