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Post-transcriptional control of gene expression: a genome-wide perspective

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Gene expression is regulated at multiple levels, and cells need to integrate and coordinate different layers of control to implement the information in the genome. Post-transcriptional levels of regulation such as transcript turnover and translational control are an integral part of gene expression and might rival the sophistication and importance of transcriptional control. Microarray-based methods are increasingly used to study not only transcription but also global patterns of transcript decay and translation rates in addition to comprehensively identify targets of RNA-binding proteins. Such large-scale analyses have recently provided supplementary and unique insights into gene expression programs. Integration of several different datasets will ultimately lead to a system-wide understanding of the varied and complex mechanisms for gene expression control.

The multifaceted control of gene expression

The characteristics of organisms result largely from the dynamic interplay between DNA or RNA and the regulatory apparatus. The control of gene expression is a fundamental process to bring the genome to life, and it pervades most of biology, from cell proliferation and differentiation to development. It is well recognized that gene expression is regulated at several levels (Figure 1). Cells need to integrate intrinsic and environmental information and coordinate multiple regulatory mechanisms of gene expression to properly exert biological functions. Mis-regulation of gene expression at any level can lead to disease. Gene transcription has received the most attention, both through traditional studies [1] and via recent genome-wide approaches such as expression profiling [2], location analyses of transcription factors [3] and global chromatin remodelling [3]. This bias has both historical and technical reasons: transcriptional control is the most basic and intuitively important step of gene expression, and is straightforward to study with established methods. However, post-transcriptional regulation, including the processing, export, localization, turnover and translation of mRNAs (mRNAs), adds substantial complexity to the control of gene expression. The various steps in the pathway from DNA sequence to proteins seem

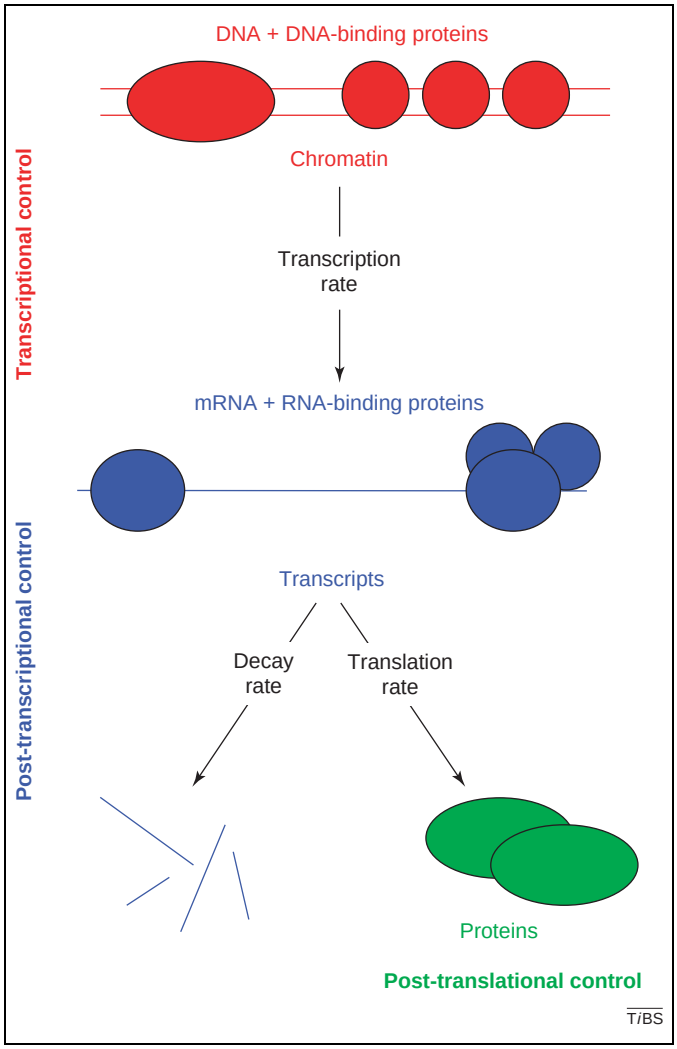

Figure 1. Typical steps in the control of gene expression. The rates of gene transcription and of mRNA decay determine the steady-state levels of transcripts. Transcript levels, together with translation rates, then determine the amount of protein produced. Whereas transcription is controlled by DNA-binding proteins, post-transcriptional regulation such as mRNA decay and translation is mediated by RNA-binding proteins that form ribonucleoprotein complexes with transcripts. There seems to be a high level of coordination and inter-dependence between the various steps of gene expression. Note that post-transcriptional gene expression is also regulated at other levels (e.g. mRNA processing, export and localization), and proteins are further controlled at post-translational levels (e.g. protein modification and degradation).

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to be connected and coordinated with each other [4,5]. Post-transcriptional control is mediated by various combinations of RNA-binding proteins (RBPs) that determine the fate of the tagged transcripts and that seem to coordinately regulate specific subsets of mRNAs [6–8]. Small interfering RNAs and microRNAs, together with protein-effector complexes, can also control the degradation and translation of target transcripts [9]; it is possible that a substantial network of these small RNAs regulates a large subset of mRNAs in a combinatorial manner. The application of various genome-wide approaches is increasingly providing valuable information on post-transcriptional aspects of gene expression, and complements more traditional approaches. Here, we focus on recent global studies of mRNA stability, translation and RBPs, and discuss the unique insight that can be gained by using such systematic approaches.

