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Gene expression as a circular process

Cross-talk between transcription and mRNA degradation in eukaryotes; International University of Andalusia (UNIA) Baeza, Spain

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Studies on the regulation of gene expression in eukaryotes over the past 20 years have consistently revealed increasing levels of complexity. Thirty years ago it seemed that we had understood the basic principles of gene regulation in eukaryotes. It was thought that regulation of transcription was the first and most important stage at which gene expression was regulated, and transcriptional regulation was considered to be very simple, with DNA-binding activators and repressors talking to the basic transcription machinery. This simple model was overthrown when it became clear that other stages of gene expression are also highly regulated. More recently, other dogmas have started to collapse. In particular, the idea that a linkage between the different steps in gene expression is restricted to processes ongoing in the same compartment has fallen out of favor. It is now evident that functional and physical linkage occurs in eukaryotes. We know that factors contributing to transcription in the nucleus can be found in the cytoplasm, and that RNA binding proteins that contribute to RNA decay in the cytoplasm are present in the nucleus. However, shuttling of such factors between nucleus and cytoplasm has traditionally been thought to serve a simple regulatory purpose, for instance, to avoid untimely activation of a transcription factor in the nucleus. Alternatively, it was thought to be necessary to recruit RNA binding proteins to the relevant RNAs. The notion that is now emerging is that factors thought to have evolved to specialize in regulating a single step of gene regulation in one cellular compartment may be contributing to the regulation of mRNAs at multiple steps along the lifecycle of an mRNA.

The meeting that took place this November at the International University of Andalusia (UNIA) in Baeza, Spain, "Gene expression as a circular process: cross-talk between transcription and mRNA degradation in eukaryotes," brought together for the first time researchers studying this emerging concept of the circuitry of gene expression regulation in eukaryotes. Specifically, they explored the concept that the disparate processes of mRNA synthesis and destruction are in fact interconnected processes that collaborate to regulate gene expression.

That mRNA levels are buffered in eukaryotic cells has been key to the realization that the different stages of gene expression talk to each other. Buffering is defined as the compensatory changes in mRNA synthesis or decay to off set changes in the other to arrive a constant steady-state mRNA level. One critical study in this regard has been that of Itay Tirosh and his collaborators who observed that levels of specific mRNAs were similar in different species of yeast despite very different rates of mRNA degradation. [1] In Baeza, Tirosh presented evidence that buffering of mRNA levels is a conserved phenomenon in eukaryotes since mice and human have similar mRNA levels of given genes despite different transcription rates. His detailed analysis of the widespread compensatory evolution of mRNA decay and transcription rates leading to a buffering of mRNA levels in different yeast revealed the importance of *cis* and *trans* sequences. In *trans*, they map to transcription factors, RNA binding proteins, and components of the transcription and mRNA degradation machines, but mostly to genes encoding Rpb4 or components of the Ccr4-Not complex. In *cis*, they define transcription factor binding sites in promoters rather than mRNA stability motifs, suggesting that the evolutionary changes in *cis* affect transcription directly, and only indirectly mRNA stability. In additional experiments, he was able to find that *cis*-coupling maps to promoter elements rather than 5′UTR elements. The global idea is hence that transcription factors may be talking to Rpb4 and/or the Ccr4-Not complex to ensure buffering of mRNA levels in eukaryotic cells. Along these lines, Mordechai Choder discussed published experiments in which he found that two reporter genes, identical except for the promoter elements, produce identical mRNAs but with different decay rates.[2] His findings indicate that promoter elements dictate mRNA decay rates.

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From Transcription to mRNA Decay

Several participants discussed the communication of the transcription process to subsequent stages of gene expression. These presentations together raised a model whereby integral RNA Polymerase II (RNAPII) must make it through the transcribing unit for the newly produced mRNA to be appropriately controlled during the subsequent stages of gene expression. Integrity of RNAPII, efficient backtracking, and appropriate rescue from backtracking for resumption of elongation, are essential in this regard. Sebastián Chávez explained the importance of backtracking for the overall capacity of the cell to buffer mRNA levels[3], and presented his findings that a cellular component, Sfp1, promotes backtracking across the genome. *SFP1* was identified in a genetic screen for mutations that reduced the sensitivity of TFIIS mutants to elongation stress. Sfp1 is particularly important at highly transcribed genes, such as ribosomal protein genes. Sfp1 lowers RNAPII elongation rate and prevents loss of RNAPII from the transcription unit before it reaches the end. It is therefore important for global mRNA synthesis and stability, hence for cellular mRNA levels. Faster elongating RNAPII is more susceptible to encounter problems during elongation, and reducing elongation rates via backtracking or other mechanisms may allow for mRNA regulatory factors to assemble onto elongation complexes.

