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Eukaryotic mRNPs May Represent Posttranscriptional Operons

Hypothesis

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Summary

Genomic array analysis of endogenous mammalian ribonucleoproteins has recently revealed three novel findings: (1) mRNA binding proteins are associated with unique subpopulations of messages, (2) the compositions of these mRNA subsets can vary with growth conditions, and (3) the same mRNA species can be found in multiple mRNP complexes. Based on these and other findings, we propose a model of posttranscriptional gene expression in which mRNA binding proteins regulate mRNAs as subpopulations during cell growth and development. This model predicts that functionally related genes are regulated posttranscriptionally as groups by specific mRNA binding proteins that recognize sequence elements in common among the mRNAs.

Prokaryotic Operons Are Efficient but Not Evident in Higher Eukaryotes

Operons are clusters of genes physically ordered in the genome in a manner enabling them to be regulated as groups (reviewed in Beckwith, 1996; Gralla and Collado-vides, 1996). The clustering of genes into operons allows prokaryotic organisms to coordinately express proteins involved in a common process, while greatly facilitating the ability to respond efficiently to environmental changes (Figure 1A). The colinearity of an operon's DNA and its encoded polycistronic (polygenic) mRNA allows a single transcriptional event to facilitate the production of a set of functionally linked proteins. Because transcription and translation are physically coupled in prokaryotes, operons provide a highly efficient method of regulating the transfer of genetic information from DNA into protein. Additionally, prokaryotes have evolved feedback loops and higher order regulatory mechanisms (i.e., regulons) that represent interactive operational networks necessary for efficient growth under different environmental conditions (reviewed in Niedhardt and Savageau, 1996). While operons provide a major paradigm of genetic regulation in prokaryotes and in certain lower eukaryotes such as *C. elegans* (Huang et al., 2001), the identification of an analogous system in higher eukaryotic systems has been less obvious. Although it would appear equally advantageous for multicellular organisms to utilize such an efficient regulatory mechanism, most of the genes physically linked into operons in prokaryotes are unexpectedly dispersed as independent genes in higher eukaryotes (Kenmochi et al., 1998; Uechi et al., 2001; Yoshihama et al., 2002).

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Although highly efficient, the clear disadvantage of the prokaryotic operon is the constraint placed upon gene expression by physically coupling the production of multiple proteins in a fixed group. For example, the presence of a nonsense mutation in an operon can result in polarity effects that disrupt downstream functions. Also, the ability to independently regulate the production of proteins with multiple functions is limited when genes are confined to a polycistronic architecture. In eukaryotes, many proteins have become multifunctional (Hentze, 1994; Jeffery, 1999). Thus, producing a protein contained within an operon for a new function would be inefficient because the other proteins in the operon would have to be expressed concurrently (Figure 1A).

How Do Mammalian Cells Efficiently Coordinate Gene Expression without Operons?

The coordinated regulation of multiple genes is needed in higher eukaryotes to accomplish complex phenotypic functions such as cell growth and differentiation (Niehrs and Pollet, 1999; Qian et al., 2001). This includes the production of proteins for generalized housekeeping functions and proteins for specialized functions that can be tissue or developmental specific (Ideker et al., 2001; Michelson, 2002; Wen et al., 1998). In development, the orchestration of multifaceted networks is essential for efficient performance of both generic and specialized gene functions (Davidson et al., 2002; Niehrs and Pollet, 1999). It is likely that gene regulatory networks require coordination between transcriptional and posttranscriptional processes (Keene, 2001). Most studies of gene regulation in higher eukaryotes have focused on transcription and are based upon the tenet that regulatory networks are the result of interactions among promoters, transcription factors, and enhancers. In eukaryotes, transcription is not directly coupled to translation and the two processes are physically separated by the nuclear membrane. While transcription is a significant contributor to eukaryotic gene expression, several studies have shown that there is often a poor correlation between mRNA levels and protein production when cells are functionally perturbed (Gygi et al., 1999; Ideker et al., 2001). The discordance between mRNA and protein levels under these conditions occurs because eukaryotic mRNAs undergo posttranscriptional processing and regulation. Although it is accepted that promoters residing on dispersed genes participate in the coordinated production of gene products, posttranscriptional regulation must also function to maintain coordinated protein production (Gygi et al., 1999; Keene, 2001; Klausner et al., 1993).

