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Post-transcriptional RNA Regulons Affecting Cell Cycle and Proliferation

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Abstract

The cellular growth cycle is initiated and maintained by punctual, yet agile, regulatory events involving modifications of cell cycle proteins as well as coordinated gene expression to support cyclic checkpoint decisions. Recent evidence indicates that post-transcriptional partitioning of messenger RNA subsets by RNA-binding proteins help physically localize, temporally coordinate, and efficiently translate cell cycle proteins. This dynamic organization of mRNAs encoding cell cycle components contributes to the overall economy of the cell cycle consistent with the post-transcriptional RNA regulon model of gene expression. This review examines several recent studies demonstrating the coordination of mRNA subsets encoding cell cycle proteins during nuclear export and subsequent coupling to protein synthesis, and discusses evidence for mRNA coordination of p53 targets and the DNA damage response pathway. We consider how these observations may connect to upstream and downstream post-transcriptional coordination and coupling of splicing, export, localization, and translation. Published examples from yeast, nematode, insect, and mammalian systems are discussed, and we consider genetic evidence supporting the conclusion that dysregulation of RNA regulons may promote pathogenic states of growth such as carcinogenesis.

Keywords

postranscriptional regulation; RNA-binding proteins; translational control; p53; DNA damage; cancer

1. Introduction

Post-transcriptional RNA regulons consist of RNP complexes that coordinately regulate the production of functionally related proteins [1,2]. The primary functional role of RNA regulons is to respond to endogenous and exogenous cell signals efficiently and

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economically in order to help regulate components of biochemical pathways or macromolecular protein complexes; for example, subunits of cellular pumps, organelles, or other machines that function together in cellular locations. RNA regulons consisting of RNAs and RNA-binding proteins have been widely documented to coordinate post-transcriptional processes in many species; the general topic has been broadly reviewed in recent years [3–17]. Therefore, this article will focus on RNA regulons/PTRs identified to date that are involved in coordinating the production of proteins regulating cell cycle initiation and progression.

The concept of Post-transcriptional RNA Operons and Regulons, generally referred to as PTRs or RNA regulons, invokes the classical bacterial operons discovered by Jacob and Monod [18] as a didactic tool to make it easily understood. However, there are significant differences between the classical operons and regulons of bacteria and RNA regulons. These differences are not bacterial versus eukaryotic, as RNA regulons have recently been described in archaea and bacteria [19–24]. Instead, they are due to the fundamental differences in the organization of DNA and RNA in the cell. While DNA genes in bacterial operons are constrained by covalent architecture and physical location in the nucleus, RNAs are diverse in quantity and location, affording them multiple combinatorial interactions and transient regulation. This variability in quantity and localization of RNAs affords RNA regulons many additional properties. First, multiple copies of each mRNA are capable of joining many different RNA regulons simultaneously based on different recognition elements in the RNA (RREs). Second, RNA regulons are based on reversible interactions between RNA binding proteins (RBPs) and RNAs, and RNAs are disposable. Therefore, RNA regulons are highly dynamic and the RNPs can be remodeled rapidly because the RNAs themselves are combinatorial. Third, RNA regulons offer the property of localized dynamic regulation. This is particularly important in eukaryotes, as cellular compartmentalization requires greater control of localization among different organelles. In sum, multiple copies of each mRNA can use their multiple recognition elements or RREs to join other groups of functionally related mRNAs in time and space in order to perform multiple functions in combination. These overlapping mRNA networks have modular coherence because the RNPs that form the RNA regulons can be remodeled with different functional states depending on signal inputs [25–29].

These properties of cytoplasmic, functionally related RNPs we termed “quasi-genomes” to describe their oft remote locations in cells [1,2]. The combinatorial use of functionally related mRNAs is highly plastic in real-time but that plasticity is not available to classical operons and regulons in bacteria. Perhaps a better RNA regulon analogy may be to music, where mRNAs equate to individual notes from each instrument that must be brought together in different coordinated combinations in time and space to produce a melodically orchestrated symphony. Moreover, imaginative composers can rearrange the notes and instruments to produce different musical scores just as RNA regulons provide an avenue of evolutionary opportunity for proteins to gain new functions by rewiring existing RNA recognition elements or proteins, or evolving new ones [30–32]. A good example of RNA-protein rewiring have been observed with PUF3 family RBPs, with changes in classes of regulatory targets within fungal species [31,32] as well as across many other metazoan species [30,33]. Presumably as a consequence of these myriad advantages, RNA regulons

have evolved to play functionally important roles in nearly all species across the three domains of life [3]. Indeed, the dynamic plasticity of RNA regulons are just beginning to be understood [1,2,7,34–37].

It was previously assumed that every mRNA species functioned independently in the eukaryotic cell and was regulated independently by distinct RBPs. But, as noted above, we now understand that with few exceptions every mRNA is a member of one or more RNA regulon(s) and most RBPs regulate large numbers of mRNAs [3,38–41]. This is because each mRNA has multiple copies and each one of those copies has the potential to associate with other mRNAs that encode proteins that are functionally related [17]. Thus, mRNAs are themselves combinatorial, meaning that different combinations of mRNAs can form different RNA regulons depending on the needs of the cell in time and space [2]. The first studies to indicate that RBPs can target multiple growth regulatory mRNAs used *in vitro* RNA binding methods and Selex procedures with random RNAs [42–46] or human brain extracted RNAs [38]. The first *in vivo* evidence indicating global coordination by endogenous RNA regulons was derived developing the RNA immunoprecipitation (RIP) followed by microarray procedure with mammalian neuronal ELAV/Hu proteins known to be involved in cell growth and differentiation [25]. This study used both DNA arrays and multichannel RNase protection assays to verify mRNAs encoding cell cycle factors among the mRNA target sets of ELAV/HuB and HuR including n-myc, l-myc, b-myc, max, and cyclins A2, B1, C, D1 and D2, but not cyclin D3, cyclin B2 [25]. These findings were consistent with concomitant findings by Gorospe and colleagues showing that cyclin A and B mRNAs were bound and stabilized by HuR during cell proliferation [47]. These findings were later confirmed using the global RIP-chip procedure of Tenenbaum et al. with microarrays [48].

The use of global post-transcriptional procedures like RIP-chip/seq [25,49], temporal RNA decay and global nuclear run-on (reviewed in [13]), polysome profiling and cell synchronization have been used to identify cellular and developmental RNA regulons [3–15]. RNA regulons have been shown to coordinate cellular processes during cellular homeostasis and normal replicative events such as cell cycle, as well as during developmental programs, biological activations, or environmental perturbations. Many RNA regulons have also been discovered using genetic approaches while others were observed as dynamic patterns of mRNA co-regulation during a regulatory event. The trypanosome life cycle field has employed this latter method extensively, followed by identifying the key RBPs or global coordinators [5,16,28]. Through these techniques, many examples of RNA regulons have emerged over the past decade at nearly every step of post-transcriptional RNA processing, including splicing, export, translation, RNA stability and localization [6,10].

A major unresolved question, however, is whether RNA regulons are directly or indirectly coupled to one another at each step along the path of RNA processing and how mRNA coordination via RNA regulons maintains a consistent mRNA target set as the information flows from the chromatin to the ribosome [6]. In other words, how is mRNA traffic directed through the RNA processing pathway? It would seem efficient for mRNAs encoding functionally related proteins to flow together from step to step in a pathway when they are destined to function together at the final steps of gene expression. Much of the information

regarding the coupling of each of these processes is based upon single mRNAs. However, evidence for coupling of post-transcriptional interactions globally is beginning to accumulate, and among the most compelling examples are from experiments with *Saccharomyces cerevisiae* RNA regulons.

