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Toward a Genome-Wide Landscape of Translational Control

Ola Larsson[1], Bin Tian[2], and Nahum Sonenberg[3]

[1]Department of Oncology–Pathology, Karolinska Institute, Stockholm SE-171 76, Sweden

[2]Department of Biochemistry and Molecular Biology, UMDNJ–New Jersey Medical School, Newark, New Jersey 07103

[3]Department of Biochemistry and Goodman Cancer Research Centre, McGill University, Montreal, Quebec H3A 1A3, Canada

Correspondence: ola.larsson@ki.se

Genome-wide analysis of translational control has taken strides in recent years owing to the advent of high-throughput technologies, including DNA microarrays and deep sequencing. Global studies have unraveled a principal role, among posttranscriptional mechanisms, for mRNA translation in determining protein levels in the cell. The impact of translational control in dynamic regulation of the proteome under different conditions is increasingly appreciated. Here we review genome-wide studies that use high-throughput techniques and bioinformatics to assess the role of mRNA translation in the regulation of protein levels; we also discuss how genome-wide data on mRNA translation can be obtained, analyzed, and used to identify mechanisms of translational control.

The gene expression pathway leading to protein production consists of many mechanistic layers that are subject to regulation. They are commonly grouped into transcriptional or posttranscriptional types. Some posttranscriptional mechanisms, including RNA splicing, mRNA editing, and posttranslational modification, determine the identity and activity of the protein products, whereas others control protein levels by regulating transport of mRNA from the nucleus to the cytoplasm, mRNA stability, translation, and protein stability. Determining how posttranscriptional regulation contributes to protein levels and, more precisely, how regulation of translation impacts gene expression have attracted substantial attention during the last decade.

POSTTRANSCRIPTIONAL MECHANISMS SUBSTANTIALLY AFFECT GENE EXPRESSION LEVELS AT A GENOME-WIDE SCALE

Several studies have examined the extent to which posttranscriptional mechanisms affect protein expression by comparing mRNA and protein levels, either in one cell state or across different conditions. This is typically based on Pearson or Spearman correlation coefficients, denoted as r_p or r_s, respectively (for examples, see Fig. 1). Both range from −1 to 1, where 0

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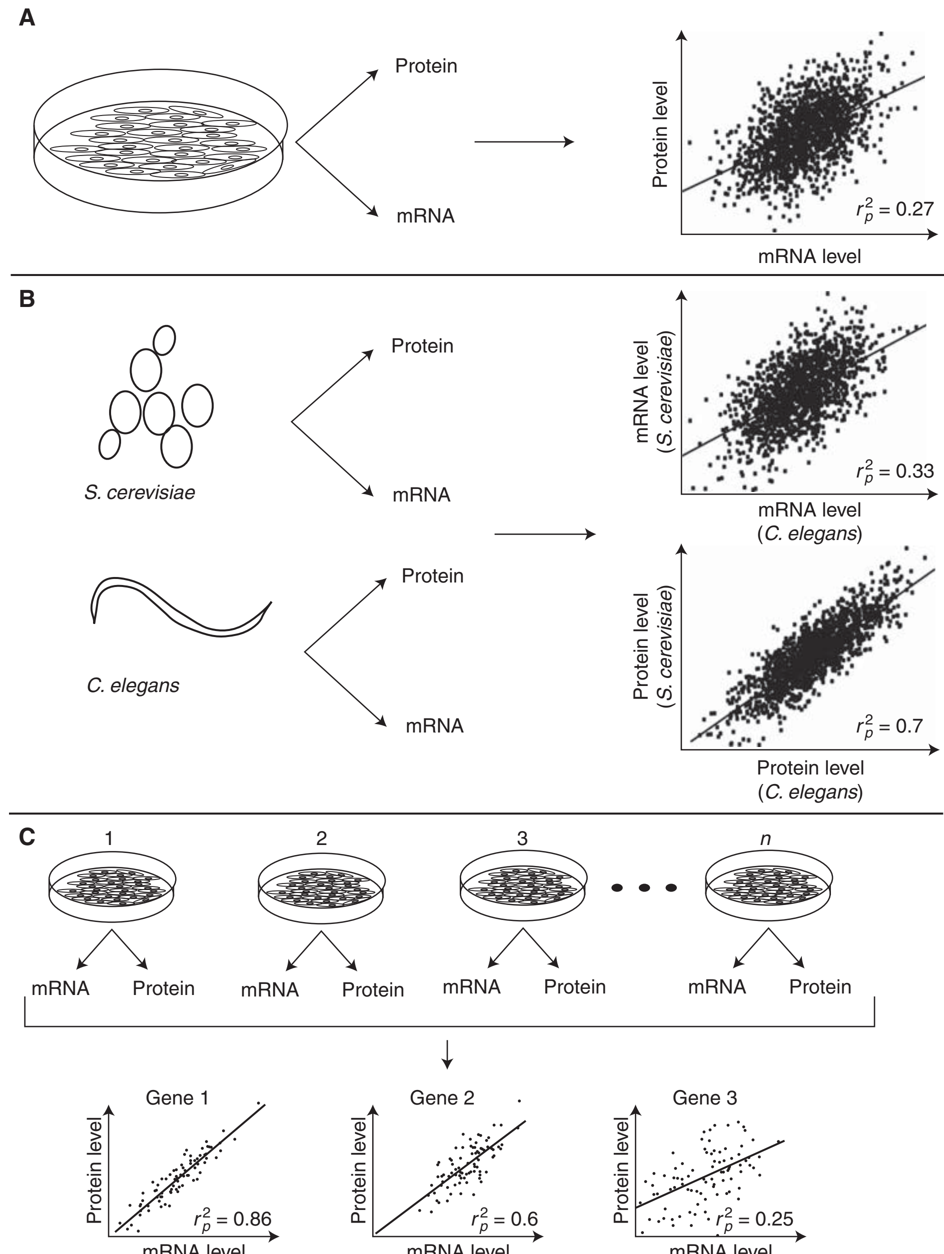

