RNA regulons: coordination of post-transcriptional events

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Abstract | Recent findings demonstrate that multiple mRNAs are co-regulated by one or more sequence-specific RNA-binding proteins that orchestrate their splicing, export, stability, localization and translation. These and other observations have given rise to a model in which mRNAs that encode functionally related proteins are coordinately regulated during cell growth and differentiation as post-transcriptional RNA operons or regulons, through a ribonucleoprotein-driven mechanism. Here I describe several recently discovered examples of RNA operons in budding yeast, fruitfly and mammalian cells, and their potential importance in processes such as immune response, oxidative metabolism, stress response, circadian rhythms and disease. I close by considering the evolutionary wiring and rewiring of these combinatorial post-transcriptional gene-expression networks.

RNA operon
A ribonucleoprotein structure in which multiple mRNAs are coordinately regulated by RNA-binding proteins and small non-coding RNAs. The combination of multiple mRNAs in an RNA operon can change dynamically following biological perturbations.

Living systems depend on the dynamics of gene expression to regulate cell survival, adaptation to stress, homeostasis, cell fate and differentiation, in response to environmental signals[1]. Cells have evolved mechanisms to generate and use transcripts with optimal efficiency to meet their needs throughout their life cycle, while retaining the robustness to re-adapt. The ability of bacteria to respond rapidly and efficiently to incoming signals is in part due to the organization of genes into DNA operons[2], with genes that work in the same pathway being physically linked to facilitate rapid temporal and spatial coordination of their expression. Among eukaryotic genomes, which are much more complex than those of bacteria, DNA operons are rare, and gene coordination is thought to involve activation and repression at multiple transcription promoter sites[3–8]. Additional possibilities for gene regulation and further diversification of eukaryotic proteins are provided by split genes and alternative RNA splicing.

Until recently, most gene-expression studies in eukaryotes have measured steady-state mRNA levels, rather than the actual synthetic rates of transcript production, thereby failing to account for different states of translational activation and different stabilities of individual mRNA species[9]. New methods have more recently revealed that global coordination of gene expression also depends on post-transcriptional events[10–12]. RNA-binding proteins (RBPs) organize nascent RNA transcripts into groups in order to percolate them together down the chain of splicing, nuclear export, stability and translation so that proteins are efficiently produced to meet the needs of the cell[9] (FIG. 1). This apparent coordination, especially at the level of mRNA stability and translation, formed the basis of the post-transcriptional 'RNA-operon' theory, according to which *trans*-acting factors combinatorially regulate multiple mRNAs along a coordinated pathway of RNA processing, allowing cells to respond with unusual agility to environmental cues[10,13] (FIG. 1). This model provides a simplifying principle that helps to explain the higher-order organization and dynamics of functionally related mRNAs at several levels of post-transcriptional regulation, many of which do not exist in bacteria. But, like bacteria that use polycistronic mRNAs and higher-order DNA regulons to coordinate protein production, RNA operons co-regulate monocistronic mRNAs that can each function as members of more than one RNA operon, forming higher-order 'RNA regulons' (FIG. 2). Here I describe recent evidence for the co-regulation of multiple mRNAs in budding yeast, fruitfly and mammalian cells, discussing the advantages of such dynamic higher-order coordination and its implications for growth, development and disease.

RNA operons

Post-transcriptional RNA operons (and regulons) function on the basis of a ribonucleoprotein (RNP)-driven process in which multiple functionally related mRNAs are coordinately regulated by *trans*-acting factors — primarily RBPs, but also non-coding RNAs or metabolites[10,13]. These factors interact with multiple regulatory elements within mRNAs, termed USERs (untranslated sequence elements for regulation). Although most

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USERs lie in the non-coding regions of mRNAs, some reside in coding regions. USERs determine the RNP association and mode of regulation of each mRNA (FIG. 2a). Moreover, the *trans*-acting factors, such as RBPs or microRNAs (miRNAs), can regulate the same mRNA by cooperating or competing for a regulatory outcome at more than one USER. A single miRNA species can also target multiple mRNAs, and combinations of miRNAs and RBPs probably dictate the coordinated outcomes of post-transcriptional gene expression by interacting with multiple USERs[14,15]. Each mRNA can join different RNA operons as determined by 'USER codes' that govern its fate (FIG. 2b). Because each mRNA can be a member of more than one RNA operon, if the protein encoded by the mRNA evolves more than one function, it can be dynamically co-regulated with other mRNAs independently to serve a different functional role[10]. Therefore, the genetic information that is represented by multiple copies of each mRNA species, rather than by the gene itself, can be used combinatorially at several levels, and can be regulated as part of multiple RNPs, simultaneously or sequentially. In essence, RNA operons represent modular RNP units that coordinate multiple mRNAs to ensure an efficient yet flexible use of the genetic information. Not unlike DNA operons that can be regulated by catabolite repression, RNA operons can be activated or repressed by RBPs through signals such as phosphorylation[2,16–18]. In contrast to more constrained polycistronic bacterial transcripts that contain multiple ORFs, one can think of RNA operons as organized collections of individual monocistronic transcripts, which can use their independence to respond to cellular needs with greater agility.

Interconnected steps of mRNA processing

Post-transcriptional regulation of gene expression begins even before the synthesis of a transcript is completed; recent evidence suggests that RNA processing is coordinated from that point forward[9,11,12]. Certain RBPs associate with nascent transcripts and provide protection in the form of heterogeneous nuclear ribonucleoprotein (hnRNP) assembly, but also prepare a transcript for splicing[19,20] (FIG. 1). The splicing factors include serine–arginine (SR)-rich RBPs and related factors that help to mark exons and introns for the engagement with the spliceosome[21,22]. The progressively organized RNP complexes assemble functionally related transcripts for export to the cytoplasm (FIG. 2a), where their stability and translation are regulated[12,23].

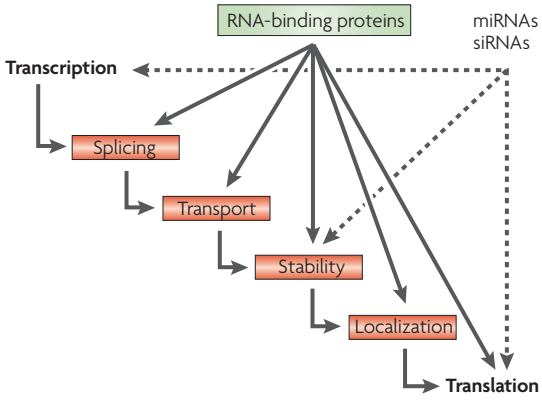

Figure 1 | **Interconnected steps of post-transcriptional regulation and its potential coordination.** In eukaryotic cells, mRNAs undergo several steps of regulation from transcription to translation. The coordination of multiple mRNAs is regulated by RNA-binding proteins and small non-coding RNAs at different levels. miRNAs, microRNAs; siRNAs, small interfering RNAs.