2. Global RNA regulons in budding yeast

Silver and colleagues presented the first solid confirmation of the RNA regulon model in 2003 [50]. They demonstrated that functionally related mRNAs associated with two yeast RNA export factors, Yra1 and Mex67 coordinately export multiple functionally related mRNAs to the cytoplasm [50,51]. For example, Yra1 associated largely with cell-cycle regulatory factors and proteins involved in carbohydrate metabolism, Mex67 associated with mRNAs encoding membrane proteins, cell-wall components, and RNA-regulatory factors. This latter set of Mex67 exported mRNAs continues to be a consistent finding that many RBPs interact with mRNAs encoding regulatory RBPs, translation factors, and transcription factors. This concept of broad regulation of regulatory factors has been termed “regulators of regulators” as RNA regulons [25,52,53]. While Yra1 associated with approximately 1,000 mRNAs and Mex67 associated with approximately 1,150 mRNAs, they shared 349 overlapping transcripts. This was consistent with results from Tenenbaum et al. [25] showing that RBPs such as HuR and eIF4E associated with unique as well as overlapping transcripts. This would be expected if each mRNA has more than one RRE or USER code, thus, demonstrating that networks of mRNAs could be modular and overlapping [2]. An exciting aspect of the work by Hieronymus and Silver [50] was the connection between the Yra1 mRNA export factor and the Abf1 transcription factor. Not only does the Abf1 transcription factor regulate the Yra1 gene, but the Abf1 mRNA is exported by Yra1 protein. This was confirmed genetically suggesting regulatory crosstalk between transcription and export.

A longstanding curiosity has been whether mRNAs exit the nucleus randomly as they are passed from the RNA processing apparatus through nuclear pores or whether they are organized into functionally coherent subpopulations. An early study from the Cole lab demonstrated mRNA-specific regulation in RNA export. Using yeast mutants of the nucleoporin Rip1p, mRNAs encoding certain heat shock proteins were efficiently exported despite global polyA⁺ mRNA export suppression under stress conditions [54]. A unique nuclear pore complex containing Rip1p along with several nucleoporins exports heat shock mRNAs to the cytoplasm. Differential mRNA export of this type likely involves distinct RNA-binding surfaces, although it is possible that some of the Nup proteins themselves either possess RNA-binding properties or are coupled with specific RBPs, possibly coupled to the splicing apparatus [55]. While several examples of pre-messenger RNA splicing regulons and RNA export regulons have been published, demonstrating coupling of these RNA regulons at these steps along the pathway of RNA processing has remained challenging. As discussed below, a subsequent study from the Silver lab using *Drosophila* cells [56], also showed export of heat-shock factors whose mRNAs are uniquely exported like those of Saavedra et al. [54] but have known roles in splicing via the PCI domain-containing protein-2 that subsequently associates with polysomes. However, it is not known

from this work whether the subsequent events downstream of the heat-shock specific export mechanism are coordinated as an RNA regulon(s).

Brown and colleagues discovered that nuclear-encoded mitochondrial mRNAs bound to the PUF3 RNA-binding protein are exported out of the nucleus to mitochondria, undergo derepression and translation, allowing the new functionally related proteins to enter the mitochondrion [34]. Derepression of factors already positioned for activation at a remote site is an efficient and accurate means to coordinate a group function. Several subsequent studies confirmed and extended these findings using genetic and biochemical methods [57–59]. Moreover, the Brown laboratory followed up this initial discovery by identifying a total of 46 RBPs of *S. cerevisiae* that also bound to unique modular subsets of mRNAs encoding functionally related proteins [60].

In addition to these early confirmations of the RNA regulon model, many additional distinct biological processes, pathways and macromolecular complexes across the three domains of life have been shown to be regulated together at the mRNA level, and in nearly every case regulatory RBPs involved in coordinating these functions were identified [3,10,13,61] (see review articles noted above). Below, we focus on cell cycle functions in yeast and mammalian cells that are potentially coordinated as RNA regulons at the levels of RNA export and translation/stability.

3. Nuclear export RNA regulons during the cell cycle

Many published examples of splicing events functioning as RNA regulons have suggested that discrete subsets of mRNAs are coordinately spliced, associated with RBP complexes such as the exon junction complex and eventually exported to the cytoplasm [62–64]. In addition, there are definitive examples of very early nuclear RNA imprinting such as the exon-junction complex that helps determine the final fate of a given mRNA, as well as the fate of functionally related subsets of mRNAs localized for translation in the cytoplasm [15,65,66]. Several studies demonstrated the association of export proteins with mRNA subsets in organisms from yeast to flies to mammals, some of which include mRNAs encoding cell cycle and proliferation factors [9,50,51,56,67].

For example, Guthrie and colleagues demonstrated that hnRNP-type RNA export factors Nab2, Nab4 and Npl3 in *S. cerevisiae* each bind to their own unique specific subset of mRNAs [67]. Using the RIP-chip procedure, they found that Npl3 preferentially binds to ribosomal protein mRNAs, while Nab4 strongly prefers mRNAs encoding factors involved in amino acid metabolism. The latter observation was consistent with genetic mutants of Nab4 that also showed a desensitized growth phenotype when exposed to amino acid stress. As with other RIP-chip studies, they identified a common set of motifs: AAAAG prevalent in Nab2 and UAUAUAA or ACACTACA in Nab4 mRNA targets. The authors concluded that there are multiple routes of mRNA export for functionally related mRNA subsets, and hypothesized that Npl3 may be responsible for the bulk mRNA export given that many hnRNP proteins associate with it, but that these balances may shift during stress conditions. In addition, the authors suggest coupling of RNA export with 3' end processing via interactions between Nab2 and polyA-binding protein. It was not clear whether the 3' ends

of mRNAs encoding the functionally related proteins would be coordinately cleaved in a simultaneous or sequential order by cleavage factor 1 in concert with their coordinated export to the cytoplasm via these hnRNP proteins. In any case, this is landmark paper presents evidence that these important proteins can export distinct combinations of mRNA cargos.

As noted above, using a genome-wide RNA interference screen, Silver and colleagues identified 72 factors required, some required for differential nuclear export in *Drosophila*, and examined specific mRNAs encoding heat shock factors that are in turn, coupled to polysomes and translation via a PCI domain protein that associates with RNA [56]. These findings, while not global, have strong implications for coupling of RNA regulons from splicing to translation.

4. Nuclear export RNA regulons in mammalian cell cycle

4.1. eIF4E RNA regulon of cell cycle and proliferation

Specific nuclear pore components have been shown in mammalian cells to be unique for export of mRNA subsets encoding proteins regulating cellular growth, DNA damage and cell cycle regulation [68–74]. For example, the well-studied cap binding protein, eIF4E, is a major regulator of protein synthesis and has mitogenic and oncogenic properties discovered by Sonenberg and colleagues and confirmed in many laboratories [15,75–78]. The overexpression of eIF4E increased Ras activation and it could be reversed by expression of GTPase activating protein, a known negative antagonist [75]. More recently, Borden and colleagues reported that eIF4E associates in the nucleus with RNPs that mediate nuclear export of discrete mRNA subsets as a RNA regulon via a CRM1-dependent mechanism [68]. The mRNA targets of this RNA export regulon were identified by a RIP-chip/seq type immunoprecipitation and included several cyclins, mdm2, c-myc and other cell cycle-related factors. These mRNAs were found to have a specific stem-loop RRE they termed “4E-SE” functioning as a USER. The authors examined the 3D structural features of the stem-loop and confirmed its structure using nuclease probes, and demonstrated that the 4E-SE was required to form stable RNP complexes using cyclin D1 as their model. They concluded that eIF4E, acting as a RNA regulon, could modulate the coordinated expression of these cell cycle proteins efficiently, thereby impacting proliferation and cell survival.