The regulatory benefits afforded by operons may be recapitulated in higher eukaryotic cells by utilizing regulatory features at both the DNA and the mRNA levels. At the DNA level in both prokaryotes and eukaryotes, transcription is coordinated whether genes are in operons or dispersed throughout the genome (Lieb et al., 2001; Moller et al., 2002; Warner, 1999). But at the mRNA level, polycistronic mRNAs have been replaced in higher

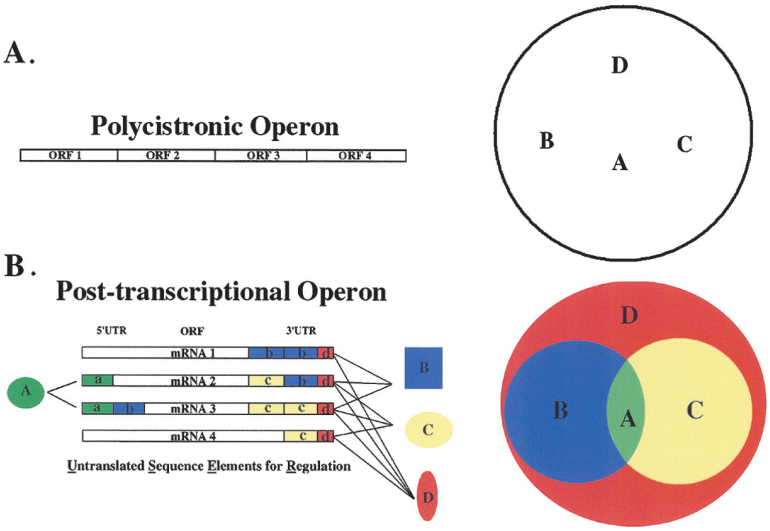

Figure 1. Combinatorial Organization of mRNA Subpopulations in Eukaryotic Ribonucleoproteins

(A) Representation of a polycistronic transcript from a prokaryotic operon containing open reading frames coding for four genes (ORFs 1–4).

(B) Four monocistronic mRNAs that contain different combinations of USER codes are represented (colored boxes a–d). mRNAs containing a specific USER code can be bound by a specific mRNA binding protein (colored shapes A–D). This model illustrates the potential for genetic information to be shuffled combinatorially as mRNA subsets. In this example, USER codes are placed in the 5′ and 3′ untranslated regions (UTRs) but could also reside in the coding regions. An important feature of the model is that a given mRNA binding protein may regulate its own mRNA, thus affecting the regulation of its larger subset of mRNAs. Venn diagrams (shown at the right) depict subpopulations of mRNAs that can be organized in various combinations by the USER codes.

eukaryotes with monocistronic transcripts that contain regulatory 5′ and 3′ untranslated regions (UTR) (Table 1; Figure 1B). Even when regulation is predominantly mediated by transcription, all transcripts must still pass commensurately through the posttranscriptional infrastructure in order to generate the intended protein products. Recent advances in the use of genomic array technologies have revealed that mammalian mRNA binding proteins interact with subsets of messages and, following functional perturbation, these subsets can change in a dynamic manner (Brown et al., 2001; Eystathioy et al., 2002; Keene, 2001; Tenenbaum et al., 2000). Our posttranscriptional operon model helps explain how subsets of transcripts may be organized to coordinate the expression of gene products needed collectively for a biological process (Figure 1B). In addition, it suggests that higher eukaryotes have acquired the ability to posttranscriptionally coordinate the regulation of subsets of monocistronic mRNAs by utilizing related sequence elements present in the 5′ and 3′ UTRs of the transcripts. Posttranscriptional regulation of these otherwise independent mRNA species is codified by various conserved *cis* sequences we have termed untranslated sequence elements for regulation (i.e., USER codes).