Figure 1. Approaches to examine the contribution of posttranscriptional regulation to protein expression. (*A*) Comparison of mRNA and protein levels from a single condition. Measured mRNA and protein levels are compared to indicate posttranscriptional regulation across genes using r_p^2, which describes the fraction of the observed protein levels that are explained by mRNA levels. (*B*) Cross-species analysis of mRNA and protein levels. mRNA and protein levels are obtained in parallel from two species, and cross-species levels are compared. Higher correlation of protein levels across species as compared with mRNA levels indicates posttranscriptional regulation that maintains conserved protein levels. (*C*) Parallel measurements of mRNA and protein levels across many conditions. Per gene mRNA and protein levels are compared across conditions to estimate dynamic regulation of gene expression by posttranscriptional mechanisms. As indicated by r_p^2, gene 3 shows more posttranscriptional regulation as compared with gene 1. Simulated numbers are shown.

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indicates no correlation and −1 and 1 indicate perfect negative and positive correlations, respectively. Whereas Pearson correlation is based on actual values, Spearman correlation uses ranks (ordered by values) and is therefore less influenced by extreme values (outliers). A squared Pearson correlation value (r_p^2; the coefficient of determination) describes how much of the variance of one factor is explained by that of the other factor. For example, a Pearson correlation between protein and mRNA levels of 0.6 indicates that 36% (0.6^2) of the protein levels can be explained by mRNA levels. Thus, a lower r_s or r_p^2 between mRNA and protein levels indicates more posttranscriptional regulation.

Comparison of mRNA and protein levels across genes under one condition (e.g., steady-state growth) has been conducted in species ranging from bacteria to humans. These studies attempted to assess intrinsic posttranscriptional regulation of gene expression (Fig. 1A). Pioneering studies in yeast established genome-wide differences in expression levels between mRNAs and proteins, although they assessed only a fraction of all yeast genes (Futcher et al. 1999; Gygi et al. 1999). Subsequent studies based on measurements of more genes estimated r_p^2 from 0.14 to 0.73 (Lu et al. 2007; Schmidt et al. 2007; Ingolia et al. 2009) or r_s of 0.57 and 0.58 (Ghaemmaghami et al. 2003; Beyer et al. 2004). In bacteria, r_p^2 between 0.20 and 0.47 has been reported (Nie et al. 2006; Lu et al. 2007; Jayapal et al. 2008). Moreover, in a recent single-cell study in *Escherichia coli*, mean mRNA copies and protein copies showed r_p^2 of 0.29 and 0.59 using deep sequencing of RNA (RNA-seq) and fluorescence in situ hybridization (FISH), respectively (Taniguchi et al. 2010). These studies indicate that posttranscriptional regulation is used in unicellular organisms, although estimates of its extent vary substantially across studies.

Similar studies performed in multicellular organisms, including *Arabidopsis thaliana* (Baerenfaller et al. 2008), *Drosophila melanogaster*, and *Caenorhabditis elegans* (Schrimpf et al. 2009), all indicated substantial posttranscriptional regulation (r_p^2 between 0.27 and 0.46 [Baerenfaller et al. 2008] and r_s of ∼0.6 [Schrimpf et al. 2009]). Moreover, a recent study of a human cancer cell line reported a modest r_p^2 of 0.29 from more than 1000 genes (Vogel et al. 2010). Interestingly, these investigators built a model that predicts protein levels from measured mRNA levels and a variety of mRNA properties that affect posttranscriptional regulation, such as the length of the 3′ untranslated region (UTR). The r_p^2 between the predicted and the measured protein levels increased dramatically as compared with the initial r_p^2 between mRNA and protein levels (from 0.29–0.67). Because the data used to derive the model were also used to generate the predictions, creating the possibility of data overfitting (meaning that the prediction outcome is heavily influenced by the data used to construct the model), future studies will be needed to assess the generality of the model. Despite this limitation, the study suggests that mRNA sequence features systematically impact protein production through posttranscriptional control.