4.2. Nup96 mRNA subsets encoding cell cycle regulators

A study in the Blobel and Matunis labs revealed that a polycistronic cDNA encodes a precursor protein containing both the Nup98 and Nup96 nucleoporins [79]. Further analysis indicated that this precursor protein is cleaved by a protease to yield these two nucleoporins that were later found to have related functional roles in nucleocytoplasmic transport. Interestingly, both proteins have roles in cell cycle, p53 pathways and carcinogenesis in many species [69,71,73,80,81]. In acute myeloid leukemia, a translocation creates a fusion protein between Nup98 and a homeodomain DNA binding domain that blocks the normal functioning of Nup96, and thereby, disrupting its putative tumor suppressor function as discussed below in section 4.3. from the data of Singer et al. [73]. Nup98 can shuttle into and out of the nucleus and appears to interact directly with the chromatin to affect

transcription of cell cycle genes in *Drosophila* [71,81]. In any case, both Nup96 and Nup98 are suggested to function as part of post-transcriptional RNA regulons coordinating transport of mRNAs encoding cell cycle, development and oncogenesis.

Nup96 has been shown to have several important functions and is a component of the Nup107–160 complex that is cell cycle regulated. In some cases, components of the complex have different stoichiometries in the nuclear pore during stages of the cell cycle [69]. Case in point, at mitosis the Nup96 subunit is downregulated preferentially as a requirement for T cells to exit G1/S phase of the cell cycle and in heterozygous mutant Nup96 mouse T cells that express lowered levels of Nup96, cell cycle progression increased. Moreover, levels of cyclin A were also downregulated early in mitosis, while cyclin B was increased in these T cells. In the Nup96^{+/-} heterozygous mouse cells, decreased Nup96 increased the rate of cell cycle progression that correlated with failure to export specific mRNAs that encode cell cycle regulators. These mRNA targets of Nup96 associated with cell cycle dysfunction included cyclin D3, CDK6 and IκBα that accumulated in the cytoplasm while the nuclear/cytoplasmic ratio of GAPDH, ICAM-1 and α-tubulin did not change when Nup96 levels were lowered [69,80]. The cytoplasmic predominance of cyclin D3 and CDK6 mRNAs resulted in increased production of their proteins and premature activation of S phase.

While it appears that Nup96 suppresses export of these mRNA specific subsets, these investigators suggested that Nup96 could be a potential RBP. However, further studies are needed to define how these cell cycle-specific mRNAs are selected from the up-stream processing apparatus and moved through the pipeline. These findings led Wozniak and Goldfarb [80] to suggest that these findings fit the criteria for a RNA regulon and speculated that Nup96 itself could be the transacting RBP that controls the RNA regulon. How discrete subsets of functionally related mRNAs are able to locate and associate with the nuclear pore subunits is not known but could involve a coupling of export to the nascent splicing apparatus that are themselves function as an early organizational step in the RNA regulon pathway of coordination [6,62].

4.3. Nup98 mRNA subsets encoding cell cycle regulators

Nup98 is another astounding example of a potential RNA export regulon that is involved in cell cycle and p53 regulation in mammalian cells. Prives and coworkers reported that Nup98 is required for the expression of the p21 (CDKN1A) oncogenic protein on the post-transcriptional level and thereby affects several proteins in the p53 pathway [73]. In an RNA interference screen, Nup98 appeared to be involved in targeting of p53 regulated genes. Previous studies demonstrated a link between Nup98 and leukemia via an unrelated mechanism [81]. However, it is exceedingly interesting to note that Nup98 and Nup96 (discussed above in section 4.2.) are products of a cleavage event of a precursor protein [79–81]. The p21 regulation reported in the Prives Lab showed that Nup98 forms an RNP complex with p21 mRNA via its 3' UTR and prevents degradation of the p21 message and can be rescued by blocking the RNA degradation exosome system. Using a RIP procedure, the 3' UTR of p21 was shown to directly bind to Nup98 in HepG2 cells, and further analysis suggested a C-rich RRE that was also present in other mRNAs. For example, their data

based on computational analysis indicated that mRNA encoding the 14-3-3 σ protein, another p53 pathway factor, is similarly bound to Nup98 and protected from degradation. Moreover, the authors confirmed using RIP-seq and qPCR that the 14-3-3 σ protein has a similar C-rich RRE for binding. They went on to demonstrate that Nup98 is required for cells to undergo p53 mediated senescence and could partially protect cells from DNA damage-mediated apoptosis. Nup98 as well as 14-3-3 σ levels of expression were both decreased in concert following DNA damage as demonstrated using murine hepatocellular carcinomas and in a cohort of patient samples [73]. One important conclusion is that if Nup98 is a tumor suppressor, it would be advantageous for cancer cells to inactivate Nup98 during their evolution. It will be interesting to see how the functions of Nup98 are compromised among diverse collections of carcinoma or leukemia cells.

In summary, the findings that Nup98 can function as a RBP that binds to a subset of p53 target genes and regulate the stability of their mRNAs is both exciting and unique. It is a potentially separate oncogenic pathway whereby Nup98 can regulate p53 genes as a tumor suppressor. The authors suggest that this may represent a p53 post-transcriptional RNA regulon that couples with p53 transcriptional regulation to coordinate otherwise normal functions which are potentially dysregulated at several stages along the gene expression pathway. It will be interesting to use genome wide RIP-chip/seq and PAR-CLIP methods to determine the global set of mRNAs that interact with Nup98 under various growth conditions. Other more diverse examples of RNA regulons and RBPs affecting cell cycle and DNA damage responses are discussed in the next section.

5. Coordinated RNA targeting in cell cycle, p53 and DNA damage

5.1. RNA regulons responding to the DNA damage response

Among the most prevalent mutational events in cancer involves the checkpoint kinase, Ataxia Telangiectasia Mutated (ATM) [82]. The ATM response activates when DNA undergoes double stranded breaks by irradiation, oxidative stress or other forms of physical or chemical assault causing the kinase to phosphorylate a series of cell cycle checkpoint regulators including check point kinase 2 or Chk2 and other proteins in the p53 pathway involved in the DNA damage response and repair. This response helps to minimize the effects of DNA damage by delaying the cell cycle or inducing apoptosis to eliminate damaged cells. Several RBPs including HuR, AUF1, TIAR, RPL26, nucleolin and others are documented to respond to induced stress and DNA damage [70,72,74,83–85], in some cases, becoming relocalized to stress granules. The role of stress granules resulting from oxidative stress is believed to temporarily store RBPs and their RNA cargo in order to protect the mRNAs until the DNA is repaired [86]. For example, after exposing mammalian cells to UVC light the levels of p53 mRNA did not change, but translation of its mRNA to protein was increased. The increased production of p53 under these conditions was shown to involve the HuR RBP acting on the 3' UTR of the p53 mRNA. These results were confirmed using RNA interference against HuR to demonstrate the expected inhibition of p53 production. It is logical to expect that when the synthesis of p53 protein is altered, the downstream transcription targets of p53 would be affected accordingly.

Gartenhaus and coworkers took advantage of these observations to examine whether HuR participates in the ATM response on a global level via RNA operons and regulons [29]. The Chk2 kinase has been shown to phosphorylate HuR at residues 88, 100, and 118, and thereby alter its specificity of binding to the SIRT1 mRNA following oxidative stress induction. A previous model was generated in the Gorospe lab proposing that ATM and Chk2 cooperate to regulate the binding of specific mRNA subsets by HuR [87]. Using B-lymphocyte cell lines and a RIP-chip ribonomics approach, followed by confirmation of individual target effects, Gartenhaus and collaborators concluded that modular networks of HuR regulated targets encoding functionally related proteins of the p53 and other growth pathways are coordinated globally in responses to DNA damage and stress [29]. For example, this study identified numerous mRNA targets of HuR that encoded proteins involved in cell proliferation, cell cycle, cell arrest, MAP kinase regulation and stress responses. Among them was p21, FOXO3, MEK1, MEK2 and DUSP10 whose mRNA binding was directly assessed for downstream ATM/Chk2 signaling using HuR mutants at Chk2 phosphorylation sites S88A, S100A and T118A, as well as testing the effects of Chk2 inhibitors in the presence or absence of irradiation. Overall, this network of mRNA targets represented an ATM/Chk2 cascade affecting the phosphorylation state of HuR that subsequently targeted functionally related mRNA subpopulations encoding factors involved in cell proliferation and cell cycle regulation.