One of the first clues that synthesis and decay of mRNAs may be linked came from studies on the Rpb4/7 module of RNAPII in yeast by the Choder laboratory.[4] Loss of contact between Rpb4/7 and the core polymerase impaired the deadenylation of certain mRNAs. At the meeting John Panepinto described the importance of Rpb4 for stability of an ER stress mRNA, *KAR2*, in Cryptococcus neoformans, suggesting that Rpb4/7 is an integral component linking decay and synthesis also in other species. Mordechai Choder reminded us that Rpb4/7 must be associated with the polymerase in the nucleus to be recruited to mRNA 3-'UTRs and contribute to regulation of the mRNAs during their cytoplasmic life. A molecular mechanism for how Rpb4/7 connected these two processes is unknown, but the presentation from Joe Reese suggested that Rpb4/7 is required for Ccr4-Not to interact with RNAPII during, and stimulate transcription elongation. Interestingly, Rpb4/7 may not be the only structural feature of RNAPII important for transcription and decay. Francesco Navarro reported that the foot domain of RNAPII is essential not only for efficient transcription elongation, but also subsequent mRNA decay.

Integrity of RNAPII during transcription is obviously quite dependent upon assembly of RNAPII. Surprisingly, Martine Collart revealed that components of the Ccr4-Not complex themselves are essential for proper assembly of newly produced RNAPII in the cytoplasm and for efficient association of Rpb4 with newly produced mRNAs in the nucleus, which will consequently affect mRNA decay in the cytoplasm.

From mRNA Decay Back to Transcription

Many talks concerned how components of the mRNA degradation machine in turn are connected to transcription in the nucleus. As discussed above, Itay Tirosh and his collaborators determined that perturbation of mRNA decay rates lead to compensatory changes in mRNA synthesis rates.[1] This phenomenon was also observed by Patrick Cramer and his collaborators a year later using the cDTA method.[5] These important observations have raised the crucial question of which components of the decay machinery are most highly relevant for the buffering of mRNA levels. Patrick Cramer presented his results from a systematic testing of synthesis and decay rates in mutants of non-essential components of the decay machinery. The 5′ to 3′ exonuclease Xrn1 is clearly the most central component required for buffering.[6] Cramer reported how if Xrn1 was prevented from entering the nucleus by ligand-induced tethering to ribosomes using the anchors away technology, then the rate of mRNA degradation was minimally changed, but synthesis rates were significantly altered. Inversely inactivating the exonuclease activity of Xrn1 through active site mutations reduced mRNA degradation rates, but the synthesis rate was unchanged. In either case, mRNA buffering capacity was compromised when Xrn1 function was altered. He also reported on the roles of 46 yeast factors in regulating synthesis and decay. Of all the 46 mutants, only a mutant of Xrn1 lost the ability to buffer gene expression fully. Surprisingly, mutants in many decay factors, including Ccr4, showed little to no changes in buffering. After *xrn1*, other mutants of the Ccr4-Not complex, namely *caf1* and *caf40*, or mutants of factors involved in non-sense mediated decay, had the greatest effect. It is possible that some decay factors, such as Ccr4, participate in coordinating decay and synthesis of a fraction of the genome or only do so under stress conditions (see below).

The very essential and primary buffering role of Xrn1 could be explained by Choders' observation that the nuclear import of many decay factors depends upon Xrn1: an enzyme-dead Xrn1 mutant that remains associated with RNAs but does not degrade, means that several degradation factors are not imported into the nucleus, and the impact on transcription is more severe than the knockdown of Xrn1. This supports the notion that the buffering of mRNAs levels by compensatory changes in synthesis and decay rates requires the shuttling of these factors in and out of the nucleus. Consistently, Carol Wilusz presented work from the Wilusz lab in which they observed that the knockdown of Xrn1 in mammalian cells or sequestering it in the cytoplasm on the highly structured flavirus sfRNA reduced mRNA decay rates but did not increase mRNA levels. These observations are consistent with a buffer mechanism requiring the shuttling of Xrn1 as part of the crosstalk between synthesis and decay. Additionally, her results suggest that Xrn1 is a target of cellular pathogens.

The role of many mRNA decay factors in transcription regulation was supported by data from Choder and his collaborators who showed that all decay factors tested shuttle between the nucleus and the cytoplasm, in a manner dependent upon Xpo1 and Mex67, so upon mRNA export. He reported that decay factors such as Lsm1, Xrn1, and Dcp2 are all found on chromatin, and they bind promoters about 30 bp upstream of transcription start sites.[7] Michael Kracht in turn described how in mammalian cells a component of the decapping machine, Dcp1a, mediates the impact of the IL-1 signaling pathway on both mRNA decay and mRNA transcription of specific genes.