Cross-species comparisons provide an alternative approach for assessing the importance of posttranscriptional regulation (Fig. 1B). In such studies, mRNA and protein levels from one species are compared with their orthologs in a different species. Assuming that the measurement error is similar for mRNA and protein levels, a higher cross-species correlation between protein levels than between mRNA levels would suggest a role of posttranscriptional control in maintaining conserved protein levels. Based on this reasoning, Schrimpf et al. (2009) reported r_s of 0.79 and 0.47 for protein and mRNA levels, respectively, when comparing *C. elegans* with *D. melanogaster*. A follow-up study examined all paired comparisons between seven species and found a higher cross-species correlation for protein levels in 17 out of 21 comparisons (Laurent et al. 2010). These comparisons included mRNA measurements obtained by RNA-seq, which is thought to better reflect relative mRNA levels across genes as compared with DNA microarrays (Laurent et al. 2010). Cross-species comparisons thus provide further support for the idea that posttranscriptional mechanisms substantially contribute to determination of protein levels.

A common critique of correlation-based mRNA/protein comparative studies is that the

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magnitude of systematic and random variations inherent in mRNA and protein analysis tools, such as DNA microarrays, RNA-seq, and mass spectrometry, is often unknown. This is of significance because more variation will lead to lower correlation, giving the appearance of a greater degree of posttranscriptional regulation. This ambiguity in the interpretation of r_s or r_p^2 has been addressed in some studies by assessing how much variation affects the r_s or r_p^2 values. Another caveat is that the half-lives of proteins and their cognate mRNAs often differ, which can reduce r_s or r_p^2 if the mRNA or protein levels are obtained under non-steady-state conditions. Indeed, in mouse NIH/3T3 cells, mRNAs show a median half-life of 9 h, whereas proteins have a median half-life of 46 h (Schwanhausser et al. 2011), making protein level regulation linger over a longer time period relative to the corresponding mRNA. Caution is therefore needed when interpreting r_s or r_p^2 values.

DYNAMIC REGULATION OF GENE EXPRESSION AT THE POSTTRANSCRIPTIONAL LEVEL

In addition to the studies discussed above, which evaluate intrinsic protein levels in the cell, progress has been made in understanding the role of posttranscriptional mechanisms in dynamic regulation of gene expression. Three approaches have been applied to determine whether the protein product levels of individual genes can change independently of their mRNA levels.

In the first approach, mRNA and protein levels are measured under two conditions, and differences between the conditions are calculated for mRNA and protein levels separately. These per gene differences in mRNA and protein levels are then compared across all genes. This approach using yeast produced r_s of 0.21 or 0.45 (Griffin et al. 2002; Washburn et al. 2003). A similar study of two human cell lines reported an r_p^2 of 0.41 (Tian et al. 2004). Importantly, the latter study estimated the maximum obtainable r_p^2 to 0.81 (given the variation in measurements of mRNA and protein levels) using a simulation approach, suggesting a substantial contribution from posttranscriptional mechanisms in the dynamic regulation of gene expression (r_p^2 of 0.41 vs. 0.81).

The second approach involves parallel measurement of mRNA and protein levels at several time points following a treatment. This approach also allows for assessment of the extent to which differences in half-lives between mRNAs and proteins can affect the result. A study using yeast monitored the mRNA and protein levels in untreated cells and at six time points following treatment with rapamycin (Fournier et al. 2010). The investigators found that for proteins whose expression had changed, their mRNA levels at 1 h after treatment showed the maximum correlation with protein levels 6 h after treatment—thus, a delayed adjustment of protein levels to mRNA levels. Yet, the r_p^2 only reached a maximum of 0.36 throughout the experiment. In a similarly designed experiment of mouse embryonic stem cell differentiation with four time points, Lu et al. (2009) concluded that only about half of the proteins that changed their levels also displayed concordant mRNA level changes. A proportion of the proteins that initially did not show concordant protein and mRNA levels did, however, show concordant levels at a later time point.