Regulation of mRNA stability

Transcripts are subject to multiple levels of control, one of them being mRNA turnover that is regulated by several different pathways (Box 1). Decay rates can be specified by control elements that are usually located within the 3′-untranslated regions (UTRs) of mRNAs and are recognized by various RBPs [10,11]. Degradation of transcripts occurs at distinct cytoplasmic sites (processing bodies) in both yeast and human cells [12].

Although most expression-profiling studies focus on transcriptional control, it is actually the mRNA steady-state levels that are measured – these reflect not only the production but also the stability of transcripts. Recently developed techniques to globally assess mRNA stability are providing important insights into this level of regulation (Figure 2). Genome-wide mRNA turnover has been determined in bacteria [13,14], yeast [15,16], plants [17] and humans [18,19] by measuring mRNA levels at different times after RNA polymerase II inactivation. Together, these data show that decay has an important role in the control of mRNA levels [20]. Median mRNA half-lives seem to scale linearly relative to the length of the cell cycle, increasing from bacteria to yeast to humans [19]; the significance of this intriguing correlation will require further investigation. Transcript half-lives in yeast vary from a few minutes to two hours. Similar to transcription rates, decay rates seem to be precisely controlled: mRNA half-lives often correlate among components of a common macromolecular complex or among members of the same functional class [15], thus defining decay regulons. For example, transcripts encoding core metabolic proteins have long half-lives, whereas transcripts encoding transcription factors or members of the ribosome-biogenesis machinery are markedly unstable [15,16,19,21]. Short transcript half-lives enable both more rapid and more dramatic changes in mRNA levels in

Box 1. Multiple pathways for regulated mRNA decay

Transcripts levels can be modulated via mRNA degradation, which is controlled by several different pathways [10,11]. In eukaryotic cells, polyadenylated transcripts are degraded via exonucleolytic or endonucleolytic pathways (Figure Ia). The two exonucleolytic pathways are initiated by deadenylation of the poly(A) tails, which is a key step for controlling mRNA stability. Some transcripts will then be degraded from their 5′ ends by the exonuclease Xrn1, following enzymatic removal of their 5′-methyl guanosine cap (decapping). Alternatively, the exosome complex [25] can degrade transcripts from their 3′ ends before decapping. Other transcripts are degraded after endonucleolytic cleavage without prior deadenylation, e.g. during mRNA decay involving the RNAi machinery [9].

In addition to regulating transcript turnover, the cell also applies quality-control mechanisms to ensure that faulty transcripts are destroyed [25] (Figure Ib). To avoid the accumulation of truncated proteins, transcripts with premature stop codons are degraded via a process known as nonsense-mediated decay (NMD). These transcripts are decapped without deadenylation and are degraded from their 5′ ends. Another mechanism ensures the degradation of transcripts lacking proper stop codons (non-stop decay). In that case, faulty mRNAs are recruited to the exosome and degraded without decapping. These mRNA surveillance pathways are traditionally distinguished from pathways controlling mRNA turnover, although there are several overlaps, and recent evidence suggests that NMD also has regulatory roles (see main text).

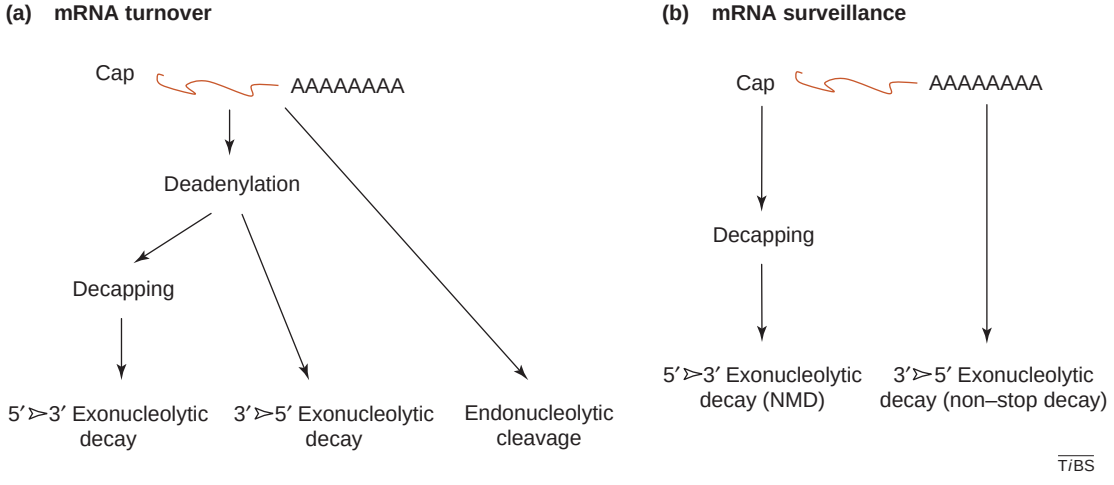

Figure I. Different pathways for **(a)** mRNA turnover (regulation of transcript levels) and **(b)** mRNA surveillance (degradation of faulty transcripts).