Table 1. Sequence Elements Identified in Processed mRNA and Their Interacting Proteins

mRNA *cis* Element	Location	mRNA	RNA Binding Proteins
Iron response element (IRE)	5′UTR 3′UTR	H and L-ferritin, transferrin receptor	Iron regulatory proteins, Aconitase, Transferrin
Male specific lethal (MSL-2)	5′UTR 3′UTR	msl-2	Sex-lethal (SXL)
Internal ribosome entry site (IRES)	5′UTR	picornavirus, cellular mRNAs	PTB, UNA, PCBP-2, La/SS-B
5′-terminal oligopyrimidine tract (TOP)	5′UTR	ribosomal proteins, translation factors	La/SS-B, CNBP
AU-rich elements (AREs)	3′UTR	early response gene, cytokines, others	ELAV/Hu proteins, TTP
Selenocysteine insertion sequence (SECIS)	3′UTR	selenoprotein	SECIS binding protein
Histone stem loop	3′UTR	histone	Stem loop binding protein (SLBP)
Cytoplasmic polyadenylation elements (CPEs)	3′UTR	developmental, embryonic mRNAs, Myb	CPEBP
Nanos translational control element	3′UTR	nanos, hunchback	Smaug repressor, other factors
Amyloid precursor protein element (APP)	3′UTR	APP	Multiple cytosolic proteins
Translational regulation element (TGE)/ direct repeat element (DRE)	3′UTR	tra-2 ad GLI	Direct repeat factor
Bruno element (BRE)	3′UTR	oskar	Bruno
15-lipoxygenase differentiation control element (15-LOX-DICE)	3′UTR	lox	LOX-binding proteins, hnRNPK and E1
G-quartet element	5′UTR 3′UTR	FMRP, MAP-1B, Rab6,Sec-7 Munc, V1a/ GPC, others	FMRP

See reference list and http://bighost.area.ba.cnr.it/srs6bin/wgetz?-page+LibInfo+-id+42RGj1Ipf1K+-lib+UTRSITE and http://bighost.area.ba.cnr.it/BIG/UTRHome/

Multiple Elements in an mRNA Can Determine Different mRNA Fates via Interactions with Distinct RNA Binding Proteins

Mammalian RNA binding proteins have been shown to associate with multiple mRNAs as discrete subsets of the total cellular mRNA population, both in vitro (Gao et al., 1994; Levine et al., 1993) and in vivo (Brown et al., 2001; Eystathioy et al., 2002; Takizawa et al., 2000; Tenenbaum et al., 2000). The acquisition of shared regulatory USER codes in the UTRs of monocistronic mRNAs provides a means to mimic the coordinated regulatory advantages of clustering genes into polycistronic (polygenic) operons. This mechanism also allows for the advent of new regulatory pathways as proteins evolve multifunctional properties. For example, various eukaryotic ribosomal proteins have extraribosomal functions (Vilardell et al., 2000b; Wool, 1996), suggesting that instances have arisen in which it was advantageous to regulate their expression independently of the constraints of an operon. For mRNAs being regulated independently of one another, one would predict that organisms exploit alternative uses for a protein, as well as the ability to utilize a protein in more than one cellular compartment for different functions (Hentze, 1994; Jeffery, 1999). We contend that the evolution of monocistronic mRNA transcripts with flanking regulatory UTRs provides a significant adaptive advantage for higher eukaryotes by providing a means to separately regulate the expression of a protein as it acquires a new function. Whether regulated transcriptionally or posttranscriptionally, this would allow a protein to evolve new functions independently of its ancestor protein without the need for gene duplication because the mRNA encoding the new function could be separately regulated. Additionally, and as described below, the evolution of monocistronic mRNAs would allow genetic information to be regulated in a variety of combinations as subsets of transcripts (quasi-genomes). Thus, a posttranscriptionally regulated, monocistronic genetic organization allows mRNAs to be expressed either independently or in concert with other genes as needed (Figure 1B). A prediction of this posttranscriptional regulatory model is that multiple USERs present on an mRNA would allow the protein product to be localized at more than one intracellular site and/or to be expressed at different times. Recent studies have shown that specific mRNA populations can be coimmunoprecipitated with different mRNA binding proteins that are known to recognize distinct USERs within the mRNA species present in the subset (Tenenbaum et al., 2000, 2002; Brown et al., 2001).