The third approach minimizes the potential bias arising from differences in mRNA and protein half-lives by studying mRNA and protein levels under steady-state conditions (Fig. 1C). In a recent study, 1066 mRNA and protein levels were measured in 23 human cell lines (Gry et al. 2009). The average r_s between mRNA and protein levels was 0.20 and 0.25 using cDNA microarrays or Affymetrix GeneChips, respectively. As a comparison, the average r_s between mRNA levels obtained from cDNA microarrays and Affymetrix GeneChips was 0.52. This is substantially higher than that observed between protein and mRNA levels (i.e., 0.2 or 0.25 as compared with 0.52), indicating that mRNA measurement error is not likely to explain the low correlations between protein and mRNA levels. In an extensive study using the approach shown in Figure 1C, mRNA and protein levels in livers from 97 inbred mice were measured (Ghazalpour et al. 2011). Out of 396 genes, only 21%

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showed significant correlations between mRNA and protein levels. By replicating the experiment, the researchers stratified the genes based on their signal-to-noise ratio, thereby also assessing the impact of random variation on the reported correlations. As expected, the mean r_s increased as the signal-to-noise ratio increased and reached a maximum of ~0.4. Thus, in this extensive study in which differences in half-lives between mRNAs and proteins are likely to have a minimal impact and only genes that could be measured with high confidence were analyzed, the results still support a substantial role for posttranscriptional mechanisms in dynamic regulation of protein levels.

GENOME-WIDE ANALYSIS OF TRANSLATIONAL ACTIVITY

The studies described above all indicate substantial posttranscriptional controls in different systems. A detailed, in-depth examination of posttranscriptional regulation was recently conducted by Schwanhausser et al. (2011), using a multi-omics approach in NIH/3T3 cells (Fig. 2A). They assumed a model in which mRNA levels are determined by transcription and mRNA stability, whereas protein levels are determined by mRNA levels, translational activity, and protein degradation (Fig. 2B). Accordingly, per gene translational activity and transcription could be inferred from measurements of mRNA levels, mRNA stability, protein levels, and protein degradation. Notably, the investigators used independently replicated data to assess the extent to which protein levels predicted by the model compared with the measured levels from the replicates. This allowed the researchers to determine the relative contribution of different gene expression mechanisms while avoiding overfitting. Strikingly, a principal role for mRNA translation among posttranscriptional mechanisms, was identified in determining intrinsic protein levels, strongly suggesting that most of the discrepancies between mRNA and protein levels result from translational control.

More direct evidence supporting the widespread role of translational control comes from studies of the global association between mRNAs and ribosomes. Because mRNAs that have a higher translational activity are associated with more ribosomes, the polysome microarray technique has been used to study genome-wide mRNA translation. For polysome preparation, translation elongation is inhibited by cycloheximide, the cytoplasmic lysate is isolated and applied to a sucrose gradient, and mRNAs associated with varying numbers of ribosomes are separated using ultracentrifugation according to their sedimentation velocity (Fig. 3A). Fractionated mRNAs are then extracted and subjected to DNA microarray analysis for

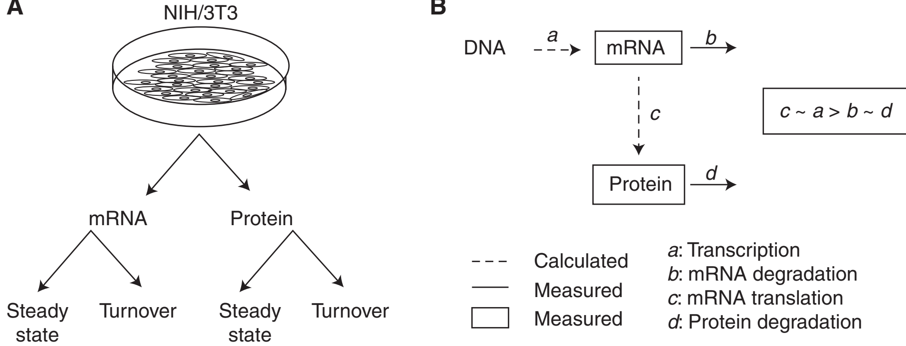

Figure 2. A multi-omics approach to examine relative contributions of posttranscriptional mechanisms to protein expression. (*A*) Levels and turnover rates for both mRNA and proteins are obtained in parallel. (*B*) A simple model is used to calculate transcription and translational efficiencies using measured levels and turnover rates for both mRNA and proteins.

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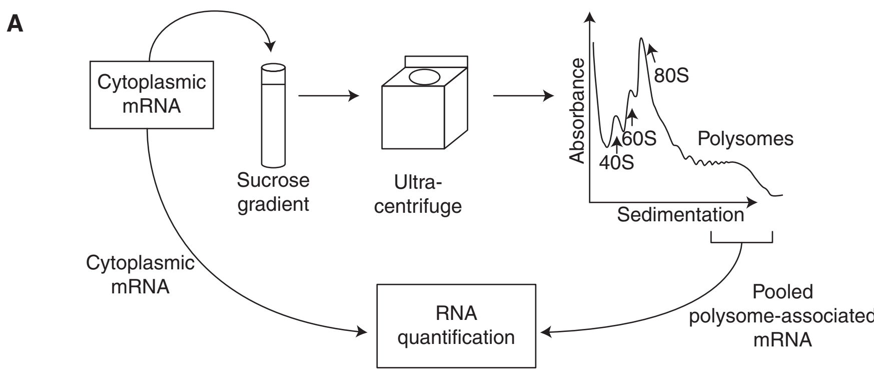