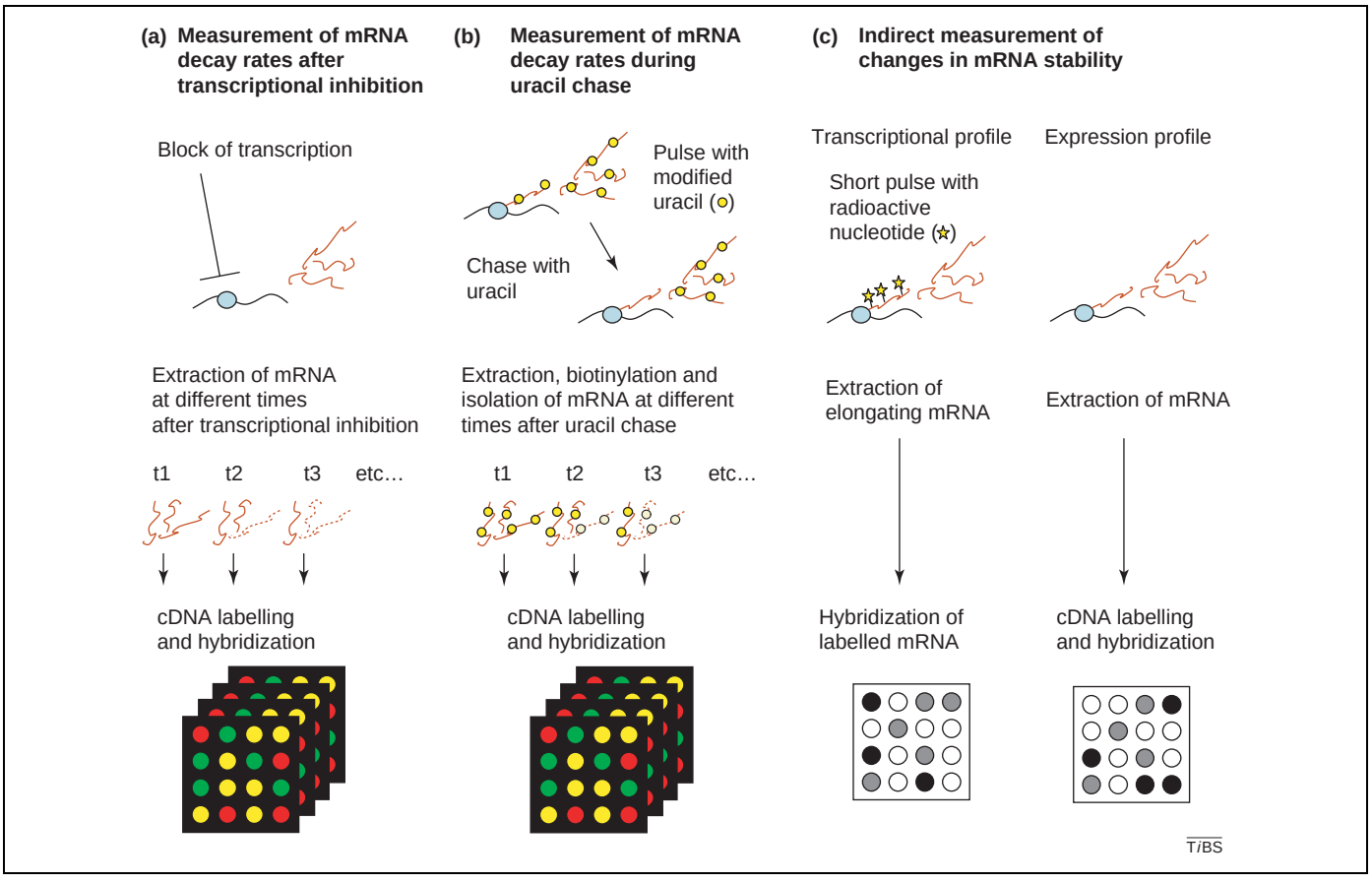

Figure 2. Methods to assess genome-wide mRNA turnover. Transcription and mRNA decay both contribute to steady-state mRNA levels. To measure the contribution of mRNA decay to overall mRNA levels, decay must be separated from mRNA synthesis. Three main strategies have been used for this. **(a)** Determination of mRNA-decay rates after inhibition of transcription using drugs or mutants of RNA polymerase II. At different times after the transcriptional block, transcripts are isolated and quantified using DNA microarrays [20]. (c) A recent alternative method for global measurement of mRNA-decay rates [67] takes advantage of the salvage enzyme uracil phosphoribosyltransferase (UPRT) to incorporate 2,4-dithiouracil into RNA. This modified uracil can be biotinylated for isolation and/or detection. This enables determination of mRNA-decay rates with microarrays using a pulse/chase approach, without the need to inhibit transcription. (c) Indirect determination of changes in mRNA stability by measuring steady-state mRNA levels and global transcriptional activity (using a run-on approach combined with filter arrays) under different conditions. Changes in transcript levels but not in transcriptional activity indicate that mRNA stability is regulated [22–24].

response to different conditions, which might be an advantage for transcripts encoding regulatory proteins [19]. Interestingly, similar mRNA-turnover patterns are found among orthologous genes in yeast and humans [21]. Taken together, these results indicate that the regulation of mRNA stability is a widespread, tightly regulated and conserved mechanism for the control of gene expression.

Other genome-wide studies have determined mRNA stability indirectly by combining measurements of mRNA levels and transcriptional activity to identify changes in mRNA stability under different conditions (Figure 2c). Fan *et al.* [22] analysed the variation of mRNA stability in human cell lines that had been subjected to stress-inducing agents. Strikingly, ~50% of the affected transcripts showed altered abundance due to changes in mRNA stability rather than changes in transcription. Similarly, a significant contribution of mRNA decay to transcript levels was observed during endoplasmic-reticulum stress [23]. Garcia-Martinez and colleagues [24] assessed the effect of a carbon-source shift on transcription and mRNA stability in yeast, and provided evidence for a general and transient stabilization of mRNAs during the transcriptional reprogramming that follows the shift. In addition, clusters of functionally related genes showed coordinated changes in mRNA stability, supporting the hypothesis of decay regulons [15]. These findings indicate that transcript turnover is an important target of regulation in response to perturbations.