Using genomic arrays to sample populations of mRNAs associated with individual RNA binding proteins, we observed redundancy of certain mRNAs in endogenous mRNP complexes (Eystathioy et al., 2002; Keene, 2001; Tenenbaum et al., 2000). These findings are consistent with the presence of multiple USER codes in mRNAs that allow them to interact with different RNA binding proteins (Table 1; Figure 1B). Examples of messages found in more than one type of mRNP complex include the c-*myc* and β-actin mRNAs. Several regulatory elements in c-*myc* have been identified including a 5′ UTR IRES (van der Velden and Thomas, 1999), an AU-rich element (ARE) present in the 3′ UTR (Shaw and Kamen, 1986), and an element in the coding region (Doyle et al., 1998; Kislauskis et al., 1994). In addition, multiple *cis* elements have also been identified in β-actin mRNA (Duret and Bucher, 1997; Ross et al., 1997). Indeed, there are numerous examples of mRNAs that contain multiple untranslated sequence elements that potentially could function as USER codes and be regulated by *trans* acting mRNA binding proteins. Many of these UTR elements are conserved and have been identified by phylogenetic comparison and are believed to function in various regulatory capacities (Duret and Bucher, 1997; Duret et al., 1993; Hardison, 2000; Pesole et al., 2001, 2002, and references therein). Examples of these UTR sequence elements can be found at http://pbil.univ-lyon1.fr/acuts/ACUTS_list.html. Some of these conserved UTR *cis* sequences contain well-characterized elements like the ARE, CPE (cytoplasmic polyadenylation element), Nanos translational control element (TRE), Bruno element (BRE), and the TOP (terminal oligo-pyrimidine) sequence, which are known to interact with mRNA binding proteins and are likely functioning as USER codes (Amaldi and Pierandrei-Amaldi, 1997; Dahanukar and Wharton, 1996; Kim-Ha et al., 1995; Mendez and Richter, 2001; Shaw and Kamen, 1986).

If a shared USER code exists among a subset of monocistronic mRNAs, the model would predict that they could be regulated as a group, thus providing a posttranscriptional eukaryotic counterpart to polycistronic prokaryotic operons. Additionally, instances are expected to arise in which other USER codes present in a specific mRNA within a subpopulation could allow it to be regulated independently of other transcripts in that subset. Examples of both have been observed among the mRNA targets of the ELAV/Hu proteins (Tenenbaum et al., 2000) and Fmrp (Brown et al., 2001) RNA binding proteins. Altering putative USER sequences and analyzing the effects on the mRNAs associated within an mRNP complex is one way to further test this aspect of the model.

Many of the proteins involved in making the translational machinery are expressed as a result of coordinated transcription (Lieb et al., 2001). However, in order to ensure proper production of these proteins, coordinate regulation must also be maintained at the posttranscriptional level (Warner, 1999). Interestingly, TOP sequence elements (Table 1) have been found in the 5′ UTRs of all mammalian ribosomal protein mRNAs and in many other translation factors (Amaldi and Pierandrei-Amaldi, 1997; Meyuhas, 2000; Yoshihama et al., 2002). Amaldi and coworkers have also provided evidence that several RNA binding proteins interact with TOP sequences (Crosio et al., 2000; Pellizzoni et al., 1996, 1997, 1998). The TOP sequence may serve as a USER code that allows the subset of ribosomal protein mRNAs to be regulated posttranscriptionally as a group. Other USER codes embedded in some of these mRNAs may allow their expression to be additionally regulated for separate functions (Wool, 1996). Thus, individual transcripts, while representing the genes from which they are derived, are predicted to assort and reassort in a manner that allows their gene products to participate in multiple complex biological processes.