Transcription. According to the current model of coordination of gene expression, transcription factors work together in various combinations to activate or repress promoters, in the context of a specific chromatin structure[1,4]. Despite the need for coordination of transcription, it is still not entirely clear how this can be achieved at numerous sites over long distances on chromosomes. Low-level illegitimate transcription of 'tissue-specific' genes in other tissues was first reported in mammalian cells nearly 15 years ago[24], indicating that low-grade spontaneous transcription is ongoing as a result of continuous and sometimes random initiation. More recent studies have shown that such illegitimate initiation is due to leakage, perhaps resulting from the spreading of chromatin modifications across neighbouring genes[25]. Other recent studies have shown that much of the tissue-specific expression that occurs is non-functional, and might reflect the need for cells to retain flexibility to respond to developmental signals[26,27].

Stochastic transcription in eukaryotes might at least in part be explained by the discovery of gene-expression neighbourhoods[28–30]. A genome-wide study reported that the *Drosophila melanogaster* genome has hundreds of sets of 15–30 genes that reside in the same chromosomal domains and are co-expressed temporally[31]. These genes are not functionally related and are transcribed regardless of whether their products are needed or not, as if most of them were 'carried along for a ride'[31].

An alternative to coordination at the level of transcription was suggested for germ-cell development, in which most of the burden of gene regulation is transferred to, "…an RNA-centric program of post-transcriptional regulation…" in the cytoplasm to, "…ensure that their genome remains plastic."[32] Seydoux and Braun suggest that a DNA-centric programme of gene expression, which might be appropriate for somatic cells, is too limiting for germ cells, which must maintain their totipotency[32]. RNP granules serve as regulatory hubs that direct the differentiation of germ cells into gametes. At the same time, the transcriptional capability of a stable chromatin structure is retained for subsequent somatic-cell differentiation. Indeed, it is difficult to understand how transcription can be the sole coordinating process of gene expression, given its spontaneous and stochastic nature[33,34]. Importantly, transcriptional noise can be compensated for later, at the level of translation. Post-transcriptional RNA operons could provide a more stringent selective mechanism for gene expression in the face of stochastic transcriptional initiation[25,27].

Gene-expression neighbourhoods
Chromosomal domains containing 15–30 tandem genes that are expressed together owing to localized chromatin modifications.

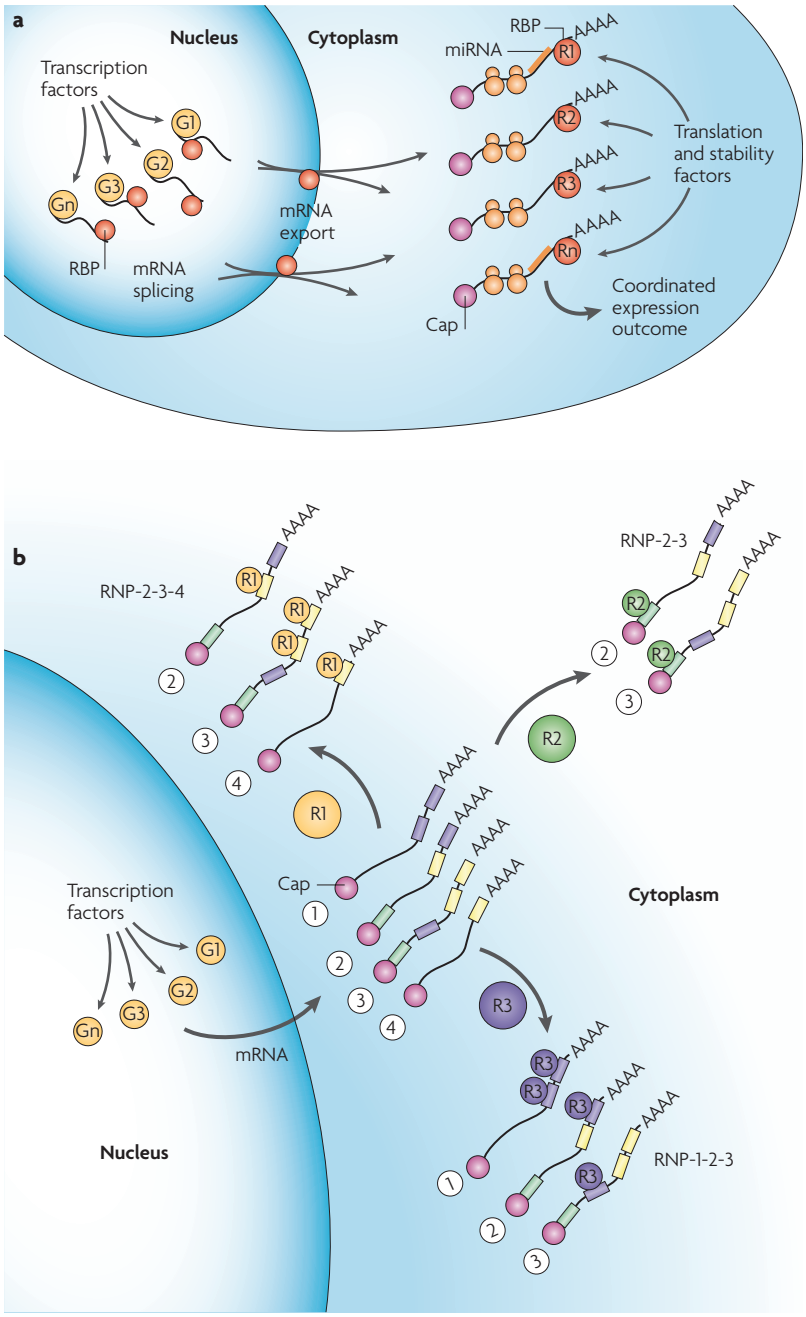

Figure 2 | **Coordination of subsets of eukaryotic mRNAs from transcription to translation. a** | Transcription factors activate the transcription of a set of genes (G1–Gn). While they are still being transcribed, the resulting mRNAs are bound by RNA-binding proteins (RBPs), spliced and exported to the cytoplasm[10]. The RNA operon regulates mRNA stability and translation, guaranteeing the co-regulated expression of a set of proteins that function in the same metabolic pathways or form a macromolecular complex[10]. RBPs and microRNAs (miRNA) can affect the stability of transcripts, activate or repress their translation or recruit the transcripts to ribosomes or processing bodies in the cytoplasm. **b** | The four mRNAs shown (labelled with numbered white circles), when grouped in different combinations, form three different RNA operons, labelled as ribonucleoprotein (RNP)-2-3-4, RNP-2-3 and RNP-1-2-3. The make-up of each operon is determined by the binding of RBPs (labelled R1, R2 and R3) to specific sequence elements, which leads to both co-regulation within each RNA operon and overall coordination of all three operons as higher-order combinatorial regulons. The four transcripts contain different combinations of RBP-binding elements. Therefore, mRNAs that contain more than one binding element can be members of more than one RNP complex[45].