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The recruitment of decay factors to promoters suggests that they may regulate transcription initiation. However, this may not be the entire story. Sebastián Chávez was able to define that the transcriptional effect of Xrn1 is also observed at the level of elongation, since Xrn1 mutants accumulate RNAPII in the body of genes that is not competent for run-on and does not have the propensity to become backtracked. Hence, Xrn1 may play a more important role on long or difficult to transcribe genes.

Regulation of mRNAs by RNA Binding Proteins

The concept of RNA regulons has been around for a while,[8] and with it, the notion that mRNAs are somehow imprinted in the nucleus. An interesting new concept that was discussed at this meeting is that RNA binding proteins can impact processing in the nucleus such as to define the subsequent fate of the mRNAs that they regulate in the cytoplasm.

While some talks implicated the promoter regions of genes in the regulation of transcription coupled decay, the 3′UTRs of genes have long been known to be a major contributor of mRNA regulation and play a role in defining RNA regulons. Talks by Jack Keene and Robert Schneider addressed the roles of AU-rich binding proteins in the regulation of mRNAs during cellular responses and human disease. Interestingly, both described a mechanism for coupling transcription and decay involving interesting feedback loops, where AU-rich elements control the abundance of mRNAs encoding transcriptional regulators, splicing factors, and mRNA decay factors. This idea was also put forth by Patrick Cramer who provided evidence that impairing the decay of the mRNA of the yeast global transcriptional repressor Nrg1 led to the downregulation of numerous genes. Collectively, these results provide an alternative mechanism from the imprinting of mRNAs by decay factors during synthesis. Feedback control of mRNAs by AU-rich element binding factors Cth1/2 from yeast was also described. Cth1/2 bind to the 3′-UTRs of genes controlling iron-dependent pathways, including their own mRNAs. Sergi Puig described how Cth1/2 autoregulation is critical for cells to recover from changes in iron availability and this may be dependent on the ability of Cth2 to shuttle between the nucleus and cytoplasm using the mRNA export pathway, thus in association with mRNAs. Interestingly, Cth2 binds its target RNAs in the nucleus and controls their polyadenylation and export. Cth2 has long been known as an mRNA decay factor that functions in the cytoplasm and its additional nuclear roles highlights, again, the cross talks between the nuclear and cytoplasmic stages of gene expression.

One of the first factors identified to regulate mRNAs through the 3′-UTR is the Cytoplasmic Polyadenylation Element Binding protein (CPEB), which is well known for controlling poly(A) tail elongation and translational activation in the cytoplasm. However, Raul Mendez found that this cytoplasmic factor moonlights as a pre-mRNA processing factor that controls the formation of the 3′ ends of mRNAs in the nucleus. Depleting CPEB1 from cells caused the utilization of downstream polyadenylation sites within genes. Furthermore, the CPEB-dependence requires the presence of a CPE element within the gene, suggesting the effect is direct. Thus, a factor once thought to regulate translation and poly A tail length in the cytoplasm has unexpected functions in the nucleus that will determine the subsequent fate of the mRNA in the cytoplasm.

Keeping with the theme of translational regulators affecting gene expression in the nucleus, Maria Vera from the Nudler and Singer labs used live imaging studies using fluorescent eEF1A, a translation elongation factor, and RNA FISH to show that eEF1A and the Hsf1 transcription factor co-localize to the *HSP70* gene in the nucleus under stress and that eEF1A travels with RNAPII during transcription of heat shock genes. She showed that the partial knockout of eEF1A leads to less transcription of *HSP70*. Furthermore, eEF1A associates with the 3′-UTR of the heat shock factor mRNA to regulate its export and ultimately translation in the cytoplasm. She proposed this might be how the *HSP70* mRNA is translated when all others are repressed during heat shock. This specific function of eEF1A at ribosomes during translation, and in the nucleus during transcription to subsequently affect the fate of mRNAs in the cytoplasm is reminiscent of the role of Ccr4-Not complex subunits during translation of subunits of RNAPII that impacts production and stability of mRNAs described by Martine Collart at the meeting.