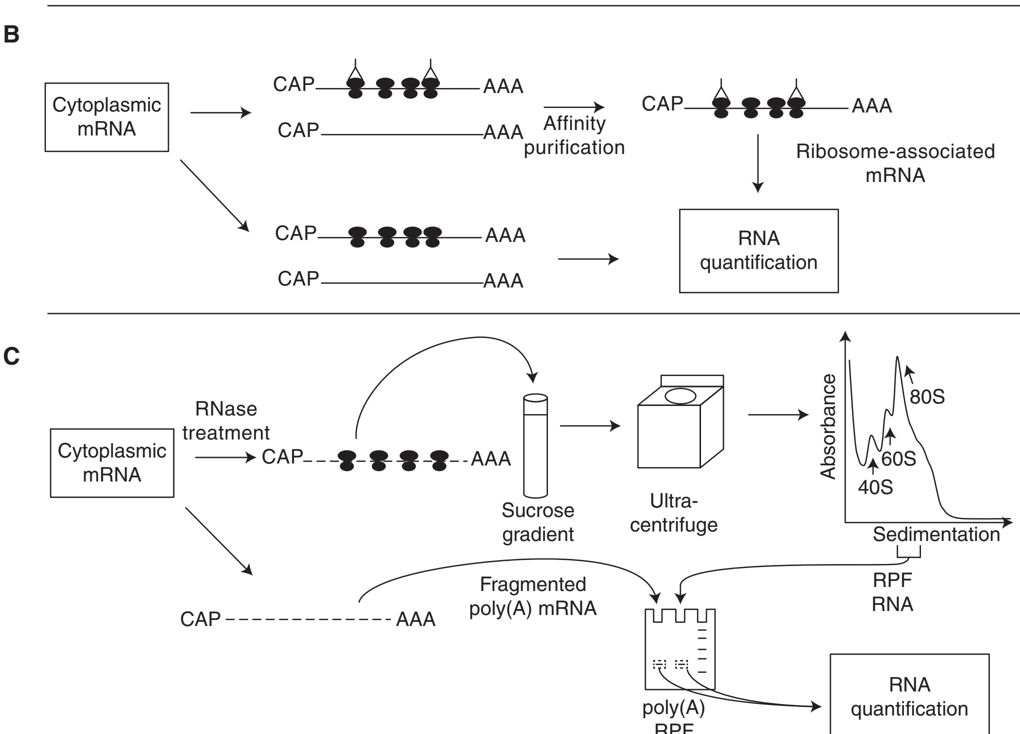

Figure 3. Techniques to obtain genome-wide data on mRNA translation. (*A*) The polysome technique. Polysome-associated mRNA is prepared in parallel with cytoplasmic mRNA and quantified using DNA microarrays or RNA-seq. (*B*) The affinity purification technique. mRNAs that are associated with tagged ribosomes are purified using an antibody-based affinity purification approach. Cytoplasmic mRNA is prepared in parallel, and both samples are quantified using DNA microarrays or RNA-seq. (*C*) The ribosome profiling technique. RPFs generated by RNase treatment are isolated from monosomes and subsequently purified by gel based on size. A randomly fragmented sample from poly(A) mRNA is prepared in parallel, and both samples are sequenced.

identification and quantification. Although a large number of fractions can be obtained to examine mRNA profiles across the entire polysome range (Arava et al. 2003), most studies pool fractions because of the high cost of DNA microarrays (Johannes et al. 1999; Zong et al. 1999; Chen et al. 2002, 2011; Jechlinger et al. 2003; Preiss et al. 2003; Kitamura et al. 2004, 2008; Provenzani et al. 2006; Spence et al. 2006; Genolet et al. 2008; Parent and