These dynamic datasets were similar to earlier studies in which the Gartenhaus group compared HuR and AUF1 RBPs and demonstrated changes in overlapping functionally related mRNA subsets using MCT-1 oncogene-mediated transformation of immortalized breast epithelial MCF10A cells [88]. This work demonstrated the combinatorial binding of multiple RBPs to some of the same mRNAs as suggested in other studies [25,27,34,74], and others), but also showed that overlapping mRNA targets of HuR and AUF1 involved in cell cycle and cell signaling pathways were differentially expressed during tumorigenesis [88]. In sum, these global mRNA targeting studies with ELAV/Hu proteins have consistently identified many of the same mRNA targets encoding cell proliferation and cell cycle proteins in support of the RNA regulon model and many of its dynamic properties.

5.2. RNA regulons targeting p53 and DNA damage at translation

A striking study from the Tofilon laboratory comparing transcriptional with translational regulation of genes expressed in glioblastoma cell lines following gamma irradiation revealed that a response at the level of the ribosome determines the changes and obviously the differences in protein production [89,90]. The initial study compared three independently derived cell glioma lines before and after 7 Greys of irradiation using mRNA analysis by microarray [89]. By quantifying mRNAs that increased or decreased in each of these cell lines after irradiation, definitively demonstrated that about 500–600 RNAs shifted on polysome gradients from the inactive to the translationally active regions, while only about 100 mRNAs changed in the transcriptome (total mRNA) data. Moreover, comparing the three cell lines showed zero overlap among the transcriptome mRNAs that changed, but approximately 300 RNAs that changed consistently among the translome datasets. These dramatic results are compelling and unexpected, and were confirmed and extended in a subsequent paper that compared other types of cancer by these criteria [90].

Lu et al. [89] used western blotting to confirm that the protein products of the mRNAs found to increase or decrease their positions on polysome gradients corresponded to the observed up or down shifts of the mRNAs. As one might expect, the identification of these mRNAs and their encoded proteins showed that they were functionally related. For example, the key proteins included primarily cell cycle regulators, cell death, repair and cell proliferation factors [89]. As with other studies of this type, many DNA and RNA regulatory proteins were included as well reminiscent of the regulators of regulators findings. The authors went on to suggest that these results are consistent with the post-transcriptional RNA regulon model, and discussed translation factors such as eIF4E and 4E-binding proteins as well as RBPs like fragile X mental retardation and CUGBP that could be involved in globally coordinating these growth regulatory mRNAs.

5.3. Global translational dynamics during the cell cycle

While many RNA regulons have been described in the literature to date, those coordinating cell cycle and cell proliferation, and those implicated in oncogenesis are compelling. Moreover, the global role of translational RNA regulons are particularly exciting because the coordinated production of functionally related proteins is the most important end game for biological systems [15]. A recent study by Ruggero and colleagues provided strong evidence for translational events determining the expression of cell cycle progression, lipid metabolism and the DNA damage/repair pathways at each stage of the cell cycle [91]. Several previous reports characterizing changes in mRNA populations throughout the cell cycle have identified responsive gene sets.

To examine translational regulation during the cell cycle, Stumpf and coworkers [91] used ribosomal profiling/occupancy and high-throughput sequencing of RNA from synchronized HeLa cells to identify mRNAs unique to G1, S phase, and mitosis. The authors found that 12% of the transcripts in the cell change ribosome occupancy (higher or lower) at some stage of the cell cycle. As expected, during mitosis there were fewer changes in mRNA levels in either G1 or S phases together or separately. They identified “core” groups of mRNAs that change in all phases of the cell cycle. The patterns of clustering transcripts with ribosome occupancy were distinctly different than the mRNAs expressed by the transcriptome, suggesting to the authors that the two processes are uncoupled during the cell cycle. While there were features of the 5' UTR such as GC content of certain transcripts noted these were not deemed to be the chief determinants of cell cycle translational changes. On the other hand, specific mRNAs encoding known regulators of cell cycling were identified. These included increases in cyclin CCNE2 mRNA translation in G1 and S phases, and decreases in occupancy of the E2F1 transcription factor mRNA, possibly resulting from microRNA functions.

The authors noted distinct changes in ribosome occupancy of mRNAs encoding mitotic checkpoint factors and strong increase in translation of the RICTOR subunit of the mTOR complex 2 from G1 to S phase that was shown to be dependent upon its 5' UTR. They noted a total of 353 differentially expressed mRNAs between the three phases of G1, S and M. There was clear evidence that these transcripts encoded functionally related cell cycle regulatory proteins. Thus, the authors constructed modular network models that coordinate

the translation of nuclear transport, metabolism, cohesins, condensins and DNA repair components. In sum, this is a very interesting study that demonstrates dynamic changes in the translome during the cell cycle consistent with our own concept of RNA regulons [15].

5.4. Developmental RNA regulons of p53 and cell cycle pathways

There have been various examples of RNA regulons found in cell lines and several that appear to coordinate various processes in tissues and organs [3,10,13]. Indeed, one would expect that RNA regulons would be an efficient mechanism to optimize multicellular organismal development and several examples are in the literature. With respect to cell cycle regulation in developing systems, we will consider a *Caenorhabditis elegans* GLD1 RBP that coordinates cell cycle mRNAs in developing embryos, a mouse Pumilio 1 RBP that coordinates components of the p53/MAP kinase/cell cycle pathways during spermatogenesis, and the DND1 (dead end homolog 1) RBP that affects cell cycle arrest and differentiation of testicular teratomas in mouse.

The *C. elegans* GLD1 RBP is a member of the STAR family of RBPs that are regulators of germ cell development and can function as tumor suppressors. GLD1 mutants affect sex determination, mitosis-meiosis decisions and germ cell identity and have phenotypes involving cell division, cell cycle and DNA replication, and appear to function as translational repressors. Rafal Ciosk and coworkers used RIP-chip to quantify GLD1 RBP-mRNA target binding strengths in whole worms and by confirming with cell lines to identify mRNA targets and establish approximate binding affinities [92]. Using cells transfected with epitope tagged GLD1, extracts of transgenic adult worms were used for the RIP procedure and mRNA targets quantified by microarray. These experiments took advantage of the earlier methods that allow one to use cell type-specific RBPs to RIP mRNAs away from endogenous RBPs in the same tissues [25,52]. The data were highly reproducible ($r = 0.96$) and nontagged controls were used, and the previously demonstrated heptanucleotide repeat UACUAAAC/U with minor sequence variants.

Approximately, 950 mRNA targets were identified that encoded functionally related proteins that participate in cytokinesis, cell division, reproduction, cell cycle and DNA replication in a manner consistent with the RNA regulon model. Among the targets, it seems that GLD1 may relay its tumor suppression by translationally repressing cyclin E mRNA, and possibly B-type cyclins as well [93]. Overall, these functional groups were deemed to be consistent with meiotic cell cycle progression, sex determination and cell fate specification phenotypes observed in earlier studies [92,94]. Not only were the authors able to monitor dynamic changes in individual targets in the embryos, they were also able to quantify apparent binding affinities of the targets in the extracts based on binding motifs using fluorescence polarization, and to match them with the in vitro binding and translational repression of the targets by GLD1. Interestingly, a GLD1 PAR-CLIP study in *C. elegans* by Rajewsky and coworkers [95] found an approximate 70% identity with the GLD1 RIP-chip mRNA targets of Wright et al. [92] and they represented the same functionally related proteins consistent with the RNA regulon coordination model.