Nonsense-mediated mRNA decay (NMD) is a well-studied decay pathway that ensures the degradation of transcripts with nonsense mutations to protect cells from deleterious truncated proteins [25] (Box 1). Genome-wide studies have uncovered a completely new aspect of this pathway: inhibition of NMD leads to the induction of hundreds of transcripts belonging to distinct functional classes, including those responsible for amino-acid metabolism, in addition to transcripts derived from transposons and retroviruses [26,27]. NMD requires translation and is inhibited during amino-acid starvation; this raises the possibility that NMD couples the transcript levels of genes involved in amino-acid homeostasis to amino-acid availability by monitoring translational capacity as an indirect measure for amino-acid starvation [27]. Thus, these global studies revealed that NMD represents not only a quality-control system dealing with nonsense mutations but also provides a more general mechanism to regulate gene expression and to suppress genetic remnants such as transposons.

Global studies have also provided substantial insight into the mechanics of mRNA decay. First, high mRNA-decay rates correlate with the presence of AU-rich elements (AREs) in the 3′-UTRs of transcripts [18,19]. However, AREs are neither always nor exclusively found in rapidly decaying transcripts [13,19,28]. Additional sequences have been described but none of them are strong predictors for mRNA-decay rate, suggesting that the control of mRNA-decay regulons might involve the cooperative binding of multiple RBPs to different sites [19]. Second, global studies of degradation mutants have identified factors such as budding yeast Ccr4, Pan2, Pub1, Puf4 and Cth2 that regulate the stability of dozens or hundreds of mRNAs [16,28]. At the other extreme, microarray data suggest that the yeast Edc3 protein specifically regulates a single mRNA [29]. Thus, genome-wide studies can reveal both widespread and highly specialized roles of regulatory factors. In conclusion, microarray-based approaches can make crucial contributions to the characterization of mRNA turnover and to its recognition as a global regulatory process.

Regulation of translation

Another level of post-transcriptional control takes place during translation, and encompasses both global and transcript-specific mechanisms to regulate protein synthesis [30,31]. The initiation of translation is a complex, multi-step process that is rate-limiting for protein synthesis and is the main target for translational control [32]. Global regulation, which affects the translation of most transcripts, usually occurs by changes in the phosphorylation state of translation initiation factors and by adjusting the number of available ribosomes. Transcript-specific regulation, by contrast, modulates the translation of a distinct group of mRNAs and is mediated by a large diversity of mechanisms. It involves RBPs that associate with particular structural features or control elements present in the UTRs of target transcripts, and is similar to the control of RNA decay (described earlier). The regulation of translation is of particular importance under conditions that require sudden and precise changes in protein levels, including the cellular response to stress and apoptosis [33], the regulation of cell growth and its coordination with cell division [34], and during differentiation and development [35]. Cellular stresses that lead to global repression of translation are often accompanied by increased translation of selected proteins that are required for cell survival. There is growing evidence that de-regulation of translational control can lead to the development of cancer, and modulators of translation such as rapamycin, which inhibits the target of rapamycin (TOR)-signalling pathway (Box 2), provide effective anti-cancer drugs [36–39].

Translation is measured at a genome-wide level by fractionating transcripts based on the number of associated ribosomes, which reflects translation rates; the various fractions are then quantified with microarrays to obtain a holistic view of translational control (Figure 3). This ‘translational profiling’ has provided unique insights that would be difficult to obtain with more traditional approaches [40]. A range of experiments has comprehensively identified genes that are regulated at the translational level, giving insight into the extent of both global and transcript-specific translational control under different conditions [41–47]. For example, Kuhn *et al.* [47] studied yeast cells shifted to a non-fermentable carbon source. They observed a reduction in global translational activity, which was especially pronounced for genes encoding ribosomal proteins; a few specific transcripts went against this trend and were selectively mobilized into polysomes. In another example, Rajasekhar *et al.* [45] revealed that the activation of oncogenic Ras- and Akt-signalling pathways in mouse cells leads to widespread recruitment of ribosomes to specific transcripts, reflecting an immediate regulatory effect of this signalling that was substantially greater than its effect on transcriptional regulation.

Other papers have reported various characteristics of translational control using more than ten mRNA fractions to obtain ribosome profiles at high resolution [48–50]. In rapidly growing yeast cells, 70–80% of the transcripts are associated with polysomes, whereas ~85% of the ribosomes are actively involved in translation [48,49]. This suggests that the transcriptome and translational capacity are well balanced and coordinated. Depending on the transcript, the density of associated ribosomes varies widely, ranging from one ribosome per 30 nucleotides (corresponding to the length of mRNA protected by a ribosome) to less than one ribosome per 1000 nucleotides.

Box 2. Cellular systems for integrated gene expression control

The conserved TOR-signalling pathway and its control of ribosome biogenesis provide a good example for cellular integration of different levels of gene expression. TOR proteins are central regulators of cell growth in response to nutrients and growth factors. TOR signalling controls the protein-synthesis machinery at multiple levels [68]: (i) transcriptional regulation of rRNA and genes encoding ribosomal proteins, involving the control of all three RNA polymerases; (ii) control of 35S-precursor processing and of ribosomal mRNA stability; and (iii) regulation of general translation, probably via the translation initiation factor eIF4E. Thus, the TOR pathway regulates different levels of gene expression to co-ordinately adjust ribosome production and protein synthesis in response to external stimuli. Integration of transcriptional and post-transcriptional regulation of ribosomal components might enable a rapid response and fine-tuning to changing conditions.