Splicing. An emerging theme of mRNA targeting by nuclear splicing factors such as U2AF, SR, PTB and others is that these RBPs interact not only with unspliced mRNAs, but also with mature mRNAs, the products of which regulate cell growth, cell cycle and differentiation[21,35–37]. Ribonucleoprotein–immunoprecipitation–microarray (RIP–chip) analyses showed that the mRNA targets of U2AF2 and PTB represent distinct subsets of spliced transcripts, rather than precursor transcripts in the nucleus and in the cytoplasm[37]. These target mRNAs are functionally related — those that are targeted by U2AF2 predominately encode transcription factors and cell-cycle regulators, whereas those that are targeted by PTB encode proteins involved in protein transport and trafficking, as well as apoptosis. Thus, these nucleocytoplasmic shuttling RBPs, not unlike the RNA-binding ELAV (embryonic lethal, abnormal vision)/Hu antigen proteins, interact with precursor mRNAs and potentially carry the mature spliced mRNAs to the cytoplasm, where they help to regulate their localization, stability and translation[37–39] (FIG. 2a). The fact that some mRNAs are only regulated by one of these factors, whereas others can be regulated by both, demonstrates the combinatorial RNA modularity that is afforded by a post-transcriptional RNA-operon mechanism.

An adaptation of RIP–chip, using UV crosslinking and immunoprecipitation (CLIP), identified mRNA targets of the brain-specific NOVA1 RBP in mice[40]. Many of these target mRNAs encode components of the neuronal inhibitory synapse, indicating that there is a functional link between them[40]. The fact that the NOVA1 RNP includes both spliced and unspliced mRNAs indicates that it probably functions at more than one level of post-transcriptional regulation, including translation in the neurites[40].

Few studies have provided insights into how splicing *per se* is organized with respect to the multiple interactions that can be coordinated. Bioinformatics approaches have the potential to advance our understanding of splice-site selection and reveal combinations of USERs that reside in different regulatory regions of precursor RNAs[20,41,42]. For example, the computational identification of NOVA1-specific exonic and intronic binding-sequence clusters was consistent with previously demonstrated exon-inclusion patterns, providing a genome-wide map of probable alternative-splicing outcomes[42].

Nuclear export. mRNA splicing is intimately connected to the export of mature transcripts from the nucleus[19,37]. A RIP–chip study in yeast showed how the RNA-nuclear-export proteins Yra1 and Mex67 associate with functionally related mRNAs in the nucleus soon after transcription, and organize them for export to the cytoplasm[43] (FIG. 3). Importantly, although some mRNAs are exported in association with only either Yra1 or Mex67, others are exported in association with both, demonstrating one of the tenets of the post-transcriptional RNA-operon model: the combinatorial association of two (or more) *trans*-acting factors (such as RBPs) results in the export of a distinct mRNA subpopulation[10,13] (FIG. 2b).

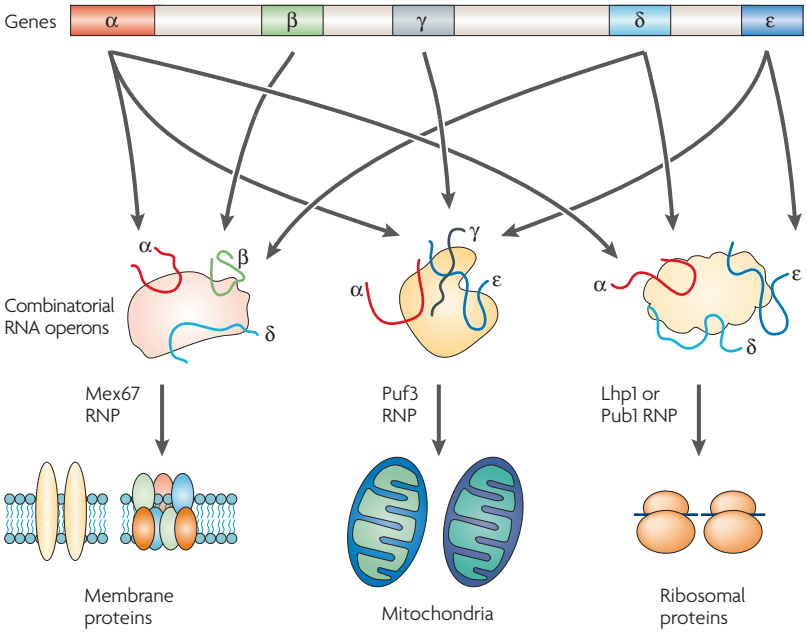

Figure 3 | **Formation and dynamics of ribonucleoprotein (RNP) complexes.** Dispersed eukaryotic genes produce transcripts that are then assembled into RNP complexes. mRNAs that are associated with each specific RNP complex are translated to form protein–protein complexes. However, four yeast RBPs — the export protein Mex67 (REF. 43), the Pumilio protein Puf3 (REFS 93,95), Pub1 (REF. 61) and the La protein homologue in yeast, Lhp1 (REF. 123), have been shown to associate with unique but also overlapping groups of transcripts, such as those involved in ribosome and membrane biogenesis. This outcome illustrates the combinatorial property and dynamics of post-transcriptional RNA operons, as depicted in FIG. 2b.

Another recent study of three yeast RBP export factors — Nab2, Npl3 (also known as Nop3) and Nab4 (also known as Hrp1), which function as hnRNP shuttling proteins — identified three discrete subpopulations of mRNAs, encoding proteins that are involved in transcription, ribosomal biogenesis and intermediary metabolism, respectively[44]. mRNAs in the same group have common sequence elements that, when mutated, result in growth defects that are consistent with an impaired function of the pathway in which these mRNAs are involved. Once these organized groups of transcripts enter the cytoplasm, they are presumably co-regulated at the levels of mRNA stability and translation.

The export protein antigen peptide transporter TAP1 forms a heterodimer with NTF2-like export factor 1 (NXT1) and interacts with the constitutive transport element (CTE) that is contained in introns of several unspliced RNAs. For example, in the RNA genome of the Mason Pfizer Monkey retrovirus, the CTE promotes the translation of RNA after it exits the nucleus via a non-CRM1 (also known as XPO1) pathway[35]. A subset of unspliced cellular mRNAs that possess a CTE is exported by the same mechanism to translationally active polysomes, along with TAP1, as part of a remodelled RNP. Similarly, the Wilms tumour 1 (*WT1*) gene is alternatively spliced to produce two protein isoforms: one regulates transcription and the other binds CTE-containing mRNAs in the cytoplasm and increases their translation during development[36]. Bor *et al.* proposed that the CTE is a USER that coordinates the regulation of functionally linked mRNAs, which together work as a post-transcriptional RNA operon[36].