The Capel laboratory investigated the functions of DND1 RBP in formation of mouse testicular teratomas, and its role in cell cycle arrest as well as the differentiation of

primordial germ cells (PGC). The DND1 RBP is necessary for preventing cell death of primordial germ cells in gonads, as demonstrated using PGC defects in mouse embryos with DND1 *Ter* mutations [96,97]. These investigators show that DND1 regulates mitotic arrest in male PGC cells at the level of translation acting principally on cell cycle genes. They hypothesize that the balance of positive and negative cell cycle regulators, consistent with the observation that cell cycle arrest by DND1, is able to prevent tumor formation. Using the RIP-chip procedure with DND1, they identified functionally related mRNA subsets that regulate the cell cycle and tumor-suppressor signaling pathways in mouse germ cells at stage E14.5. In particular, mRNAs encoding p21, p27, Lats2, p53 and Pten, representing cell cycle inhibitors were highly enriched in the RIP-chip study. On the other hand, Akt2, Akt3, cyclins E2, cyclin E1 and Akt1 representing cell cycle activators were not enriched. The authors conclude that these results support the notion that DND1 regulates cell cycle events that are consistent with its established phenotypes as a RNA regulon [97].

Another related example of a cell cycle regulatory RNA regulon was discovered in the Lin laboratory while investigating loss of spermatocytes in the mouse germline [72]. The Pumilio 1 mammalian gene is a member of the PUF RBP family that is known to suppress expression of mRNAs to which they bind [30,33,34]. During spermatogenesis in the testes an overwhelmingly large number of sperm cells is generated by mitosis and must be eliminated through apoptosis to keep the organ healthy. Genetic deletion of Pumilio 1 in mice causes increased activation of p53-mediated apoptosis, resulting in reduction in sperm numbers and infertility. On the other hand, deletion of p53 in the Pumilio 1 null mice decreased apoptosis and rescued this testicular hypotrophy phenotype. In this study, RIP-chip studies of Pumilio 1 targeting in testicular lysates revealed that it interacts with mRNAs that encode p53 pathway, cell cycle and MAP kinase-signaling components, and represses their translation [72]. Using a variety of cell imaging and molecular techniques, the authors confirm these targets in vitro and in vivo and report that a distinct subset of these mRNAs encode key members of the p53 and cell cycle pathways, functioning as a Pumilio 1 RNA operon/regulon.

6. Conclusions and common themes

A theme of this article is the challenging problem of understanding how each step of the RNA processing pathway is coupled physically to the next step, and whether RNA regulons at each step are maintained and/or transferred down the pathway. The traditional assumption of many biologists in the past has been that transcription is the sole coordinator of cellular events and that protein production is generally a more passive process. But while transcription is the initial process that starts gene expression, accumulating evidence supports a view that transcription and translation are intertwined and co-dependent [1,6,13,15,98,99]. Both processes appear to be coordinated independently, but operate co-dependently between the nucleus and the cytoplasm because every transcription factor must be translated and every translation factor must be transcribed. It would seem logical to propose that early events at the level of transcription actually derive some of their specificity from transcription factors that were translated in response to cellular needs such as stress responses, possibly triggered by events in the cytoplasm. Such a model seems perfectly reasonable when considering that neurons have their protein synthesis machinery

out in dendrites, far from the nucleus and many of the mRNAs in dendrites encode transcription factors [1,6,99,100]. And the same may be true of all eukaryotic cells, albeit at shorter distances. Thus, one would expect this global “coupling” to be coordinated by DNA-binding proteins and RNA-binding proteins, and many examples of such coupling have been reported [13,101]. Indeed, there may exist a biological clock that cycles with the crosstalk between the nucleus and the cytoplasm, and one could speculate that intracellular homeostasis correlates the cell cycle with the conditions in the cytoplasm possibly using the Dream complex [102]. Thus, RNA regulons would participate in communication across cellular compartments to deliver functionally related mRNAs and proteins as needed for such crosstalk [6,99].

Another theme of this article is that RBPs can in many cases function as repressors of multiple mRNAs encoding proteins involved in cell cycle regulation. Logically, one would expect that functional mutations in such repressors would allow cells to grow uncontrollably. Of course, RBPs, along with transcription factors are just one of many safeguards that support cell cycle checkpoints. Nonetheless, there are examples of mutations in RBPs that lead to tumors as illustrated above with the *DND1 Ter* mutation that fails to regulate the translational RNA regulon of cell cycle inhibitors leading to teratomas in young males [96,97]. Similarly, the Pumilio 1 protein in mouse discussed above functioning as a RNA regulon suppresses activators p53-mediated pathways and cell cycle factors in mouse spermatocytes causes growth defects that activate p53-mediated apoptosis, disrupts sperm production and fertility when functionally mutated [72]. Moreover, the tumor suppression properties of nucleoporins such as Nup98 functioning as a RNA regulon may involve its dysfunctional ability to support cell cycle checkpoints when mutated [73]. Thus, it is possible that the coordination of the RNA regulons described in this article is achieved through reversing repression of a subset of functionally related cell cycle proteins. However, when the RBP driving the regulon is mutated, dyscoordinated production of these cell cycle regulators results in defective cycling and proliferation, and increases the probability of oncogenesis. In conclusion, it will be interesting to determine the degree to which RNA regulons affect other human cancers and whether subtle regulatory defects, microRNA repression, post-translational modifications, or combinatorial RNA-protein interactions may exacerbate genetic mutations in tumors and other human diseases.

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Abbreviations

RBP	RNA-binding protein
RNP	ribonucleoprotein
RNA regulons and PTROs	Post-Transcriptional RNA Operons and Regulons
RRE	RNA recognition element