The posttranscriptional operon model is compatible with the more established transcriptional regulation of coordinated gene expression in higher eukaryotic organisms but allows for additional coordination of ex-

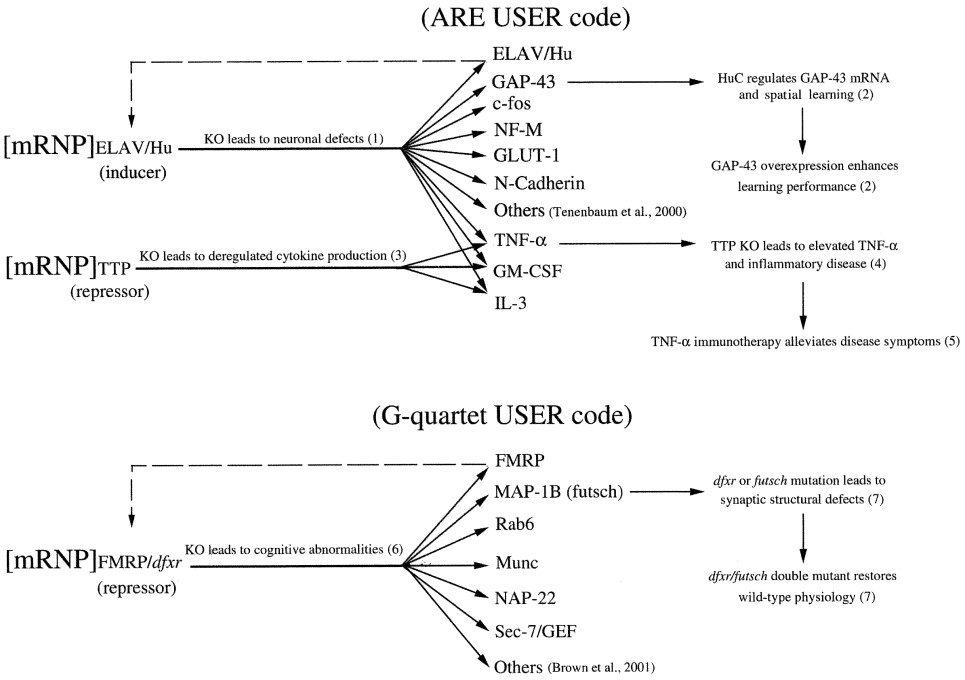

Figure 2. Cause and Effect Relationships among Three mRNA Binding Proteins and Their Downstream Targets as Validated Using Biochemical and Genetic Methods

ELAV/Hu proteins bind in vitro and in vivo to multiple early response gene transcripts and upregulate expression. GAP-43 mRNA is regulated in parallel with HuC in rodent brain in conjunction with spatial learning tests. Overexpression of GAP-43 alone results in enhanced learning performance (Routtenberg et al., 2000). Tristetraprolin (TTP) and fragile-x-mental retardation proteins (FMRP) are both repressors of expression of target mRNAs as shown biochemically and genetically. Alteration of expression of the downstream target mRNA or gene product generates the normal phenotype under the predicted conditions. While some target genes may respond differently depending upon other factors associated with the mRNP complexes, the examples shown are consistent with a posttranscriptional operon model in which multiple mRNAs are regulated as a subpopulation to generate complex phenotypes (Tenenbaum et al., 2000; Keene, 2001; Brown et al., 2001). The dotted lines indicate that the RNA binding proteins have been shown to bind their own mRNAs, thus providing the potential for feedback regulation (Abe et al., 1996; Schaeffer et al., 2001). References as shown: (1) reviewed in Keene, 1999, 2001; (2) Quattrone et al., 2001; (3) Lai et al., 1999, and Taylor et al., 1996; (4) Carballo et al., 1998; (5) Carballo et al., 2000; (6) Jin and Warren, 2000; (7) Zhang et al., 2001.