The yeast Ccr4–Not complex provides another example of integrated gene-expression regulation. This conserved macromolecular complex exists in at least two forms and regulates adaptation to environmental changes by controlling transcriptional and post-transcriptional gene expression [69]. The larger of the two forms probably contains members of the transcriptional machinery in addition to proteins involved in mRNA decay and protein degradation. The Ccr4–Not complex is thought to regulate the expression of the heat-shock gene *HSP12* at different stages [69]: (i) positively at the transcriptional level via recruitment of Taf1 by the Not5 subunit; (ii) negatively at the mRNA-stability level via Ccr4; and (iii) possibly by post-translational modification of the stress transcription factor Msn2. This suggests that a single regulatory complex can control the expression of stress-regulated genes at transcriptional, post-transcriptional and post-translational levels. In a recent report, Traven and colleagues [70] provide genetic evidence that the Ccr4–Not complex also participates in DNA-damage response at transcriptional and post-transcriptional levels [70]. These examples illustrate that the cell uses integration of gene-expression control to orchestrate the adaptation to changing conditions.

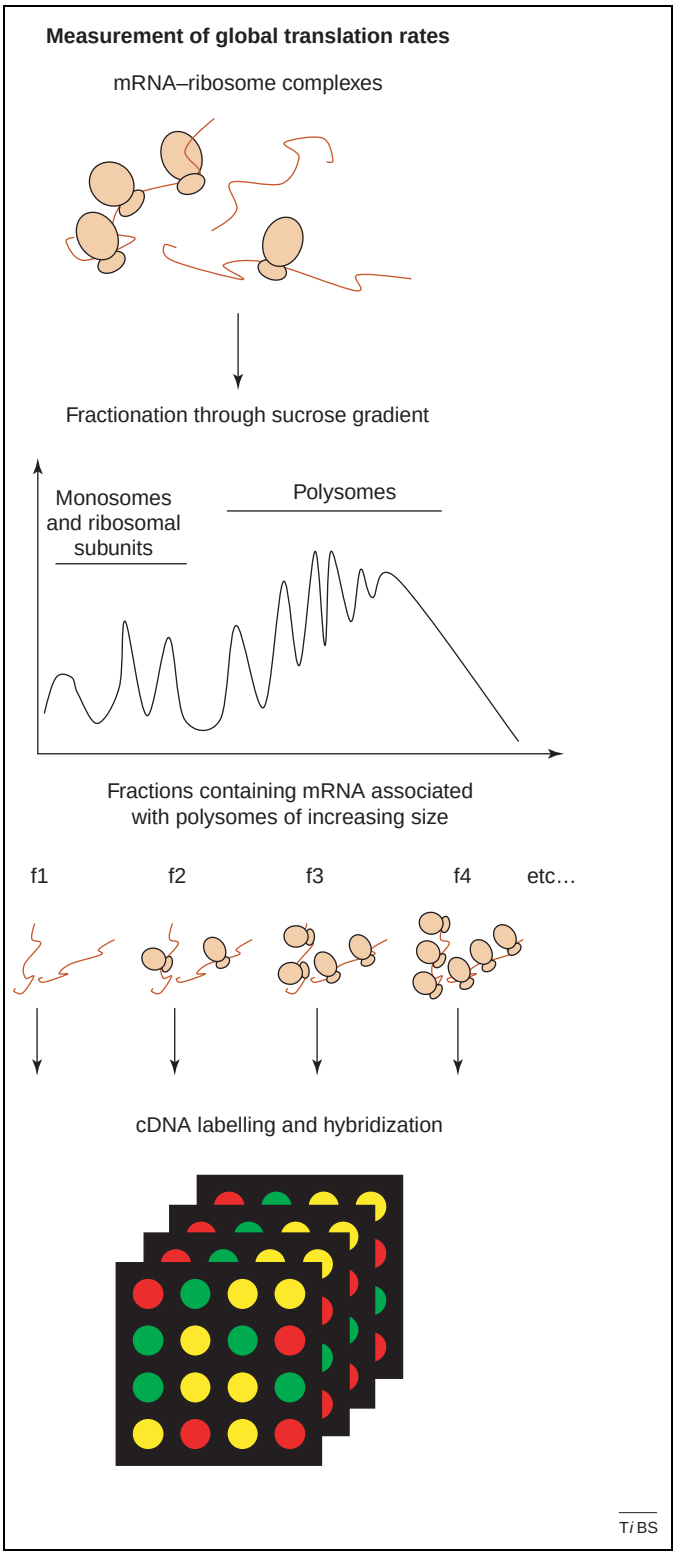

Figure 3. Genome-wide quantification of translation rates. The efficiency with which mRNAs are translated can be subject to regulation, usually at the level of translational initiation. Therefore, the number of ribosomes associated with a given mRNA is a good measure of the rate at which this mRNA is being translated. To obtain a translational profile for every mRNA, polysome preparations are separated in a sucrose gradient according to their size (which depends on the number of ribosomes they contain); the mRNAs in each fraction (or pools of fractions) are then identified and quantified using DNA microarrays [40].

Ribosomes are spaced well below the maximum packing density on most transcripts, which is consistent with initiation being the rate-limiting step for translation. Although the numbers of associated ribosomes increases with increasing transcript lengths as expected, it is surprising that ribosome density decreases with increasing transcript lengths [48,49]. This strong inverse correlation seems to be caused by less efficient translational initiation on long transcripts, the reason for which is not clear [51].

