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Eukaryotic mRNA Decay: Methodologies, Pathways, and Links to Other Stages of Gene Expression

José E. Pérez-Ortín[1], Paula Alepuz[1], Sebastián Chávez[2] and Mordechai Choder[3]

1 - Departamento de Bioquímica y Biología Molecular, *Facultad de Biológicas and ERI Biotecmed, Universitat de València, Dr. Moliner 50, E-46100 Burjassot, Valencia, Spain*
2 - Departamento de Genética, *Facultad de Biología, Universidad de Sevilla, Avda. Reina Mercedes 6, E-41012 Sevilla, Spain*
3 - Department of Molecular Microbiology, *Faculty of Medicine, Technion-Israel Institute of Technology, Bat-Galim, 31096 Haifa, Israel*

Correspondence to José E. Pérez-Ortín: *jose.e.perez@uv.es*
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Abstract

mRNA concentration depends on the balance between transcription and degradation rates. On both sides of the equilibrium, synthesis and degradation show, however, interesting differences that have conditioned the evolution of gene regulatory mechanisms. Here, we discuss recent genome-wide methods for determining mRNA half-lives in eukaryotes. We also review pre- and posttranscriptional regulons that coordinate the fate of functionally related mRNAs by using protein- or RNA-based *trans* factors. Some of these factors can regulate both transcription and decay rates, thereby maintaining proper mRNA homeostasis during eukaryotic cell life.

Introduction

Biological macromolecules have a limited life span within cells. Different reasons provoke the turnover of RNA and proteins, including chemical instability and the need to change the cell molecular repertory to cope with different situations. Proteins are more abundant than mRNAs that encode them. In mammalian cells, proteins are approximately 900 times more numerous and 5 times more stable than their corresponding mRNAs.[1] In the yeast *Saccharomyces cerevisiae*, this difference is even more evident as they are about 3000 times more abundant and 20 times more stable than their mRNAs.[2] mRNA was originally proposed to be an unstable intermediate that carries information from genes to ribosomes for protein synthesis.[3] It occupies, therefore, a central position in the central dogma of molecular biology. Structurally, an RNA molecule does not differ fundamentally from a protein. Both molecules are flexible enough to acquire various tertiary structures, and both can bind many kinds of molecules or carry out enzymatic activities. However, the ability of RNA to both store genetic information and catalyze chemical reactions has led to the hypothesis that it predates DNA and proteins. This might be the evolutionary reason for its central role in life. The distinction between coding and non-coding RNAs (ncRNAs) is somewhat artificial and is gradually fading because many "non-coding" RNAs are associated with polysomes and even can encode for small peptides.[4] Moreover, it is quite possible that "classical" mRNAs perform functions that are unrelated to their coding capacity. Nevertheless, as mRNAs have been studied for a much longer time, this review is devoted mainly to them. The emerging functions and regulatory roles of some recently discovered ncRNA merit, however, some attention.

Unlike DNA, proteins and RNAs accumulate defects because, as we currently believe, they are not subjected to repair. Instead, these defective molecules are recognized by surveillance mechanisms and are turned over.[5,6] In spite of being far less abundant and less stable than proteins, the starting set of mRNAs that a dividing cell has is mainly inherited from the previous generation and, together with inherited proteins, it forms part of cytoplasmic epigenetic heredity.[7] There is another kind of epigenetic information that acts on mRNA. The chemical modifications and decorations that mRNAs undergo co- and posttranscriptionally with RNA binding proteins (RBPs) convey information

from the nucleus to the cytoplasm and can be modified during the mRNA's life. This so-called "mRNA imprinting" confers classical genetic information flexibility, and it can even be used to transport information from one cell to other cells.[8]

In this article, we review the symmetries and asymmetries between the synthesis and degradation of mRNAs, how we can measure their stability, the ways in which those fundamental molecules are degraded, and how eukaryotic cells have used their chemical properties and kinetic laws to evolve different ways and strategies to adapt to the changing environment. Finally, we discuss the cross-talk between mRNA decay and transcription. Most examples are taken from yeasts and mammals, which are the most studied organisms in these topics. They show interesting similarities and differences, which are probably related to their different cell physiology.