mRNA-decay operons and regulons. The association of functionally related mRNAs with RBPs, together with the dynamic interchange of the components of these RNP complexes, formed the basis of the post-transcriptional RNA-operon model. But these associations provide only suggestive evidence that these mRNA subpopulations are in fact functionally coordinated. Among the strongest experimental evidence to support the co-regulation of post-transcriptional events is the demonstration that distinct classes of mRNAs that encode immune regulators such as cytokines and chemokines have similar and synchronously alterable half-lives. For example, mammalian RBPs that regulate mRNA stability, such as ELAV/Hu proteins and tristetraproline (TTP), have many common mRNA targets, including cytokines and chemokines, that must be coordinated during immune regulation[45–51]. Indeed, several studies have shown that, following activation of T cells or macrophages, the stabilities of distinct groups of mRNAs change in coordination so that the required proteins can be produced. Early work examined RNA stability using microarrays in lymphoma cell lines following transcription inhibition with the anticancer drug flavopiridol[52]. The authors classified mRNAs according to their stabilities, and suggested that this characteristic is coordinately regulated across these classes. The mRNAs studied encoded immune regulators and proteins involved in apoptosis, cell cycle and transcription. Of particular interest were the immediate-early genes encoding protooncoproteins and growth factors such as MYC, FOS and cytokines[52].

Subsequent studies found correlations in the decay rates of mRNA subsets that encode distinct functional protein groups[53]. For example, analysis of global RNA stability in primary human T cells and several T-cell leukaemia cell lines led to the categorization of mRNAs that encode related functions of immune reactivity into common stability groups on the basis of their half-lives. When transcription was blocked using actinomycin D, many immediate-early-type transcripts, including cytokine and chemokine mRNAs, showed co-regulated patterns, but each decay class also contained immediate-early transcripts[54,55]. Many of these mRNAs encoded known targets of the ELAV/HuR and TTP RBPs[50,56]. As expected, these groups of mRNAs showed simultaneous alterations in half-life as the conditions of growth and activation changed.

Another set of functionally related mRNAs that are co-regulated by RNA stability in human cells comprises the histone mRNAs. Most histone mRNAs are stabilized by a highly conserved stem–loop element in their 3′ UTRs. The stem–loop binding protein (SLBP) regulates replication-dependent histone mRNAs (H1, H2A, H2B, H3, and H4) during the S phase of the cell cycle[57]. RIP–chip analysis of SLBP targets identified approximately 30 histone mRNAs that might function as members of histone post-transcriptional RNA operons that coordinate histone production[58].

RIP–chip
A method that involves immunoprecipitation of an endogenous or tagged RNA-binding protein followed by a microarray analysis or sequencing to identify its associated RNAs.

Although the microarray studies of mRNA decay in mammalian cells are consistent with the RNA-operon model, the most convincing evidence for functional linkage among mRNA stability classes has come from *Saccharomyces cerevisiae*. Comparing gene-expression profiles following the blockage of transcription using RNA polymerase mutants revealed that mRNAs from the same stability group were functionally related, encoding components of previously known regulatory pathways including glycolysis, pheromone signal transduction and translation initiation[59]. Similar results were reported by Grigull *et al.*, who compared RNA stability in yeast cells that were treated with various chemical inhibitors of transcription with that of strains that carry temperature-sensitive mutations in RNA polymerase II[60]. These authors also analysed the effects of deleting five RBPs on the decay pattern of these mRNA classes following chemical or heat-shock-mediated blockage of transcription. A quantitative comparison of these data with those obtained by Wang *et al.*[59] revealed largely consistent results: several functionally related mRNAs were found in the same stability group, with genes involved in ribosomal biogenesis and glycolytic pathways being the most striking examples.

Deleting the *CCR4* and *PAN2* deadenylase genes, which are involved in the removal of the polyA tail from mRNA, and the *PUB1* RBP gene, led to the accumulation of ribosomal biogenesis (including nucleolar) mRNAs[60]. Consistent with these mRNA-stability studies were experiments in which RIP–chip in yeast was used to identify mRNA targets of Pub1; Pub1 coordinates the expression of mRNAs that have a role in ribosomal biogenesis, as would be predicted if they functioned as RNA operons[61].

A study of *S. cerevisiae* genes that control the cellular response to iron depletion revealed a post-transcriptional coordinating mechanism that involves the zinc-finger RBP Cth2, a homologue of human TTP that is known to regulate cytokine mRNAs in mammals[62,63]. Cth2 and TTP RBPs bind to AU-rich elements in mRNAs and lead to their degradation. Following iron depletion, Cth2 specifically degrades mRNAs that encode proteins involved in sterol and fatty-acid synthesis, the tricarboxylic acid (TCA) cycle and haeme metabolism, and those that contain iron sulphide, indicating coordinated reprogramming of protein production through RNA stability.

Biological clocks are an excellent example of biological coordination[18,64]. For example, the transcription–translation oscillation model of circadian rhythms proposes that transcription in the nucleus, followed by translation in the cytoplasm, generates a feedback loop in which positive and negative transcriptional regulators such as CLOCK, BMAL1 and PERIOD are synthesized in the cytoplasm and travel back to the nucleus to affect transcription[65–68]. Recent work in various organisms has suggested an important role for RNA stability and translation in the regulation of oscillation[69,70]. For example, in *Xenopus laevis* and mice, the RNA deadenylase nocturnin, which is rhythmically expressed in the cytoplasm of the retinal photoreceptor cells, removes the polyA tail of specific clock-related mRNAs, leading to their decay[18]. Among the most rhythmic circadian proteins is the butyrate response factor 1 (BRF1) RBP, a homologue of TTP[65]. Every 24 hours, the levels of BRF1 rise dramatically but transiently in peripheral organs and then drop, only to repeat the cycle again[67,68]. Like TTP, BRF1 degrades AU-rich transcripts of immediate-early genes, and is thought to compete for some targets with ELAV/Hu RBPs that do not seem to undergo a circadian cycle. So, during the diurnal cycle, BRF1 is proposed to function as part of a post-transcriptional operon to degrade a subset of immediate-early gene transcripts so that they can be subsequently refreshed to meet the needs of the next day[65].

Methods such as *en masse* nuclear run-on assays have been developed to distinguish transcriptional contributions from the subsequent effects of RNA stability on the steady-state levels of multiple mRNAs[9,71–73]. RNA stability is traditionally measured as an average of the entire population of a given mRNA species. Potentially, the functional state of the RNPs that associate with RNA can endow a different stability or translatability to each copy of a given mRNA, and their combined outcomes can be coordinated throughout the cell. The RNA-operon model provides a mechanism by which a given mRNA can exist in more than one RNP state[10,13,16,17] (FIGS 2b,3,4).

Translation. Although it has long been known that specific mRNAs reside in neuronal processes and in oocytes, their temporal co-regulation was not demonstrated until the advent of genome-wide methods[74–76]. For example, mRNAs encoding proteins such as FOS and cyclin B1 were found in the cytoplasm of neurons and oocytes, respectively, but most studies focused on molecular interactions between one RBP and one mRNA[77]. Moreover, until recently, RBPs and non-coding RNAs were not shown to associate with groups of localized mRNAs in neurons and oocytes; therefore, the underlying mechanisms for their co-regulation remained unknown. By contrast, the fragile X mental retardation syndrome protein (FMR1) was estimated to bind as much as 4% of all mRNAs in the brain, although the identities of its target mRNAs were unknown[78]. Thus, there was little reason to assume that so many mRNAs formed a coherent group of functionally related transcripts.