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Beretta 2008; Ramirez-Valle et al. 2008; Ceppi et al. 2009; Dhamija et al. 2010; Rivera-Ruiz et al. 2010; Di Florio et al. 2011). Commonly, fractions with two or more associated ribosomes are pooled, limiting the analysis of regulation to a shift from less than two to two or more associated ribosomes (i.e., "on–off" regulation), whereas other shifts (e.g., from three to nine associated ribosomes, i.e., "relative" regulation) are missed. An alternative approach involves pooling mRNAs that are associated with $>n$ ribosomes, where n is often 3 (Larsson et al. 2006, 2007; Mamane et al. 2007; Colina et al. 2008; Kim et al. 2009). This approach enables identification of differential translation involving both "on–off" and some "relative" regulation. Short mRNAs or mRNAs constantly associated with more than four ribosomes are, however, not studied. The polysome microarray technique has been applied to address a wide range of questions, including regulation of translation by the mTOR pathway (Rajasekhar et al. 2003; Tominaga et al. 2005; Larsson et al. 2006, 2007; Bilanges et al. 2007; Mamane et al. 2007; Kim et al. 2009; Furic et al. 2010), dynamic regulation of protein synthesis during cellular stress (Blais et al. 2004, 2006; Lu et al. 2006; Kumaraswamy et al. 2008; Dang Do et al. 2009; Matsuura et al. 2010), and the role of mRNA translation in development or differentiation (Iguchi et al. 2006; Grech et al. 2008; Parent and Beretta 2008; Sampath et al. 2008; Otulakowski et al. 2009) and disease (Larsson et al. 2008; Davidson et al. 2009; Treton et al. 2011).

A second set of techniques relies on affinity purification of ribosomes, analogous to the RNA-binding protein immunoprecipitation (RIP)–based techniques (Keene et al. 2006). These methods allow for identification and quantification of translating mRNAs (Fig. 3B). In one application, the ribosomal protein Rpl16 in yeast was modified by addition of a protein A tag to allow purification of mRNAs associated with ribosomes (Halbeisen et al. 2009). Similarly, a hemagglutinin (HA) tag was used to mark ribosomal protein Rpl22 in mouse (Sanz et al. 2009). Importantly, the HA tagging of Rpl22 was dependent on the activity of Cre recombinase, allowing analysis of mRNA translation in a selected cell type in vivo when combined with cell-type-specific Cre expression (Sanz et al. 2009). Another approach is based on the capture of Hsp70 chaperones associated with polysomes (Kudo et al. 2010). Theoretically, the efficiency of immunoprecipitation depends on the number of associated ribosomes, thereby reflecting translational activity. However, the precise number of ribosomes per mRNA is unknown.

A new technique named ribosome profiling has recently been developed (Ingolia et al. 2009), which is designed to identify open reading frames (ORFs) and quantitatively examine ribosome association with mRNAs. The technique involves two steps: isolation of mRNA fragments that are protected by ribosomes (ribosome-protected fragments [RPF]) from RNase treatment, and identification and quantification of the fragments by RNA-seq (Ingolia et al. 2009). For simplicity, this method is called RPF-seq here. Because RNA fragments are size-selected to obtain those corresponding to the expected footprint of the ribosome (Fig. 3C), RPF data reveal the locations of ribosomes on mRNA. As such, detailed quantitative examination of all steps of mRNA translation, including initiation, elongation, and termination, becomes possible. RPF-seq was first used to examine translational control in budding yeast under rich and starvation conditions (Ingolia et al. 2009). More recent studies using RPF-seq have assessed translation in several systems, including mouse embryonic stem cells (Ingolia et al. 2011), meiosis in yeast (Brar et al. 2012), and microRNA-mediated suppression of gene expression (Bazzini et al. 2012).

ANALYSIS OF mRNA TRANSLATION DATA

A major advantage in studying translating mRNA (the "translatome") over steady-state mRNA (the "transcriptome") is the ability to obtain measurements that more closely correspond to protein levels (Ingolia et al. 2009), owing to fewer intermediate regulatory steps. Accurate assessment of translational control, however, requires adjustment for the influence of other steps in the gene expression pathway,

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including transcription, mRNA stability, and mRNA transport (Larsson et al. 2010). Because individual mRNAs can be regulated substantially at the level of mRNA transport (Rousseau et al. 1996), only comparison to cytoplasmic mRNA levels will allow for the precise analysis of differential translation, whereas comparison to whole-cell mRNA will lead to joint analysis of mRNA transport and translation. A common approach to examine translational control is to calculate translational efficiency scores—$\log_2$ [(translating mRNA)/(cytoplasmic mRNA)]—and compare these between conditions to identify differential translation. Because of a mathematical necessity, translational efficiency scores may correlate with the cytoplasmic mRNA abundance instead of solely describing mRNA translation (Larsson et al. 2010). This phenomenon is called spurious correlation (Pearson 1896), which leads to increased false-positive and false-negative rates when examining differential translation (Larsson et al. 2010). Indeed, an assessment of 20 studies using the polysome microarray technique or RPF-seq showed that spurious correlations are common (Larsson et al. 2010). This shortcoming of using translational efficiency scores prompted development of a method based on analysis of partial variance (APV, implemented in the program Anota) (Larsson et al. 2010), which eliminates spurious correlations. In APV, a linear regression model (between translating and cytoplasmic mRNA data) is applied. Fold-change for mRNA translation and associated *P*-values are calculated based on differences in intercepts and residual errors (Fig. 4). In addition, the method includes a range of quality criteria to judge whether the input data set violates model assumptions (Larsson et al. 2011). Notably, this method has been successfully applied to identify differential mRNA translation using both polysome microarray and RPF-seq data (Larsson et al. 2010).