Modeling the Coordination of Decay and Transcription

Genomics-based approaches have transformed how we study gene regulatory processes. A number of participants described their use of such methods to uncover mechanisms controlling synthesis and decay. Patrick Cramer developed a comparative Dynamic Transcriptome Analysis (cDTA) to measure and model mRNA decay and synthesis rates.[5] This method allows the determination of decay and transcription rates between different conditions or samples, and thereby, can provide information about the roles of different transcription and mRNA decay factors in regulating gene expression. Another method for measuring synthesis was also presented during the meeting. Antonio Jordan from the Perez-Ortin lab reported on a new method to map nascent transcripts in yeast, BIOGRO. Based on the GRO-SEQ method developed in metazoans,[9] it maps the location of RNAPII engaged in the process of transcription and provides a more direct method to study ongoing transcription than metabolic labeling studies.[10]

Steady-state rates are important parameters to establish, but the next step is to determine how the balance and timing of synthesis and degradation rates precisely regulate mRNA levels during normal cellular programs, such as the cell cycle. Cramer measured the synthesis and decay rates of mRNAs throughout the yeast cell cycle and found that the sharp peaks of mRNA abundance is caused by synthesis, followed by a peak in decay rates, of cell cycle-regulated mRNAs. The decay of mRNAs begins with deadenylation, which proceeds with multiphasic kinetics. Cornelia H. de Moor described mathematical models to evaluate poly(A) tail length and showed data consistent with the importance of a deadenylation delay as a means for delayed mRNA degradation to control steady-state mRNA levels in response to serum and inflammation signals.

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Mapping RNA binding proteins using crosslinking and sequencing procedures (RIP-seq) is commonly used to identify mRNA targets of RNA binding proteins. Like many new techniques, efforts are underway to improve them. Jack Keene described some modifications to the Par-CLIP method, which greatly improves its accuracy by incorporating procedural and computational methods for identifying and subtracting inherent background. Commonly used crosslinking procedures require removing cells from their natural environment and subjecting them to stress. Joe Reese described a modified formaldehyde-based crosslinking technique that takes a snapshot of RNA–protein interactions in unperturbed growth conditions in yeast. With this approach he observed that Ccr4, Dhh1, an abundant RNA helicase that is not believed to be a stoichiometric subunit of the Ccr4-Not complex but co-purifies with it[11] and the sequence-specific RNA binding protein Puf5, bound to a common set of mRNAs in vivo and the crosslinking of these proteins to the mRNAs correlated positively with the decay rates of mRNAs under non-stress conditions. Under oxidative stress, Ccr4 was redistributed from housekeeping mRNAs to those undergoing dynamic regulation by synthesis and decay pathways. This suggests that Ccr4, and possibly other decay factors, have a greater importance in coordinating synthesis and decay during stress. Elisa Izzauralde reported on the co-crystal structure of the N terminus of human Not1, hCaf1, hCcr4, and Ddx6 (hDhh1) and found that Ddx6 binds to the N terminus of Not1 on an opposite face of the protein as the Caf1–Ccr4 module. Thus, the binding of Ddx6, and by analogy Dhh1, and Caf1 to the Ccr4–Not complex is compatible, and this may explain why Ccr4 is recruited to essentially every mRNA bound by Dhh1 in vivo, as described by Joe Reese.

Challenges and Perspectives for the Field

The crosstalk between gene regulatory processes, even those performed in different compartments, is well documented now. The next challenge is to define the mechanisms and the factors regulating the process. One model put forth is imprinting, where a factor remains bound to an mRNA throughout its life, imparting regulation at each step.[12] The concept of mRNA imprinting during transcription has gained considerable support, but we still need to understand the nature of the imprinting, whether it is through protein, RNA, RNA modification, or by yet another mechanism. We also need to understand whether this imprinting is “heritable” and transferrable from one cell to another. We have started to identify players that can target multiple stages of gene expression, but we do not understand how these factors select their appropriate targets, or how they function in their “moonlighting” roles. Are there master regulators or does each participant perform its function independently to control these processes? If there is a master regulator, how does it communicate to each individual actor?

While it is relatively easy to grasp how transcription can influence mRNA decay rates, for instance, by mRNA imprinting, it is much less evident how changes in mRNA decay might influence transcription. One model put forth by the Choder and Chávez teams proposes that decay factors relocate to the nucleus after release from cytoplasmic mRNAs following decay and, consequently, affect transcription directly by binding to promoters. Cramer invokes an indirect mechanism whereby a global transcriptional repressor is upregulated by reduced mRNA decay. The increase in the transcriptional repressor represses transcription. While no less significant for the physiology of cells, this is a distinct mechanism from those requiring direct communication between the processes of synthesis and decay.

Obviously, there may be mechanisms yet to be discovered. This newly emerging field of the circuitry of gene expression is fascinating in its complexity. The questions opened by many observations discussed during this meeting promise to open exciting new areas of investigation and findings in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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