pression of mRNAs as members of distinct subsets (Figure 1B). Moreover, it provides a mechanism for creating functional diversity and flexibility in response to cellular needs while utilizing transcripts from a relatively modest number of genes in various polygenic combinations. Because of the potential to generate numerous combinations of protein products, combinatorial mRNA assortment, together with alternative splicing, is predicted to regulate the expression of complex phenotypes during organ, tissue, and tumor development. As described below, subsets of posttranscriptionally regulated mRNAs can be viewed as quasi-genomes with the plasticity to be assembled or disassembled by mRNA turnover mechanisms when necessary.

Regulatory Advantages of Posttranscriptional Operons

The model suggested here provides an explanation for the finding that mRNA subsets contained within specific mRNPs change following chemical treatment to induce differentiation (Tenenbaum et al., 2000). Addition of the anti-tumor drug retinoic acid to embryonic carcinoma (EC) cells led to reassortment of the mRNA population bound to a tumor-specific mRNA binding protein, ELAV/HuB. Many early response gene mRNAs, including c-*myc* mRNA, are associated with ELAV/HuB mRNP complexes in EC cells following the induction of differentiation by retinoic acid treatment. Additionally, the induction of a muscle differentiation pathway in these same cells using dimethyl sulfoxide (DMSO) resulted in a different population of mRNAs associated with ELAV/HuB mRNP complexes (Tenenbaum et al., 2002). By shifting the overlapping populations depicted in the Venn diagram of Figure 1B, one can envision the potential for reassortment of mRNA subsets involved in functional perturbations.

As a further analogy to operons, RNA binding proteins can function as both inducers and repressors of gene expression at the posttranscriptional level (Figure 2). For example, ELAV/Hu proteins have been shown to upregulate mRNA stability and protein production of many downsteam target mRNAs (Jain et al., 1997; Fan and Steitz, 1998; Peng et al., 1998; Levy et al., 1998; Antic et al., 1999; Keene, 1999). The neuronal ELAV/Hu proteins, HuB, HuC, and HuD, have been shown to play a role in neurite formation in mammalian systems, presumably through their interactions with mRNAs encoding neurofilament proteins, tau and the growth-associated protein (GAP-43), neuronal cadherin, and others (reviewed in Keene, 1999; Tenenbaum et al., 2000; Quattrone et al., 2001). A direct role of HuC in neuronal plas-

ticity and learning was demonstrated by HuC antisense knockdown in the hippocampus that caused both the mRNA encoding GAP-43, as well as GAP-43 protein, to be downregulated (Quattrone et al., 2001). Moreover, induced spatial learning in both mice and rats under two independent learning performance tests resulted in increased expression of HuC and concomitant upregulated GAP-43 production (Figure 2). Previous work has shown that increased expression of GAP-43 in transgenic mice results in enhanced learning (Routtenberg et al., 2000).

ELAV/Hu proteins have been shown to bind with high affinity to mRNAs encoding cytokines such as GM-CSF, interleukin-3, and TNF-α that contain AREs in their 3′ UTRs. In addition, another family of RNA binding proteins containing CCCH zinc fingers includes tristetraprolin (TTP) and butyrate-response factors (BRFs) that have been shown to participate in mRNA stability (Lai et al., 2001). For example, TTP can bind avidly to the ARE of TNF-α, interleukin-3, and GM-CSF mRNAs and repress protein expression by downregulating mRNA stability (Carballo et al., 2000; Lai and Blackshear, 2001; Lai et al., 1999). A knockout mouse lacking TTP derepresses cytokine production, giving rise to extremely high levels of TNF-α that lead to inflammation and rheumatic disease (Carballo et al., 1998; Taylor et al., 1996). While additional in vivo studies are needed to confirm this supposition, these data suggest that the ELAV/Hu and TTP may function as opposing inducers and repressors of cytokine transcripts (Figure 2).