When 100 brain mRNAs containing 3′ UTRs with AU-rich elements were shown to bind to the neuronal ELAV/HuB RBP *in vitro*, it was suggested that ELAV/HuB, and possibly other members of the ELAV/Hu RBP family, could coordinate mRNA expression during neuronal differentiation[79]. It was subsequently demonstrated that ELAV/HuB proteins stabilize and/or increase translation of the mRNAs to which they bind[38,48,80]. RIP–chip-based quantification of the mRNAs that dynamically associated with ELAV/HuB in differentiating P19 embryonal carcinoma stem cells indicated that there was a temporal flux of specific mRNAs in the RNPs, which was not solely due to changes in the mRNA steady state that resulted from transcription[45] (FIG. 4). Taken together, these results led to the suggestion that ELAV/HuB was a master regulatory RBP that

En masse nuclear run-on assay
A method in which newly synthesized mRNAs are dynamically radiolabelled in isolated nuclei before they are post-transcriptionally processed. The transcript levels are then measured by microarray analysis.

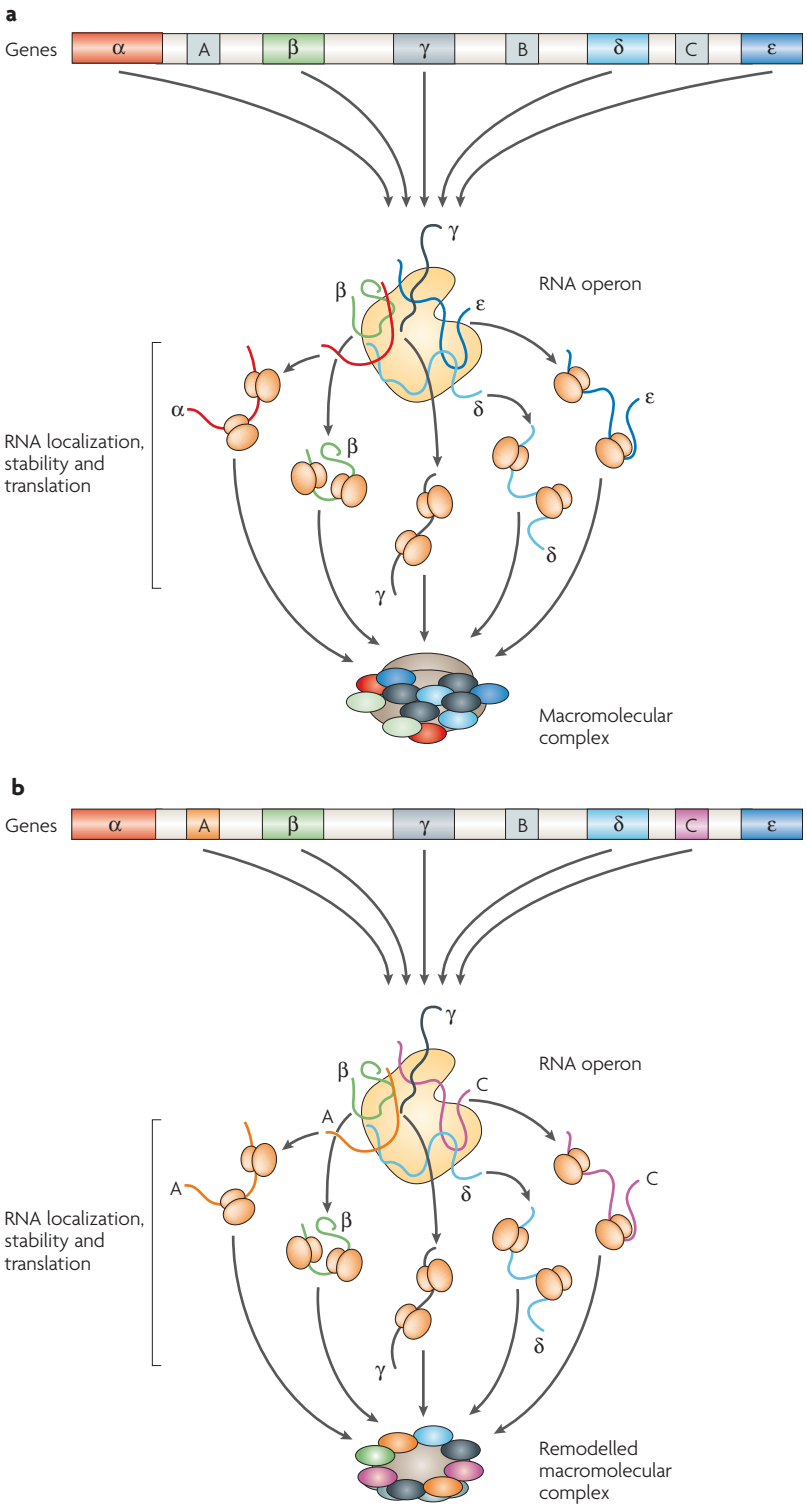

Figure 4 | **Dynamics of post-transcriptional co-regulation. a** | RNAs transcribed from genes that are dispersed across the eukaryotic genome (α, β, γ, δ and ε) associate in specific ribonucleoprotein (RNP) complexes to provide coordination of expression. **b** | Following a biological perturbation, transcripts from other genes, A and C, enter the RNP complex, whereas transcripts from genes α and ε exit the RNP. The dynamic remodelling of the RNP results in the formation of a different macromolecular protein complex, which shares three of the original protein components and has acquired two new components. If a crucial mRNA carries a mutated USER (untranslated sequence element for regulation), the mRNA could fail to be correctly co-expressed, leading to a defective phenotype or disease.

coordinated post-transcriptional expression of multiple transcripts following induction of neuronal differentiation; these RNPs were proposed to function as the organizational driving force of post-transcriptional RNA operons[9,10]. Further evidence that discrete subsets of mRNAs can both directly and indirectly associate with or be co-regulated by specific RBPs was subsequently provided for mammalian FMR1 (REF. 81), GW182 (also known as TNRC6A)[82], αCP2 (REF. 83), HuR[84], EIF4E[85,86], histones[58] and many others[11,12,87–92].