Identification of RBP targets

As outlined earlier, the fate of transcripts is regulated by RBPs, which form messenger ribonucleoprotein (mRNP) complexes by binding to specific RNA-sequence elements. RBPs control multiple aspects of mRNA metabolism and function, including the processing, export, localization, stability and translation of transcripts [6–8]. The targets of a given RBP can be identified on a genome-wide scale by co-precipitating its associated mRNAs and hybridizing them to microarrays (Figure 4). This approach is increasingly used to systematically identify the *in vivo* targets of RBPs that are involved in various aspects of post-transcriptional regulation. In a pioneering study, Tenenbaum *et al.* [52] used cDNA-array filters to identify mRNAs associated with three translational regulators. Although only a portion of the genome was analysed, they found that each RBP binds to specific subsets of mRNAs in carcinoma stem cells, and these mRNP complexes change when the cells are induced to differentiate.

Brown *et al.* [53] analysed the composition of complexes containing the RBP fragile X mental retardation protein (FMRP), mutation of which causes fragile X syndrome and mental retardation. Approximately 430 mRNAs co-precipitated with FMRP in the analysis. Interestingly, >50% of these mRNAs had abnormal polysomal profiles (i.e. an altered proportion of mRNA in polyribosome fractions). In this case, the combination of two global approaches identified defective translational regulation as a likely cause of a developmental disorder. Similar studies have been carried out with proteins involved in nuclear export: Hieronymus and Silver [54] found that two conserved mRNA-export factors bind to ~20% of all transcripts in yeast. These factors associate with different mRNA populations (although with some overlap) that are enriched in specific functional categories. Similar functional enrichments have recently been reported for other yeast export factors [55]. In another example, Blanchette *et al.* [56] found that a *Drosophila* splicing factor associates with intron-less genes and is required for their nuclear export.

The systematic identification of RNA targets could provide clues to unsuspected functions of well-known RBPs. Inada and Guthrie [57] identified mRNAs associated with the yeast La protein (Lhp1p), a conserved factor involved in the biogenesis of non-coding RNAs transcribed by RNA polymerase III. In addition to a large repertoire of expected non-coding RNAs, Lhp1p associates with specific mRNAs. One of the targets is the *HAC1* mRNA, which encodes a transcription factor required for the unfolded-protein response. Follow-up experiments showed that

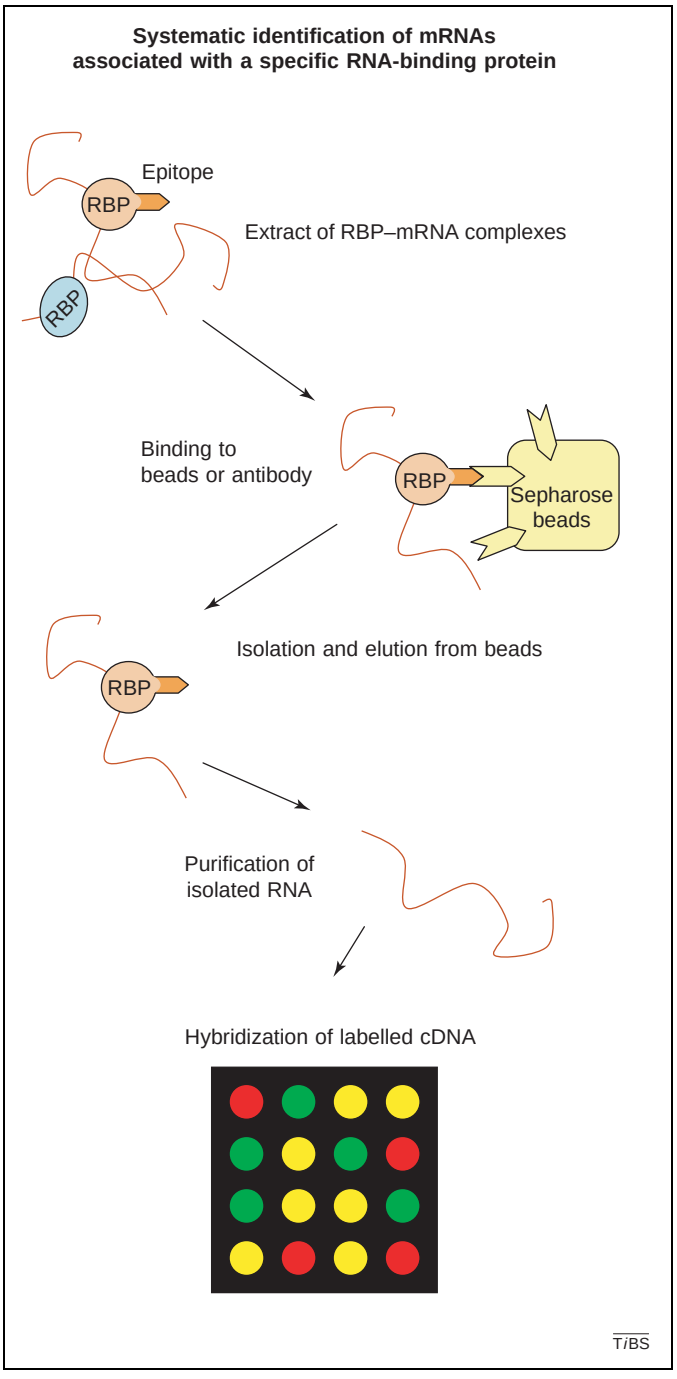

Figure 4. Identification of potential targets of a RNA-binding protein (RBP). The RNA molecules associated with a RBP represent its targets and can be identified using microarrays. To achieve this, the RBP is purified together with its associated RNAs (using an epitope tag or antibodies raised against the RBP). The RNAs from the immunoprecipitate are then isolated, labelled and hybridized to DNA microarrays [58]. This technology is analogous to ChIP-chip (chromatin immunoprecipitation on chip [3]) and is sometimes called RIP-chip (RNA immunoprecipitation on chip).