TECHNIQUES TO REVEAL *CIS* AND *TRANS* REGULATORS OF POSTTRANSCRIPTIONAL CONTROL

Posttranscriptional regulation of gene expression, including translation, is believed to involve sets of targeted mRNAs resembling the polycistronic operons present in bacteria (Spirin 1969; Keene and Tenenbaum 2002; Keene 2007). The theory posits that subsets of mRNAs can be regulated at the posttranscriptional level in a combinatorial fashion. Such selective regulation commonly involves RNA-binding proteins (RBPs) or microRNAs that associate with RNA elements within the target mRNA (Bartel 2004; Richter and Sonenberg 2005). RBP-associated mRNA elements are often defined by combinations of structure and sequence properties and usually reside in the mRNA UTR. Once associated with their target mRNA, the RBPs interact with translation initiation factors and sometimes other RBPs and/or microRNAs to positively or negatively regulate translation. Thus, active RNA elements and RBPs need to be identified to mechanistically reveal how differential translation takes place (Fig. 5).

Known RNA elements are often examined as the first step to explain differential translation. These are collected in general databases such as the UTRdb (Grillo et al. 2010), RBPDB (Cook et al. 2011), and CLIPZ (Khorshid et al. 2011) or element-specific databases such as the ARED (Halees et al. 2008) and IRESite (Mokrejs et al. 2010). Information regarding miRNA target sites can be obtained from many databases such as TargetScan (Lewis et al. 2005). Sometimes searching such databases leads to identification of active RNA elements as exemplified by identification of 5′-terminal oligopyrimidine tract (TOP) elements as targets of mTOR signaling (Bilanges et al. 2007; Mamane et al. 2007). More often, however, known RNA elements are not sufficient to explain observed mRNA translation patterns. This makes de novo discovery of regulatory RNA elements necessary.

Bioinformatic methods to uncover novel *cis* elements are based on the assumption that differentially translated mRNAs share common RNA elements (Larsson and Bitterman 2010). mRNA sequences, often in UTRs, are thus used as input to identify sequences or structures overrepresented in the regulated mRNAs, as compared with background ones (Larsson et al. 2006; Foat and Stormo 2009; Chen et al. 2011). A set of three algorithms detected ~50%

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Figure 4. Genome-wide identification of differential mRNA translation by Anota. Replicated data from translating and cytoplasmic mRNAs is used as input in Anota. Anota performs linear regression using translating and cytoplasmic mRNAs for all conditions. Treatment condition (solid line); control condition (dotted line). The intercepts of the lines on the *y*-axis are compared to derive an mRNA translation fold-change and are related to the residual error of the regression to identify differential translation. Genes 3 and 4 are differentially translated, but genes 1 and 2 are not. Simulated numbers are shown.

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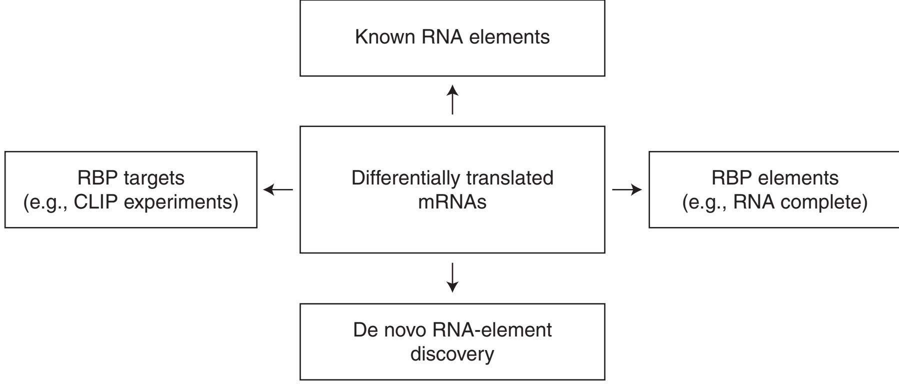

Figure 5. Approaches to examine mechanisms for differential mRNA translation.

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of known RNA elements when UTRs with specific RNA elements were mixed with randomly selected, unrelated UTR sequences (Fan et al. 2009), highlighting the potential of this approach. There are, nonetheless, several limitations. First, the precise length of each UTR is often unknown, and the active RNA elements may therefore be located outside of the studied sequences. In addition, alternative cleavage and polyadenylation, which regulates 3′-UTR length, is widespread and dynamically regulated under different conditions and across tissue types (Tian et al. 2005; Sandberg et al. 2008). Examining UTRs that are not expressed in the studied cell type can lead to false identification of RNA elements. Moreover, there is a possibility that some regulation involves several players, including RBPs, microRNAs, and RNA elements, with linear or nonlinear interactions. Such a complex situation likely makes identification of any single mechanism more difficult (Fan et al. 2009).