Probably the most definitive and highly cited example of a post-transcriptional RNA operon was that described by Gerber *et al.* These authors examined five Pumilio RBP family members (PUF: Puf1–Puf5) in *S. cerevisiae* and found that each binds a distinct subpopulation of mRNAs that encode proteins with related functions[93]. Puf3–Puf5 associate with mRNAs containing a *cis*-acting consensus-binding motif (UGUANAUA) that is similar to those defined for other Pumilio proteins, which are known to repress translation and/or stability of their target mRNAs[94]. Puf3 associates with a subset of 154 mRNAs that almost exclusively encode mitochondrial proteins, many of which are involved in protein biosynthesis. These results demonstrated the principle that mRNAs that encode components of organelles and macromolecular structures are organized, and potentially regulated in space and time, by sequence-specific RBPs. Protein-localization data suggested that Puf3 is clustered in the vicinity of yeast mitochondria (FIG. 5). Genetic and biochemical methods to further explore the functions of Puf3 provided support for a role of this protein, as well as the proteins encoded by its mRNA targets, in mitochondrial biogenesis and motility[95]. The authors suggested that Puf3 is involved in the transport of the Puf3-associated mRNAs to the mitochondria. From the identification of the Puf3-associated mRNA subset as constituting a putative post-transcriptional RNA operon to the demonstrated cellular localization and combined functions of the mitochondrial proteins encoded by that subset of mRNAs, Puf3 stands as the most complete and well substantiated example to date of a fully functional and modular post-transcriptional RNA operon.

Among the four other PUF proteins that were examined in *S. cerevisiae*, each interacted with mRNAs that encode either spindle-body components, nucleolar regulatory proteins, chromatin-remodelling enzymes or membrane proteins[93]. Overall, the five PUF proteins were shown to target over 700 mRNAs, accounting for more than 10% of the transcripts in *S. cerevisiae*. This finding is consistent with the idea that the RNP infrastructure is redundant and, in a sense, over-determined, a quality that might endow resilience and robustness to these gene-expression networks[96].

In *D. melanogaster* embryos, Pumilio regulates several mRNAs that are involved in embryo polarity and localized translational suppression. A genome-wide analysis of its target mRNAs in the ovaries and in whole embryos indicated that its targets encode functionally related proteins[97]. In the embryo, most of the targets encoded proteins that were previously known

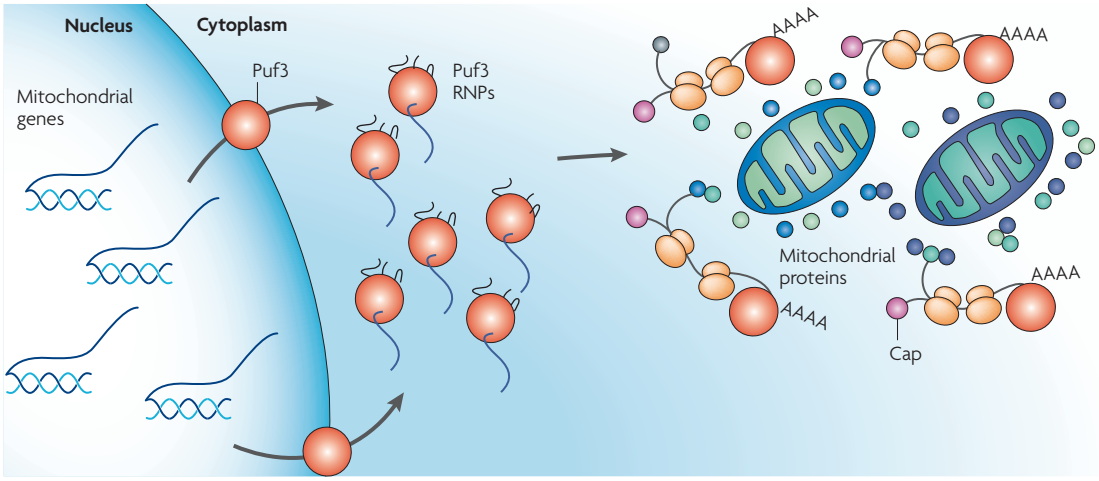

Figure 5 | **Model of the Puf3 RNA operon.** The Pumilio protein Puf3 (represented as a red circle) in *Saccharomyces cerevisiae* forms a post-transcriptional operon that is involved in the co-regulation of protein expression, functioning in motility and mitochondrial biogenesis. Puf3 associates in the cytoplasm with nuclearly encoded mitochondrial mRNAs[93]. These mRNAs are localized and possibly transported to the vicinity of mitochondria, where the Puf3 translational repression is reversed. Once translated, these proteins participate in the biogenesis and motility of the organelles[95].

to be involved in anterior–posterior patterning, such as Bicoid, Hunchback, Oskar, Nanos and Caudal. Among the mRNA subpopulations that were enriched in the ovary were those encoding 10 of the 13 protein subunits of the vacuolar protein-translocating V-type ATPase. A high percentage of the mRNAs that were identified in this subset contained the Pumilio-binding consensus sequence in their 3′ UTRs[97]. Therefore, similar to other studies, *D. melanogaster* Pumilio also seems to have a role in the localization and/or translation of distinct subpopulations of functionally related mRNAs.

In mammalian cells, the cap-binding protein EIF4E associates with the 5′ ends of mRNAs and, when deregulated, can promote malignant transformation at the level of translational control (TABLE 1). EIF4E is found in both the nucleus and the cytoplasm. Recent studies show that it promotes the export of many mRNAs (via the CRM1-mediated pathway) from the nucleus, including the cyclin D1 mRNA[85,98]. EIF4E interacts with multiple mRNAs, and this proposed RNA operon seems to be inhibited at the level of export by the growth-suppressive promyelocytic leukaemia protein, PML. Among the mRNAs that associate specifically with nuclear EIF4E, resulting in their export being blocked, are those encoding cyclins, MDM2, PIM1, MYC, FBOX1 and other proliferative factors; by contrast, mRNAs that are not associated with EIF4E in the nucleus include those encoding proapoptotic and growth-suppressor factors such as VEGF, p53, PML and CEBPA. Moreover, EIF4E-interacting mRNAs contain a common *cis* element in their 3′ UTRs — a putative structural USER code. Thus, the growth-stimulatory properties of EIF4E might involve its export of subsets of mRNAs that encode promoters of cell-cycle progression that do not necessarily correspond to most of those proteins assumed to be regulated by EIF4E at the translational level in the cytoplasm.

A complementary study investigated the possibility that EIF4E leads to a malignant phenotype in breast carcinoma cells by coordinating the expression of multiple mRNAs in a post-transcriptional operon[86]. Given that ectopic expression of EIF4E induces resistance to apoptosis, the study used EIF4E-transfected NIH 3T3 cells that were starved of serum for 16 hours to compare changes in global gene expression on polysome gradients. During the EIF4E-mediated response to apoptotic stress, 255 mRNAs, many of which contained a novel putative 5′ *cis* structural USER element, showed increased expression. Although this study examined the response of NIH 3T3 cells at relatively late times following overexpression and serum starvation, a more recent study used a tetracycline-inducible system to examine polysome profiles at 5 hours post-EIF4E-overexpression[99]. Many of the same mRNA targets and novel subsets of functionally related mRNAs were found in the translationally activated region of the polysome profiles. One predominant example of an activated mRNA was survivin, which is highly expressed in tumours and is responsive to inhibitors of the PI3 kinase–AKT–MTOR pathway[99].