Lhp1p regulates Hac1 protein levels, possibly by controlling translation [57].

In a comprehensive study, Gerber *et al.* [58] analysed the five yeast members of the Pumilio-Fem-3-binding factor (Puf) family of RBPs [59,60]. Each of the Puf proteins binds to a specific set of 40–220 mRNAs that are enriched for particular features. For example, the targets of each Puf protein tend to encode proteins with specific intracellular localizations (e.g. mitochondria or membrane associated) or biological functions (e.g. rRNA pre-processing, chromatin modifiers and spindle-pole-body components).

The approach of systematic identification of mRNAs associated with specific RBPs shown in Figure 4 can also reveal the spatial complexity of gene-expression control by identifying mRNAs localized to specific cellular sites. Two early studies identified transcripts associated with RNA-transport components in yeast, revealing a surprisingly widespread use of asymmetric mRNA localization [61,62]. The application of this technique to other systems in which mRNA localization is common (such as development of multicellular organisms or neurons) will be of great interest.

The use of these global approaches has revealed that RBPs associate with distinct groups of 20–1000 RNAs, and there are often specific correlations between the target RNAs and the functions of the corresponding RBPs. In addition, it seems likely that most mRNAs are bound by several specific RBPs, creating the potential for combinatorial control that might be used to integrate post-transcriptional regulation at different levels. These findings raise the possibility that specific regulation of transcripts surpasses the richness and complexity of transcriptional regulation; indeed, there seem to be hundreds of RBPs encoded in eukaryotic genomes [7,60]. Future studies will address how cells exploit this combinatorial diversity in different physiological and developmental situations.

Integration, integration, integration

Protein production is controlled at multiple levels, and the resulting amounts of protein reflect cellular integration of the various regulatory layers, ranging from mRNA production to protein degradation. This provides cells with several steps at which to adjust protein levels. Although regulation at a single level might prevail in some cases, it is common for cells to co-ordinately modulate gene expression at several levels. For instance, activation of the TOR pathway leads to changes in transcription, mRNA stability and translation (Box 2). Given the biological importance for linking and coordinating multiple layers of control, understanding gene expression will require an integrated view by combining data from different aspects of regulation. Although this approach holds great promise, there are currently few studies that take into account regulation at multiple levels. Some examples of unique knowledge gained by integrating different large-scale datasets are discussed here:

Beyer *et al.* [63] combined genome-wide data on yeast transcript and protein abundance, translational status and transcript length to assess the contributions of transcription, translation and protein turnover to gene expression. Protein abundance is weakly correlated with both transcript abundance and translational activity, underscoring the need to consider the regulation at several levels. Their study found that different functional groups rely more heavily on particular levels of control. For instance, genes encoding regulatory proteins tend to be translated at very low rates. This suggests that the translation of these genes can be enhanced in response to

environmental changes ('translation on demand'), thus providing the cell with the ability to mount a fast response.

Preiss *et al.* [50] monitored changes in both the transcriptome and translation profiles in response to rapamycin and heat shock. Interestingly, transcripts induced after treatments also tend to be more efficiently translated, whereas many repressed transcripts show lowered translational fitness. A similar positive correlation between changes in transcript levels and translational efficiency was observed during the yeast pheromone response [49]. This co-regulation leads to an amplification of regulatory changes in gene expression, which has been termed 'potentiation' [50]. Potentiation points to coordination between the regulation of transcript levels and translation rates, the mechanistic basis of which is not known. One possibility is that potentiation is caused by regulating transcription and translation of the same genes via independent mechanisms. Alternatively, there could be a direct mechanistic link between changes in transcript levels and in translational status: for instance, changes in transcription could affect the composition of mRNP complexes, leading to changes in translational efficiency, or translational efficiency could be coupled to transcript turnover. Potentiation could explain how even modest changes in mRNA levels can have profound biological effects [64].

It is not clear whether there is also a correlation between the absolute transcript abundance and translational efficiency in steady-state conditions: although several studies indicate a lack of an overall correlation [44,47,49,50], a more recent paper analysing published data does report such a correlation [63]. This is an interesting issue that requires further investigation.

In another example, Fraser *et al.* [65] studied the importance and control of random fluctuations (noise) in the amount of a given protein within a single cell. They hypothesized that noise would have a larger impact on fitness if it affected essential proteins or subunits of multiprotein complexes. Experimental data and mathematical modelling show that the production of similar protein amounts can result in different noise levels, depending on the relative contributions of transcription and translation (Figure 5). Genome-wide data have been used to estimate transcription and translation rates together with associated noise levels for both essential and non-essential proteins in yeast. The results revealed that essential proteins do indeed show lower noise, indicating that noise in gene expression is an important variable that is subject to natural selection. Minimization of noise seems to be achieved by a combination of high transcription rates, high mRNA-decay rates and low translation rates.