mRNA Turnover: The Pool Model

Eukaryotic mRNA is synthesized and degraded by independent machineries that reside mainly in different cell compartments. However, "effective" mRNA molecules are only those located in the cytoplasm where ribosomes work. mRNA synthesis is exclusive of RNA polymerase II (RNA pol II) and its accessory machineries (for a recent review, see Ref. 9). The rate at which genes are transcribed is called the transcription rate (TR). It is measured as a change in mRNA concentration ([mRNA]) with time:

$$TR = d[mRNA]/dt \quad (1)$$

TR is independent on [mRNA] (i.e., zero-order kinetics), but dependent on [NTP].

For our model, we consider the cytoplasm like a pool where mRNA molecules are contained and degraded (Fig. 1). It is important to stress that the key parameter for chemical kinetics is the [mRNA] and not the mRNA amount. This difference is irrelevant for a cell with a constant volume, but this distinction becomes important when cells grow or when comparing cells with different cytoplasm volumes. mRNAs are degraded in the cytoplasm by different pathways (see below) with a specific degradation or decay rate (DR) that depends on [mRNA]. It follows a first-order kinetics:[10]

$$DR = d[mRNA]/dt = k_d[mRNA] \quad (2)$$

k_d is usually replaced with an mRNA half-life (HL) because HL is more intuitive, and the experimental parameter determined by most methods is used to calculate mRNA stability (see below). HL is the time required to reduce [mRNA] to half the original value. From the integration of Eq. (2) into that case:

$$HL = \ln 2/k_d \quad (3)$$

If cells are growing (either the average cell volume or cell number increases), [mRNA] is reduced by dilution in a way that depends on the total cells' volume increasing rate (μ). Thus, the dilution effect:

$$d[mRNA]/dt = \mu[mRNA] \quad (4)$$

Therefore, both degradation and dilution cooperate in reducing [mRNA]:

$$d[mRNA]/dt = (\mu + k_d)[mRNA] \quad (5)$$

Interestingly, given the different dependency on [mRNA], the TR and DR parts of the equilibrium (Fig. 1) differ. Synthesis of mRNAs is carried out by a single polymerase, RNA pol II, whereas degradation can be executed by several machineries that utilize at least two major exonucleases in addition to endonucleases (see later). Both the synthesis and degradation machineries are characterized by their intrinsic enzymatic features, which are reflected in their kinetic constants. Moreover, there is an asymmetry because DR is mathematically dependent on its [mRNA], whereas TR of a specific gene, however, cannot be influenced directly by the concentration of its RNA product. Yet, recently, it was proposed that TR is indirectly affected by [mRNA] (see below).

In a steady-state situation, where [mRNA] is kept constant (see the top part in Fig. 1), the equilibrium is, thus, asymmetrical:

$$TR = DR = (\mu + k_d)\,[mRNA] \quad (6)$$

This asymmetry is probably one of the reasons why cells do not use the TR and DR taps (Fig. 1) as identical alternative ways to vary [mRNA]. Functional genomics studies have shown that, for most cases, the change in [mRNA] is obtained mainly by changing TR (the pathway on the right in Fig. 1), whereas changes in mRNA stability play a relatively minor role. However, when cells need to change the [mRNA], they can, in principle, change TR, DR, or both. The change in TR has a direct effect on [mRNA]. Consequently, a positive correlation has always been observed.[2,11] Because DR is equal to TR in steady state [Eq. (6)], it is also correlated with [mRNA] but this does not mean that k_d should be positively correlated as well. This explains the apparent paradox that HL has never been found to be negatively correlated with [mRNA].[1,2,12] The final result is the same regardless of whether TR or DR is changed. The time required for it, however, is not. This is because the kinetics of the change depends on the mRNA HL. The shorter the HL (the higher the k_d), the faster the [mRNA] change. This is even true for the case of increasing the [mRNA]. Nonetheless, in this case, a greater increase in TR is needed to compensate for reduced mRNA stability (see Refs. 10 and 13 for discussion). This is another asymmetry