One of the most striking examples of mRNA co-regulation at the translational level came from tumour-cell irradiation studies. Treatment of U87 glioma cells with ionizing radiation (~7 grays) resulted in the dynamic exchange of approximately 1,000 mRNAs into or out of active polysomes, indicating translational initiation. At the same time, fewer than 100 genes showed consistent changes at the transcriptional level, and these genes differed among glioma cell types[100]. Comparison of translational initiation among these different cell lines following irradiation showed that approximately 300 mRNAs consistently entered or exited the active polysomes. Interestingly, mRNAs that were translationally regulated fell into functional groupings that were suggested to respond coordinately to irradiation as post-transcriptional

Spindle body
A microtubule-organizing region, found in yeasts and other fungi, that is thought to be homologous to the centrosome of mammals.

Polysome gradients
A method in which velocity centrifugation on sucrose gradients is used to separate ribonucleoprotein complexes from ribosomes and ribosomal subunits from assembled polysomes that have initiated protein synthesis on an mRNA.

Table 1 | **Disease implications of RNA-binding proteins**

Disease or syndrome	RNA-binding protein	References
Neurodegenerative diseases; POMA paraneoplastic neuropathies	hnRNP-P2; ELAV/HuB,C,D; NOVA1,2	49,113,120,124
Fragile X mental retardation	FMRP	78,81,90,125
Turner syndrome	Ribosomal proteins (RP)	125
Mitochondrial and metabolic disorders	mitRP; IRP1,2; PCBP1,2	113,126
Oculopharyngeal muscular dystrophy	PolyA-binding protein 2	127
Spinal muscular atrophy	SMN1,2	128
Myotonic dystrophy	CUG-BP/EDEN; CELF3,4,5,	129
α- and β-thalassaemia; cardiovascular disease	BRUNO; ELAV/Hu; hnRNP-L1; αCP1,2; ETR3	113,130
Cancer and genotoxic responses; congenital dyskeratosis	ELAV/Hu; EIF4E; CUG-BP; IMP1–3; RP; musashi; telomerase	98,103,121,126, 131,132
Immunoregulatory disorders	TTP, TIA, TIAR, HuR	48,55,102,112,118

αCP, alpha-C-rich pyrimidine RBP; EIF4E, eukayotic translation initiation factor 4E; ELAV, embryonic lethal, abnormal vision homologue; BRUNO, CELF, ETR and CUG-BP constitute an ELAV-related RBP subfamily that bind to UA or UG-rich sequences including CUG triplet expanded repeats; FMRP, fragile X mental retardation protein; hnRNP, heterogeneous ribonucleoprotein; IMP, insulin-like growth factor mRNA-binding protein; IRP, iron response protein; mitRP, mitochondrial ribosomal protein; NOVA, neurooncological ventral antigen; PCBP, poly(rC)-binding protein; POMA, paraneoplastic opsoclonus-myoclonus ataxia; SMN, survival of motor neuron protein; TIA, T-cell-restricted intracellular antigen RBP; TIAR, TIA-related RBP; TTP, tristetraproline.

GW(P) bodies
Ribonucleoprotein particles that are thought to be the sites of RNA processing (P bodies) in yeast. The GW RNA-binding protein is a major component of these bodies in mammalian cells, in which they were discovered using an autoantibody. The exact functions of GW(P) bodies remain unclear.

Stress granules
Dynamic pleomorphic structures that are found in the cytoplasm of mammalian cells following physical and chemical perturbations such as oxidative stress. The mRNAs that are present in stress granules are translationally silent.

Lupus
An autoimmune disease in which autoantibodies are generated against normal human proteins, including small nuclear ribonucleoproteins (snRNPs). Autoantibodies can be used to identify RNA-binding proteins and small RNAs contained in snRNPs.

Exosomes
Membrane-bound vesicles that are involved in cell-to-cell exchange of proteins and lipids. Recent evidence indicates that retroviruses use exosomes to augment their infectivity and that mRNAs and microRNAs associate with exosomes for cell-to-cell exchange.

Selfish DNA operons
In bacterial DNA operons, the genes are located in close proximity to each other and this organization increases the probability that they are passaged together during horizontal transfer to other cells. A cell that acquires an entire functioning set of genes is likely to gain an adaptive advantage.

RNA operons. As might be expected, several of these mRNAs encode proteins that are involved in stress, survival and antiapoptotic functions. These findings are consistent with the idea that DNA damage does not induce increased production of new transcripts, but affects the post-transcriptional regulation of the previously synthesized mRNAs that are already in the cytoplasm so that the necessary functions are provided while the DNA is being repaired[101]. Similar mechanisms have been suggested for responses to heat shock, oxidative stress and other genotoxins, all of which are important in tumorigenesis and tumour therapy[102].

Evolutionary implications of RNA operons

As cellular modules that contain functionally related mRNAs and small regulatory RNAs, post-transcriptional RNA operons probably have a role in the evolution of RNA metabolism. Given that the information content of these modules is functionally coherent, they might be key components in the horizontal transfer of genetic information within and across cell boundaries. Moreover, RNA operons themselves might evolve under such circumstances to optimally drive genetic exchange and developmental processes, which might ultimately lead to the evolution of virus-like entities.

Selfish RNA operons and the origins of RNA viruses. Various RIP–chip studies have reported that the mRNAs that encode RBPs themselves are among the most common mRNA targets of RBPs in mammalian cells. For example, ELAV/Hu proteins commonly interact with mRNAs that encode RBPs, including their own transcripts[45,103–105]. Likewise, FMR1 and TIA1 in the mouse, Pumilio in *D. melanogaster* and the PUF proteins in *S. cerevisiae* have been reported to interact with a significant number of mRNAs that encode other RBPs[81,97,104]. One interpretation of these findings is that 'regulators of regulators' within the RNP infrastructure provide a self-sustaining and self-limiting balance of RBPs that ensures the resilience of post-transcriptional RNA networks[96].