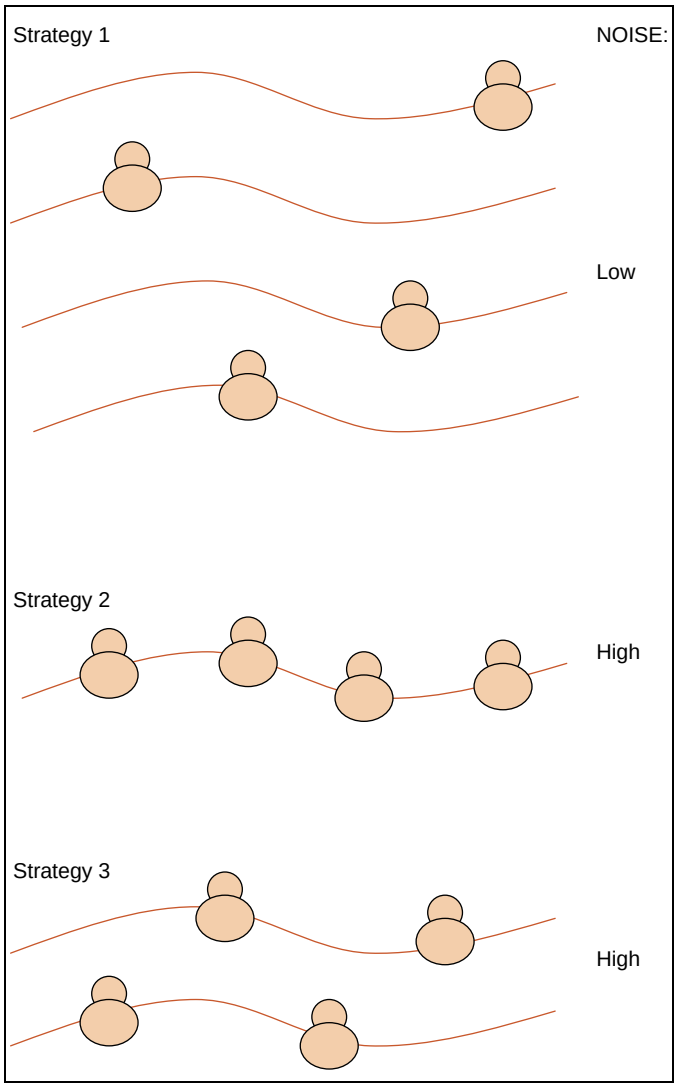

Figure 5. Effects of different strategies of gene expression on noise. Different combinations of transcription rates (represented by the number of transcripts) and translation rates (represented by the amount of ribosomes on each transcript) can be employed to produce the same average protein level (four molecules in each case). However, the extent of the random fluctuations (noise) of protein levels within a cell is specific for each strategy. Fraser et al. [65] showed that essential genes and genes encoding protein-complex subunits tend to use strategy 1, presumably to minimize noise levels. Reproduced, with permission, from Ref. [65].

Concluding remarks

It is well established that cells control the expression of certain genes at several levels after transcription. The recent application of microarray-based methods to study post-transcriptional control has enabled the determination of mRNA half-lives and translational status at a genome-wide level, in addition to the systematic identification of RBP targets. What has been the contribution of these global studies to our understanding of post-transcriptional regulation? The main conclusion from this work is that post-transcriptional regulation is not an unspecific process for most genes; on the contrary, each mRNA seems to have distinct rates of decay and translation. It is likely that these properties are mainly controlled by the binding of RBPs to regulatory regions, possibly acting in a combinatorial manner. Post-transcriptional control, together with transcription, determines not only the rate of protein production (Figure 1), but also other important features such as the amount of noise and the ability to change transcript levels in a rapid manner. An advantage of global approaches is their unbiased nature, which enables the discovery of unexpected connections. For example, in the case of NMD, use of global approaches led to the discovery of an unsuspected regulatory role for the pathway.

An interesting question – which is still largely unanswered – is how dynamic post-transcriptional controls are for each gene, and how often they are modified in response to environmental or developmental changes. Although cells commonly use transcriptional control to regulate gene expression, it is not clear yet whether modulation of mRNA stability or translational rates is similarly widespread. In the few cases in which global mRNA turnover and translation have been studied under several conditions they have turned out to be regulated, suggesting that post-transcriptional control is highly dynamic. The application of global methods to a wider range of physiological conditions will further address this issue and help to unravel how transcriptional and post-transcriptional controls are coordinated.

The analysis of mRNP complexes will be fundamental to the understanding of post-transcriptional regulation, but so far mRNA targets have been identified for only a few RBPs. This will be an important area for future research and will advance our understanding of gene expression in all its complexity. As with other global studies, the analysis of RBP targets under different conditions will be essential to determine the contribution of dynamic controls. It will also be important to combine information on RBP targets with global data on the functions of RBPs. In some cases, the target mRNAs do not provide any immediate clue to the molecular function of the corresponding RBP, or they might not even be correlated to changes in transcript levels caused by the RBP mutant [58]. Comprehensive studies of how RBP mutants affect gene expression at different levels will probably be required. The nature of the target mRNAs can also provide the basis for directed follow-up experiments, as in the case of Lhp1p (as described) [57].

To gain the most from genome-wide studies, it will be increasingly important to integrate data on different aspects of gene expression regulation. In principle, integration of steady-state mRNA levels and translation rates should provide a clearer quantitative picture of protein levels [66], although the complexity of the data and the absence of information on protein turnover make this difficult to achieve. The combination of large-scale datasets can be hampered by the fact that the experiments are often performed by different laboratories under different conditions and by the noisy character of high-throughput data. However, the studies discussed here demonstrate that global datasets can be integrated to lead to biologically significant conclusions. They show how the flexibility to regulate gene expression at different levels can be used by the cell to respond rapidly to environmental changes (translation on demand), amplify responses (potentiation) or reduce noise in protein levels.

Global studies of post-transcriptional control have provided us with a glimpse into the richness and sophistication of mRNA regulation. The next few years will bring an ever more comprehensive view of how cells regulate gene expression at every level.

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