Several high-throughput techniques have been developed to study RNA–RBP interactions. In vitro methods include RNAcompete, whereby a library of RNAs is synthesized and used in pull-down experiments with RBPs followed by detection using DNA microarrays (Ray et al. 2009), and SELEX-seq, whereby random RNA aptamers are selected based on interaction with a specific RBP and are deep-sequenced (Dittmar et al. 2012). Although these methods do not directly address which mRNAs are targeted by the RBP under investigation, they elucidate binding specificities.

In vivo methods generally involve immunoprecipitation of RBPs from cells and identification of coimmunoprecipitated RNAs (Darnell and Richter 2012). Indeed, identification of RNA elements by ribonucleoprotein immunoprecipitation (RIP) followed by microarray (RIP-chip) has been successful for a number of RBPs (Gerber et al. 2004; Hogan et al. 2008). RBPs can also be UV-cross-linked to their binding RNAs in vivo, allowing purification of the RBP:RNA complex under denaturing conditions, for example, SDS-PAGE (polyacrylamide gel electrophoresis). Deep sequencing of RNA isolated by cross-linking and immunoprecipitation (HITS-CLIP) has been used to study RBPs (Licatalosi et al. 2008; Darnell et al. 2011) and microRNAs (Chi et al. 2009). The photoactivatable-ribonucleoside-enhanced cross-linking and immunoprecipitation (PAR-CLIP) technique, which also has been used to study microRNAs and RBPs (Hafner et al. 2010; Hoell et al. 2011; Lebedeva et al. 2011; Mukherjee et al. 2011), is similar to HITS-CLIP but allows cross-linking at a longer wavelength. Both HITS-CLIP and PAR-CLIP allow precise detection of the binding site because cross-linking sites lead to mutations and deletions in sequencing reads (Hafner et al. 2010; Zhang and Darnell 2011). A comparison between HITS-CLIP and PAR-CLIP indicated that experimental conditions, such as the extent of RNase digestion,

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need to be optimized to minimize the sequence bias of the identified RNA fragments (Kishore et al. 2011). Another CLIP-based approach, iCLIP, identifies sequencing reads that terminate at the cross-linked sites (Wang et al. 2010; Konig et al. 2011; Tollervey et al. 2011). One potential caveat of all of these approaches is that binding of an RBP to mRNA may depend on other RBPs. Moreover, separating transient interactions with limited effect on regulation from stable interactions that substantially affect regulation is a challenge (Mukherjee et al. 2011). Nevertheless, systematic studies of many RBPs will likely soon be available and could be very useful to mechanistically dissect genome-wide patterns of differential mRNA translation.

CONCLUDING REMARKS

Decades of research based on single genes have established that mRNA translation can profoundly control protein levels and thereby directly determine biological outcomes (Costa-Mattioli et al. 2009; Sonenberg and Hinnebusch 2009; Jackson et al. 2010; Silvera et al. 2010; Spriggs et al. 2010; Blagden and Willis 2011). Over the last few years, genome-wide analyses have shown that posttranscriptional regulation, translational control in particular, plays significant roles in determining protein levels in the cell. With the rapid development of current methodologies, especially deep-sequencing-based methods, we can expect many major discoveries to come from genome-wide studies. Integrating data on mRNA translation with information regarding RBP–mRNA interactions is an emerging area of research. In addition, given the widespread occurrence of mRNA isoforms in higher species, resulting from alternative initiation, splicing, and polyadenylation (Wang et al. 2008), sorting out the translational efficiency for each mRNA isoform will shed important light on the compendium of protein isoforms and reveal the connection between translational control and mRNA processing. On the other hand, to harness the power of genomics fully, we will need to better adopt high-throughput techniques and use rigorous data analysis approaches. It is exciting that a genome-wide landscape of translational control is now in sight.

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Ola Larsson, Bin Tian and Nahum Sonenberg

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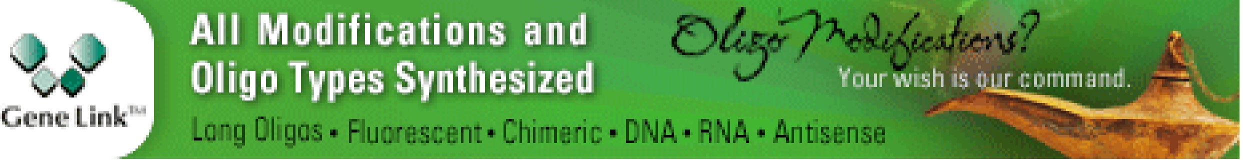

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