Another key prediction of the posttranscriptional operon model is that mutations of genes encoding downstream target mRNAs of an RNA binding protein would have phenotypes consistent with those of mutations of the RNA binding protein itself. The paradigm of downstream genetic validation has been used to establish functional linkages among members of various biochemical and developmental pathways (Davidson et al., 2002; Niehrs and Pollet, 1999). Thus, as with the HuC learning experiments described above, a phenotype associated with a defect in an RNA binding protein may be reversible by deleting or substituting the protein product of the downstream mRNA. Additional evidence for such a relationship comes from studies of mRNAs bound in vivo to the fragile-x-mental retardation RNA binding protein (Fmrp) in mouse (Figure 2). Among the mRNAs identified by immunoprecipitation and microarray analysis of mouse brain was that encoding the microtubule associated protein, MAP-1B (Brown et al., 2001). In a related study of genetic deletion of the Fmrp homolog in *Drosophila* (dFXR), Zhang et al. (2001) found that neuronal defects observed in the *dfxr* mutant fly could be reversed (suppressed) by mutating the MAP-1B gene homolog, *futsch*, as a double mutant with the *dfxr* gene. Also, overexpression of Futsch protein generated a neuromuscular junction phenotype very similar to that of the *dfxr* mutant. This study demonstrated that dFXR protein binds to *futsch* mRNA in fly head extracts and is a translational repressor of Futsch protein expression, as also suggested by the mRNA target analysis in mouse. Moreover, the larger mRNA target set elucidated in the microarray study of Brown et al. (2001) was consistent with the known functions of their protein products in neuronal development and cognitive function (Darnell et al., 2001). In most cases the mRNAs associated with mouse Fmrp contained a G-quartet binding site in the 3′ UTR. Numerous examples of functional linkages among the protein products of mRNA targets of multitargeted mRNA binding proteins are likely to emerge by applying these biochemical, genetic, and genome-wide approaches (Keene, 2001).

Genomic Plasticity at the Posttranscriptional Level via Quasi-Genomes

While the integrity of a genome must be stably maintained as a heritable source of genetic information, the information contained in the transcribed mRNA can be continuously renewed and degraded. Our model proposes that USER codes allow subsets of mRNAs to be regulated in ribonucleoprotein complexes or to otherwise be stored as subpopulations for translation (Figures 1B and 2). In this manner, subsets of related mRNAs in mRNPs or associated with the endoplasmic reticulum can be thought of as transient quasi-genomes that are dynamic (e.g., by shifting the overlapping circles of the Venn diagram in Figure 1B). When these mRNPs contain mRNAs encoding transcription factors they can feed back on the transcriptional program. Several examples of this type of regulation have been described in neuronal systems (Eberwine et al., 2001; Keene, 2001). Indeed, many ERG mRNAs that are regulated posttranscriptionally by ELAV/Hu proteins encode transcription factors including c-*myc*, c-*fos*, CREB, and zif268 (Fan and Steitz, 1998; Gao et al., 1994; Levine et al., 1993). Likewise, the very mRNAs that encode mRNA binding proteins may be similarly regulated since some mRNA binding proteins have been shown to bind their own mRNAs. Therefore, one would predict that a feedback mechanism in turn affects the larger subset of messages that the mRNA binding protein regulates (Abe et al., 1996; Brown et al., 2001; Hentze, 1994; Schaeffer et al., 2001; Vilardell et al., 2000a, 2000b). A test of this posttranscriptional regulatory model would be to delete or alter the USER elements in target mRNAs and then to monitor changes in the localization or temporal production of the encoded proteins. Additionally, alterations in the expression of a regulatory mRNA binding protein using genetics or RNA would be predicted to directly affect the expression of multiple mRNAs and thereby the functions of their encoded proteins en masse. Regardless of the precise mechanisms of gene expression that have evolved in lieu of the polycistronic operon, future models will have to account for coordinated regulation of the constellations of monocistronic mRNAs contained within the thousands of mRNP complexes in the cytoplasmic infrastructure.

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