RNPs are dynamic structures in mammalian cells. For example, GW(P) bodies and stress granules rapidly remodel and merge with one another, and then spontaneously separate[106]. Apoptotic blebs found on the surfaces of mammalian cells are rich sources of RNPs, and have been proposed to form vesicles for presentation of lupus antigens to the immune system[107]. The function of exosomes in protein exchange among cells is well established, and the 'Trojan exosome hypothesis' proposes that retroviruses can use exosomes for the transmission of retroviral particles[108]. Although it is speculative at this stage, given that each RNP granule or RNA operon is potentially a modular unit (FIG. 4), it is not impossible that they escape the cell with their RNAs intact and enter other cells using the same exosome pathway that is thought to assist in the passage of retroviruses[108]. Recently, Valadi *et al.* presented strong evidence that exosomes can transfer mRNAs and miRNAs between cells[109]. Could these dynamics provide a mechanism for cell–cell communication (as Valadi *et al.* suggest), for horizontal RNA transfer or for RNA operons to exchange cargo with other cells, serving as a mechanism for the origin of RNA viruses? Among the most common genes contained in RNA virus genomes are those encoding RBPs, such as coat proteins, matrix proteins and RNA-dependent polymerases. The main function of an RNA virus is self-replication; so, not unlike 'selfish' DNA operons, RNA operons would gain an adaptive advantage if they could escape the bounds of the cell and move to other cells (via exosomes) to survive and proliferate[110,111]. Such events would probably be strongly selected for and the ability of RNA operons to dynamically exchange their mRNA cargos using *cis*-acting adaptor elements or USERs would provide an opportunity for evolutionary improvement over time (FIGS 3,4). The fitness of a mobile RNA operon would be

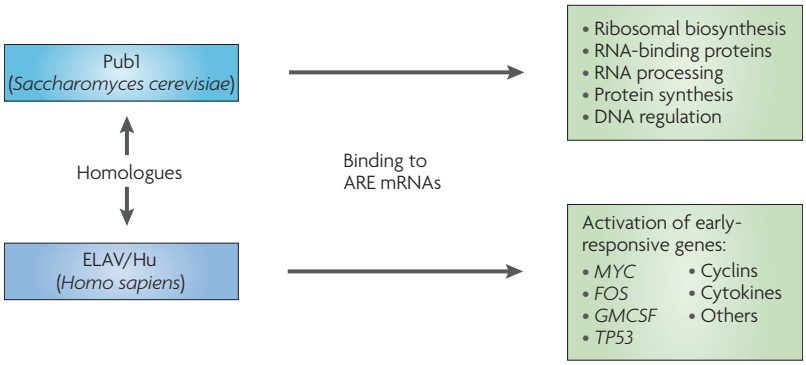

Figure 6 | **Evolutionary wiring and rewiring of RNA–protein interactions.** The RNA-binding protein (RBP) homologues Pub1 in *Saccharomyces cerevisiae* and the ELAV/Hu proteins in humans use highly conserved RNA-binding motifs to recognize AU-rich elements (ARE), which are known to regulate RNA stability. Pub1 targets mRNAs encoding proteins involved in RNA processing and translation, whereas the mRNA targets of Hu proteins encode immediate-early gene products, such as MYC and FOS, and cytokines such as GMCSF. Therefore, multiple mRNAs can be co-regulated by the acquisition of RNA-binding elements or USERs in their 3′ UTRs. By this mechanism, any mRNA can theoretically evolve to become a member of a given RNA operon.

enhanced dramatically with the evolution of a reverse transcriptase and retroposon capability that could allow their RNAs to be reverse-transcribed and integrated into the host genome[108]. The emergence of an acute 'virus' of the type we associate with pathogenesis from evolving mobile RNA operons would be rare, because the majority of such events would be more likely to produce less acute, 'inapparent' or persistent viruses.

Wiring and rewiring of RNA operons among species. Many RNA-binding motifs of RBPs and their corresponding RNA-binding sites are highly conserved in evolution[94,97]. For example, the AU-rich elements that are characterized by the AUUUA pentamer sequence tend to be recognized by the same RBPs in many species[45,61,103] (FIG. 6). Human ELAV/Hu proteins and TTP have homologues in *S. cerevisiae* that bind to the same type of AU-rich element; however, the proteins that are encoded by the mRNA targets in different species have different collective functions[46,63,65,112]. For this reason, the RBP-binding motifs and their RNA-binding elements can be seen as adaptors that can be 'rewired' during evolution[12,15]. This kind of RNA–protein platform would seem evolutionarily advantageous, and could be ancient. Thus, the RNA-operon paradigm can be seen as an evolutionary adaptation to meet different collective needs, allowing multiple mRNAs to be co-regulated in a similar way in different species. By acquisition or evolution of a functional USER element, any mRNA could conceivably become a member of a given RNA operon contributing to the fitness that is provided by its co-regulation with other genes. Moreover, the opportunity for rewiring of RNA operons as discussed above could provide an evolutionarily agile mechanism for plasticity of the RNP infrastructure.

Synexpression
Synchronous temporal or spatial expression of groups of genes during development.

Disease implications

Most diseases result from multiple complex traits or the combinatorial action of mutations[113–115]. RNA operons could help to uncover or explain some of these combined functions[88,116–118]. An RNA operon could lose a specific mRNA owing to a defect or a polymorphism in a USER region that prevents a crucial RBP from binding and regulating that mRNA (FIG. 4). For example, the mRNA that encodes fibroblast growth factor 20 (FGF20) contains two SNPs in its 3′ UTR that are strongly associated with Parkinson Disease[119]. It will be important to identify any RBP(s) that bind the wild type or fail to bind to the mutant UTR, as well as other mRNAs that might be co-regulated with FGF20 mRNA. Many SNPs have been identified in miRNA coding regions and in their predicted mRNA target sites, indicating that genetic differences at sites of post-transcriptional regulation might be more important than previously thought[14]. Thus, dysregulation of an RNA operon or failure of an mRNA to be properly expressed could be relevant to complex human diseases[114,120,121]. The well-known example of fragile X mental retardation results from loss of expression of the entire FMR1 RBP[78,81,90]. Other RBPs have also been implicated in human disease (TABLE 1). As more proteins are found to be multifunctional in human cells, understanding the coordinated expression of their mRNAs with other mRNA subsets could reveal disease-relevant combinations of functionally related mRNAs and proteins. Finally, targeting mRNAs using RNAi has become an active area of biotechnology research, and understanding RNA operons could lead to combination therapies that can realign defective post-transcriptional RNA networks.

Conclusions

The investigation of coordination of mRNA processing as an RNP-driven process is in its infancy, but the studies described above have indicated its importance in many biological processes. Taken together, these findings demonstrate that mRNAs are not left to the vicissitudes of random events in the nucleus and cytoplasm, but are instead members of a highly organized infrastructure that functions from the point of transcription to protein synthesis. As knowledge of post-transcriptional RNA operons and regulons advances, it might help to define new functions for multifunctional proteins as previously unknown members of pathways or protein complexes. These functional relationships might include regulatory interconnections within pathways and higher-order connections across pathways that are difficult to discern using traditional methods of biomolecular investigation. Indeed, exploring the diverse functions of multifunctional proteins across RNA regulatory networks is an even greater challenge, given that we have prejudiced ourselves by assigning functions to proteins on the basis of their original route of discovery. Similar to other global investigative approaches that are being developed as part of systems biology, the study of post-transcriptional RNA coordination has the potential to reveal new principles of cell–cell interaction and mechanisms of synexpression during development[122]. Post-transcriptional RNA operons represent one of many globally interconnected levels of gene-expression regulation; perhaps many others remain to be discovered.

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Competing interests statement

The author declares competing financial interests: see web version for details.

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