USER code

untranslated sequence element for regulation code

References

1. Keene JD. Ribonucleoprotein infrastructure regulating the flow of genetic information between the genome and the proteome. *Proc Natl Acad Sci USA*. 2001; 98:7018–24. [PubMed: 11416181]
2. Keene JD, Tenenbaum SA. Eukaryotic mRNPs may represent posttranscriptional operons. *Mol Cell*. 2002; 9:1161–7. [PubMed: 12086614]
3. Keene JD. RNA regulons: coordination of post-transcriptional events. *Nat Rev Genet*. 2007; 8:533–43. [PubMed: 17572691]
4. Halbeisen RE, Galgano A, Scherrer T, Gerber AP. Post-transcriptional gene regulation: from genome-wide studies to principles. *Cell Mol Life Sci*. 2008; 65:798–813. [PubMed: 18043867]
5. Ouellette M, Papadopoulou B. Coordinated gene expression by post-transcriptional regulons in African trypanosomes. *J Biol*. 2009; 8:100. [PubMed: 20017896]
6. Mansfield KD, Keene JD. The ribonome: a dominant force in co-ordinating gene expression. *Biol Cell*. 2009; 101:169–81. [PubMed: 19152504]
7. Anderson P. Post-transcriptional regulons coordinate the initiation and resolution of inflammation. *Nat Rev Immunol*. 2010; 10:24–35. [PubMed: 20029446]
8. Racher H, Hansen D. Translational control in the *C. elegans* hermaphrodite germ line. *Genome*. 2010; 53:83–102. [PubMed: 20140027]
9. Siddiqui N, Borden KLB. mRNA export and cancer. *WIREs RNA*. 2011:n/a–n/a.
10. Imig J, Kanitz A, Gerber A. RNA regulons and the RNA-protein interaction network. *BioMolecular Concepts*. 2012; 3:403–14.
11. Luna R, Rondón AG, Aguilera A. New clues to understand the role of THO and other functionally related factors in mRNP biogenesis. *Biochim Biophys Acta*. 2012; 1819:514–20. [PubMed: 22207203]
12. Simone LE, Keene JD. Mechanisms coordinating ELAV/Hu mRNA regulons. *Current Opinion in Genetics & Development*. 2013:1–9.
13. Pérez-Ortín JE, Alepuz P, Chávez S, Choder M. Eukaryotic mRNA Decay: Methodologies, Pathways, and Links to Other Stages of Gene Expression. *J Mol Biol*. 2013
14. Yuan J, Muljo SA. Exploring the RNA world in hematopoietic cells through the lens of RNA-binding proteins. *Immunol Rev*. 2013; 253:290–303. [PubMed: 23550653]
15. Jung H, Gkogkas CG, Sonenberg N, Holt CE. Remote Control of Gene Function by Local Translation. *Cell*. 2014; 157:26–40. [PubMed: 24679524]
16. De Gaudenzi JG, Carmona SJ, Agüero F, Frasch AC. Genome-wide analysis of 3'-untranslated regions supports the existence of post-transcriptional regulons controlling gene expression in trypanosomes. *PeerJ*. 2013; 1:e118. [PubMed: 23904995]
17. Keene JD. The globalization of messenger RNA regulation. *National Science Review*. 2014
18. Jacob F, Perrin D, Sanchez C, Monod J. Operon: a group of genes with the expression coordinated by an operator. *C R Hebd Seances Acad Sci*. 1960; 250:1727–9.
19. Lange C, Zaigler A, Hammelmann M, Twellmeyer J, Raddatz G, Schuster SC, et al. Genome-wide analysis of growth phase-dependent translational and transcriptional regulation in halophilic archaea. *BMC Genomics*. 2007; 8:415. [PubMed: 17997854]
20. Sittka A, Lucchini S, Papenfort K, Sharma CM, Rolle K, Binnewies TT, et al. Deep sequencing analysis of small noncoding RNA and mRNA targets of the global post-transcriptional regulator, Hfq. *PLoS Genet*. 2008; 4:e1000163. [PubMed: 18725932]
21. Ansong C, Yoon H, Porwollik S, Mottaz-Brewer H, Petritis BO, Jaitly N, et al. Global systems-level analysis of Hfq and SmpB deletion mutants in *Salmonella*: implications for virulence and global protein translation. *PLoS ONE*. 2009; 4:e4809. [PubMed: 19277208]
22. Zhang A, Wassarman KM, Rosenow C, Tjaden BC, Storz G, Gottesman S. Global analysis of small RNA and mRNA targets of Hfq. *Mol Microbiol*. 2003; 50:1111–24. [PubMed: 14622403]

23. Jørgensen MG, Thomason MK, Havelund J, Valentin-Hansen P, Storz G. Dual function of the McaS small RNA in controlling biofilm formation. *Genes & Development*. 2013; 27:1132–45. [PubMed: 23666921]
24. Holmqvist E, Vogel J. A small RNA serving both the Hfq and CsrA regulons. *Genes & Development*. 2013; 27:1073–8. [PubMed: 23699406]
25. Tenenbaum SA, Carson CC, Lager PJ, Keene JD. Identifying mRNA subsets in messenger ribonucleoprotein complexes by using cDNA arrays. *Proc Natl Acad Sci USA*. 2000; 97:14085–90. [PubMed: 11121017]
26. Grigull J, Mnaimneh S, Pootoolal J, Robinson MD, Hughes TR. Genome-wide analysis of mRNA stability using transcription inhibitors and microarrays reveals posttranscriptional control of ribosome biogenesis factors. *Molecular and Cellular Biology*. 2004; 24:5534–47. [PubMed: 15169913]
27. Mukherjee N, Lager PJ, Friedersdorf MB, Thompson MA, Keene JD. Coordinated posttranscriptional mRNA population dynamics during T-cell activation. *Mol Syst Biol*. 2009; 5
28. Archer SK, Luu V-D, de Queiroz RA, Brems S, Clayton C. Trypanosoma brucei PUF9 regulates mRNAs for proteins involved in replicative processes over the cell cycle. *PLoS Pathog*. 2009; 5:e1000565. [PubMed: 19714224]
29. Mazan-Mamczarz K, Hagner PR, Zhang Y, Dai B, Lehrmann E, Becker KG, et al. ATM regulates a DNA damage response post-transcriptional RNA operon in lymphocytes. *Blood*. 2011
30. Morris AR, Mukherjee N, Keene JD. Ribonomic analysis of human Pum1 reveals cis-trans conservation across species despite evolution of diverse mRNA target sets. *Molecular and Cellular Biology*. 2008; 28:4093–103. [PubMed: 18411299]
31. Jiang H, Guan W, Gu Z. Tinkering evolution of post-transcriptional RNA regulons: puf3p in fungi as an example. *PLoS Genet*. 2010; 6:e1001030. [PubMed: 20661438]
32. Jiang H, Guo X, Xu L, Gu Z. Rewiring of posttranscriptional RNA regulons: Puf4p in fungi as an example. *Molecular Biology and Evolution*. 2012; 29:2169–76. [PubMed: 22438588]
33. Gerber AP, Luschig S, Krasnow MA, Brown PO, Herschlag D. Genome-wide identification of mRNAs associated with the translational regulator PUMILIO in Drosophila melanogaster. *Proc Natl Acad Sci USA*. 2006; 103:4487–92. [PubMed: 16537387]
34. Gerber AP, Herschlag D, Brown PO. Extensive association of functionally and cytotopically related mRNAs with Puf family RNA-binding proteins in yeast. *PLoS Biol*. 2004; 2:E79. [PubMed: 15024427]
35. Morris A, Mukherjee N, Keene J. Systematic analysis of posttranscriptional gene expression. *WIREs Systems Biol Med* Doi. 2009:10.
36. Anderson P, Kedersha N. RNA granules: post-transcriptional and epigenetic modulators of gene expression. *Nat Rev Mol Cell Biol*. 2009; 10:430–6. [PubMed: 19461665]
37. Vicens Q, Allen MA, Gilbert SD, Reznik B, Gooding AR, Batey RT. The Cech Symposium: a celebration of 25 years of ribozymes, 10 years of TERT, and 60 years of Tom. 2008
38. Gao FB, Carson CC, Levine T, Keene JD. Selection of a subset of mRNAs from combinatorial 3' untranslated region libraries using neuronal RNA-binding protein Hel-N1. *Proc Natl Acad Sci USA*. 1994; 91:11207–11. [PubMed: 7972035]
39. Kazan H, Ray D, Chan ET, Hughes TR, Morris Q. RNAcontext: A New Method for Learning the Sequence and Structure Binding Preferences of RNA-Binding Proteins. *PLoS Comput Biol*. 2010; 6:e1000832. [PubMed: 20617199]
40. Ray D, Kazan H, Cook KB, Weirauch MT, Najafabadi HS, Li X, et al. A compendium of RNA-binding motifs for decoding gene regulation. *Nature*. 2013; 499:172–7. [PubMed: 23846655]
41. Li X, Quon G, Lipshitz HD, Morris Q. Predicting in vivo binding sites of RNA-binding proteins using mRNA secondary structure. *RNA (New York, NY)*. 2010; 16:1096–107.
42. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*. 1990; 249:505–10. [PubMed: 2200121]
43. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. *Nature*. 1990; 346:818–22. [PubMed: 1697402]

44. Tsai DE, Harper DS, Keene JD. U1-snRNP-A protein selects a ten nucleotide consensus sequence from a degenerate RNA pool presented in various structural contexts. *Nucleic Acids Res.* 1991; 19:4931–6. [PubMed: 1717938]
45. Levine TD, Gao F, King PH, Andrews LG, Keene JD. Hel-N1: an autoimmune RNA-binding protein with specificity for 3' uridylate-rich untranslated regions of growth factor mRNAs. *Molecular and Cellular Biology.* 1993; 13:3494–504. [PubMed: 8497264]
46. Butt J, Kim HY, Basilion JP, Cohen S, Iwai K, Philpott CC, et al. Differences in the RNA binding sites of iron regulatory proteins and potential target diversity. *Proc Natl Acad Sci USA.* 1996; 93:4345–9. [PubMed: 8633068]
47. Wang W, Caldwell MC, Lin S, Furneaux H, Gorospe M. HuR regulates cyclin A and cyclin B1 mRNA stability during cell proliferation. *The EMBO Journal.* 2000; 19:2340–50. [PubMed: 10811625]
48. López de Silanes I, Zhan M, Lal A, Yang X, Gorospe M. Identification of a target RNA motif for RNA-binding protein HuR. *Proc Natl Acad Sci USA.* 2004; 101:2987–92. [PubMed: 14981256]
49. Keene JD, Komisarow JM, Friedersdorf MB. RIP-Chip: the isolation and identification of mRNAs, microRNAs and protein components of ribonucleoprotein complexes from cell extracts. *Nature Protocols.* 2006; 1:302–7.
50. Hieronymus H, Silver PA. Genome-wide analysis of RNA-protein interactions illustrates specificity of the mRNA export machinery. *Nat Genet.* 2003; 33:155–61. [PubMed: 12524544]
51. Keene JD. Organizing mRNA export. *Nat Genet.* 2003; 33:111–2. [PubMed: 12560814]
52. Penalva LOF, Burdick MD, Lin SM, Sutterluety H, Keene JD. RNA-binding proteins to assess gene expression states of co-cultivated cells in response to tumor cells. *Mol Cancer.* 2004; 3:24. [PubMed: 15353001]
53. Pullmann R, Kim HH, Abdelmohsen K, Lal A, Martindale JL, Yang X, et al. Analysis of turnover and translation regulatory RNA-binding protein expression through binding to cognate mRNAs. *Molecular and Cellular Biology.* 2007; 27:6265–78. [PubMed: 17620417]
54. Saavedra CA, Hammell CM, Heath CV, Cole CN. Yeast heat shock mRNAs are exported through a distinct pathway defined by Rip1p. *Genes & Development.* 1997; 11:2845–56. [PubMed: 9353254]
55. Terry LJ, Shows EB, Wente SR. Crossing the nuclear envelope: hierarchical regulation of nucleocytoplasmic transport. *Science.* 2007; 318:1412–6. [PubMed: 18048681]
56. Farny NG, Hurt JA, Silver PA. Definition of global and transcript-specific mRNA export pathways in metazoans. *Genes & Development.* 2008; 22:66–78. [PubMed: 18086857]
57. García-Rodríguez LJ, Gay AC, Pon LA. Puf3p, a Pumilio family RNA binding protein, localizes to mitochondria and regulates mitochondrial biogenesis and motility in budding yeast. *J Cell Biol.* 2007; 176:197–207. [PubMed: 17210948]
58. Lelandais G, Saint-Georges Y, Geneix C, Al-Shikhley L, Dujardin G, Jacq C. Spatio-temporal dynamics of yeast mitochondrial biogenesis: transcriptional and post-transcriptional mRNA oscillatory modules. *PLoS Comput Biol.* 2009; 5:e1000409. [PubMed: 19521515]
59. Devaux F, Lelandais G, Garcia M, Goussard S, Jacq C. Posttranscriptional control of mitochondrial biogenesis: spatio-temporal regulation of the protein import process. *FEBS Lett.* 2010; 584:4273–9. [PubMed: 20875412]
60. Hogan DJ, Riordan DP, Gerber AP, Herschlag D, Brown PO. Diverse RNA-binding proteins interact with functionally related sets of RNAs, suggesting an extensive regulatory system. *PLoS Biol.* 2008; 6:e255. [PubMed: 18959479]
61. Haimovich G, Choder M, Singer RH, Treck T. The fate of the messenger is pre-determined: a new model for regulation of gene expression. *Biochim Biophys Acta.* 2013; 1829:643–53. [PubMed: 23337853]
62. Moore MJ, Wang Q, Kennedy CJ, Silver PA. An alternative splicing network links cell-cycle control to apoptosis. *Cell.* 2010; 142:625–36. [PubMed: 20705336]
63. Calarco JA, Zhen M, Blencowe BJ. Networking in a global world: establishing functional connections between neural splicing regulators and their target transcripts. *RNA (New York, NY).* 2011; 17:775–91.

64. Blencowe BJ. An exon-centric perspective. *Biochem Cell Biol.* 2012; 90:603–12. [PubMed: 22779775]
65. Moore MJ. From birth to death: the complex lives of eukaryotic mRNAs. *Science.* 2005; 309:1514–8. [PubMed: 16141059]
66. Lécuyer E, Yoshida H, Krause HM. Global implications of mRNA localization pathways in cellular organization. *Curr Opin Cell Biol.* 2009; 21:409–15. [PubMed: 19249199]
67. Kim Guisbert K, Duncan K, Li H, Guthrie C. Functional specificity of shuttling hnRNPs revealed by genome-wide analysis of their RNA binding profiles. *Rna.* 2005; 11:383–93. [PubMed: 15703440]
68. Culjkovic B, Tan K, Orolicki S, Amri A, Meloche S, Borden KLB. The eIF4E RNA regulon promotes the Akt signaling pathway. *J Cell Biol.* 2008; 181:51–63. [PubMed: 18391071]
69. Chakraborty P, Wang Y, Wei J-H, van Deursen J, Yu H, Malureanu L, et al. Nucleoporin levels regulate cell cycle progression and phase-specific gene expression. *Dev Cell.* 2008; 15:657–67. [PubMed: 19000832]
70. Reinhardt HC, Hasskamp P, Schmedding I, Morandell S, van Vugt MATM, Wang X, et al. DNA damage activates a spatially distinct late cytoplasmic cell-cycle checkpoint network controlled by MK2-mediated RNA stabilization. *Mol Cell.* 2010; 40:34–49. [PubMed: 20932473]
71. Kalverda B, Pickersgill H, Shloma VV, Fornerod M. Nucleoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleoplasm. *Cell.* 2010; 140:360–71. [PubMed: 20144760]
72. Chen D, Zheng W, Lin A, Uyhazi K, Zhao H, Lin H. Pumilio 1 Suppresses Multiple Activators of p53 to Safeguard Spermatogenesis. *Curr Biol.* 2012; 22:420–5. [PubMed: 22342750]
73. Singer S, Zhao R, Barsotti AM, Ouwehand A, Fazollahi M, Coutavas E, et al. Nuclear pore component Nup98 is a potential tumor suppressor and regulates posttranscriptional expression of select p53 target genes. *Mol Cell.* 2012; 48:799–810. [PubMed: 23102701]
74. Dutertre M, Lambert S, Carreira A, Amor-Guérét M, Vagner S. DNA damage: RNA-binding proteins protect from near and far. *Trends in Biochemical Sciences.* 2014; 39:141–9. [PubMed: 24534650]
75. Lazaris-Karatzas A, Smith MR, Frederickson RM, Jaramillo ML, Liu YL, Kung HF, et al. Ras mediates translation initiation factor 4E-induced malignant transformation. *Genes & Development.* 1992; 6:1631–42. [PubMed: 1516827]
76. Lazaris-Karatzas A, Sonenberg N. The mRNA 5' cap-binding protein, eIF-4E, cooperates with v-myc or E1A in the transformation of primary rodent fibroblasts. *Molecular and Cellular Biology.* 1992; 12:1234–8. [PubMed: 1532049]
77. Gingras AC, Raught B, Sonenberg N. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu Rev Biochem.* 1999; 68:913–63. [PubMed: 10872469]
78. Sonenberg N, Hinnebusch AG. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell.* 2009; 136:731–45. [PubMed: 19239892]
79. Fontoura BM, Blobel G, Matunis MJ. A conserved biogenesis pathway for nucleoporins: proteolytic processing of a 186-kilodalton precursor generates Nup98 and the novel nucleoporin, Nup96. *J Cell Biol.* 1999; 144:1097–112. [PubMed: 10087256]
80. Wozniak RW, Goldfarb DS. Cyclin-like oscillations in levels of the nucleoporin Nup96 control G1/S progression. *Dev Cell.* 2008; 15:643–4. [PubMed: 19000830]
81. Xu S, Powers MA. Nuclear pore proteins and cancer. *Semin Cell Dev Biol.* 2009; 20:620–30. [PubMed: 19577736]
82. Shiloh Y, Kastan MB. ATM: genome stability, neuronal development, and cancer cross paths. *Adv Cancer Res.* 2001; 83:209–54. [PubMed: 11665719]
83. Ofir-Rosenfeld Y, Boggs K, Michael D, Kastan MB, Oren M. Mdm2 regulates p53 mRNA translation through inhibitory interactions with ribosomal protein L26. *Mol Cell.* 2008; 32:180–9. [PubMed: 18951086]
84. Chen J, Kastan MB. 5'-3'-UTR interactions regulate p53 mRNA translation and provide a target for modulating p53 induction after DNA damage. *Genes & Development.* 2010; 24:2146–56. [PubMed: 20837656]

85. Reinhardt HC, Cannell IG, Morandell S, Yaffe MB. Is post-transcriptional stabilization, splicing and translation of selective mRNAs a key to the DNA damage response? *Cell Cycle*. 2011; 10:23–7. [PubMed: 21173571]
86. Mazan-Mamczarz K, Galban S, López de Silanes I, Martindale JL, Atasoy U, Keene JD, et al. RNA-binding protein HuR enhances p53 translation in response to ultraviolet light irradiation. *Proc Natl Acad Sci USA*. 2003; 100:8354–9. [PubMed: 12821781]
87. Abdelmohsen K, Pullmann R, Lal A, Kim HH, Galban S, Yang X, et al. Phosphorylation of HuR by Chk2 regulates SIRT1 expression. *Mol Cell*. 2007; 25:543–57. [PubMed: 17317627]
88. Mazan-Mamczarz K, Hagner PR, Dai B, Wood WH, Zhang Y, Becker KG, et al. Identification of transformation-related pathways in a breast epithelial cell model using a ribonomics approach. *Cancer Research*. 2008; 68:7730–5. [PubMed: 18829526]
89. Lü X, la Peña de L, Barker C, Camphausen K, Tofilon PJ. Radiation-induced changes in gene expression involve recruitment of existing messenger RNAs to and away from polysomes. *Cancer Research*. 2006; 66:1052–61. [PubMed: 16424041]
90. Kumaraswamy S, Chinnaiyan P, Shankavaram UT, Lü X, Camphausen K, Tofilon PJ. Radiation-induced gene translation profiles reveal tumor type and cancer-specific components. *Cancer Research*. 2008; 68:3819–26. [PubMed: 18483266]
91. Stumpf CR, Moreno MV, Olshen AB, Taylor BS, Ruggero D. The translational landscape of the mammalian cell cycle. *Mol Cell*. 2013; 52:574–82. [PubMed: 24120665]
92. Wright JE, Gaidatzis D, Senften M, Farley BM, Westhof E, Ryder SP, et al. A quantitative RNA code for mRNA target selection by the germline fate determinant GLD-1. *Cell Death Dis*. 2010; 30:533–45.
93. Biedermann B, Wright J, Senften M, Kalchauer I, Sarathy G, Lee M-H, et al. Translational repression of cyclin E prevents precocious mitosis and embryonic gene activation during *C. elegans* meiosis. *Dev Cell*. 2009; 17:355–64. [PubMed: 19758560]
94. Francis R, Maine E, Schedl T. Analysis of the multiple roles of gld-1 in germline development: interactions with the sex determination cascade and the glp-1 signaling pathway. *Genetics*. 1995; 139:607–30. [PubMed: 7713420]
95. Jungkamp A-C, Stoeckius M, Mecnas D, Grün D, Mastrobuoni G, Kempa S, et al. In vivo and transcriptome-wide identification of RNA binding protein target sites. *Mol Cell*. 2011; 44:828–40. [PubMed: 22152485]
96. Youngren KK, Coveney D, Peng X, Bhattacharya C, Schmidt LS, Nickerson ML, et al. The Ter mutation in the dead end gene causes germ cell loss and testicular germ cell tumours. *Nature*. 2005; 435:360–4. [PubMed: 15902260]
97. Cook MS, Munger SC, Nadeau JH, Capel B. Regulation of male germ cell cycle arrest and differentiation by DND1 is modulated by genetic background. *Development*. 2011; 138:23–32. [PubMed: 21115610]
98. Maniatis T, Reed R. An extensive network of coupling among gene expression machines. *Nature*. 2002; 416:499–506. [PubMed: 11932736]
99. Keene JD, Lager PJ. Post-transcriptional operons and regulons coordinating gene expression. *Chromosome Res*. 2005; 13:327–37. [PubMed: 15868425]
100. Choder M. mRNA imprinting: Additional level in the regulation of gene expression. *Cell Logist*. 2011; 1:37–40. [PubMed: 21686103]
101. Lotan R, Bar-On VG, Harel-Sharvit L, Duek L, Melamed D, Choder M. The RNA polymerase II subunit Rpb4p mediates decay of a specific class of mRNAs. *Genes & Development*. 2005; 19:3004–16. [PubMed: 16357218]
102. Sadasivam S, DeCaprio JA. The DREAM complex: master coordinator of cell cycle-dependent gene expression. *Nature Reviews Cancer*. 2013; 13:585–95.

Highlights

- Post-transcriptional regulation plays a crucial role in the regulation of the cell cycle and DNA damage responses
- Post-transcriptional RNA operons/regulons (PTROs) coordinately regulate functionally related sets of mRNAs that drive cell growth processes
- Early evidence indicates progressive steps of mRNA processing of cell cycle related messages are coupled by RNA-binding proteins
- Many cell cycle related RNA-binding proteins act as growth suppressors and their genetic modifications lead to abnormal cell proliferation

Figure 1. Depictions of RNA regulon models

A. Coordinated mRNA export showing two yeast RBP export factors Mex67 and Yra1 as they transport groups of functionally related mRNAs out of the nucleus from derived from the data of Hieronymus and Silver (2003) [50]. Adapted with permission from Keene, *Nature Genetics* 33: 111–112 (2003) [51]. B. Coordinated regulation of the yeast PUF3 RBP transporting nuclear mRNAs that encode mitochondrial proteins to mitochondria for translation and uptake from Gerber et al., (2004) [34]. Adapted with permission from Keene, *Nature Reviews Genetics* 8:533–543 (2007) [3].

Figure 2. Coupling of RNA Regulons

A model of two distinct RNA processing pathways are depicted for a set of mRNAs. The functionally related set of mRNAs in blue are spliced, exported and translated through regulatory events directed by splicing factor A, export factor B and translation factor C in RNA regulons that are coupled together through interactions between A, B and C. The functionally related set of mRNAs in red are likewise spliced, exported and translated by D, E and F, respectively, through similar coupling.

Figure 3. Cell cycle regulation by RBPs

Several RBPs have been genetically demonstrated to influence cell cycle events, including export factor NUP98 and translation factors PUM1, DND1 and GLD-1 as described in the text. Interestingly, these all act as repressors of cell growth, and therefore, have been characterized as tumor suppressors. Loss of function of these RBPs causes increased cell growth through failures of various cell cycle checkpoints.