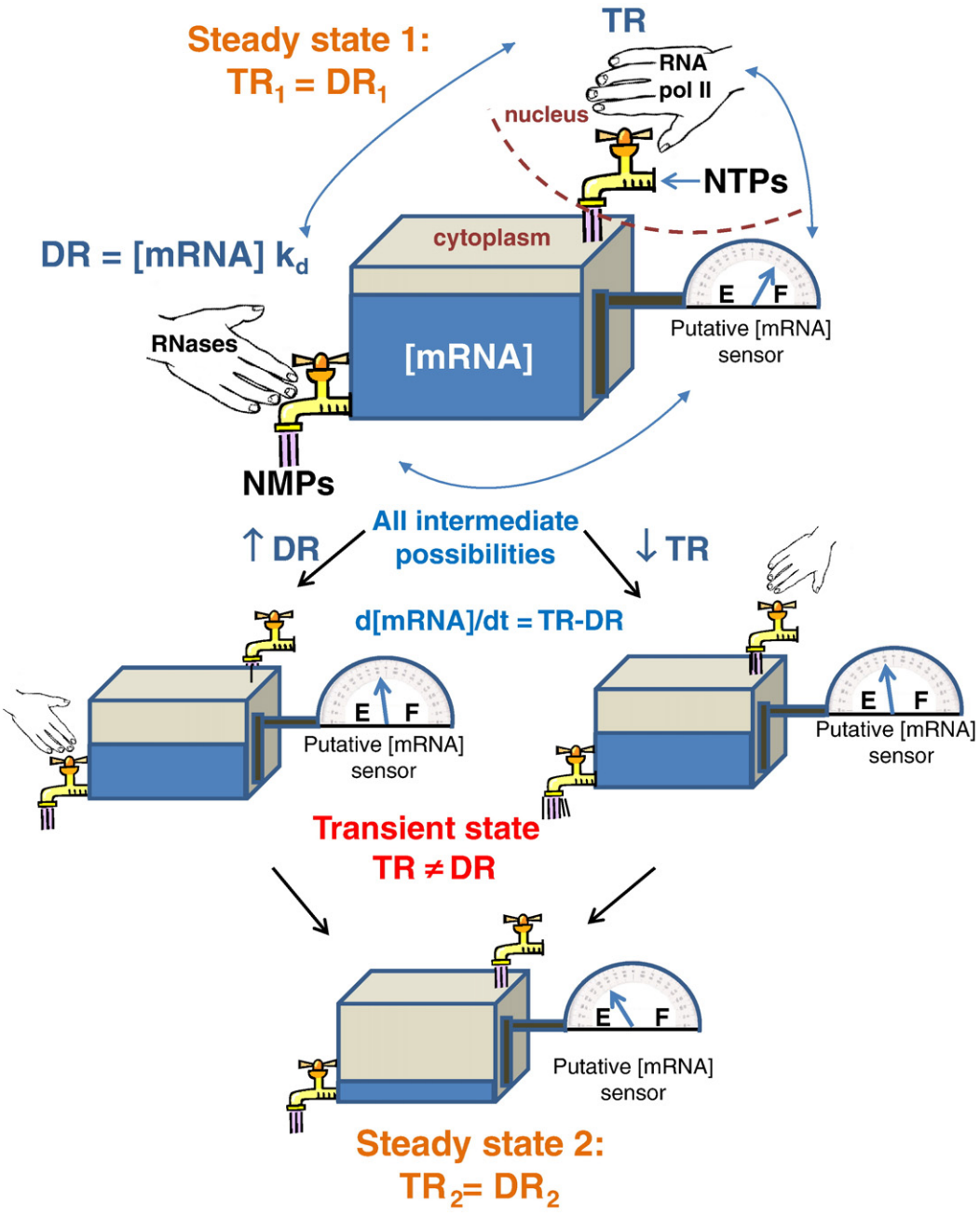

Fig. 1. The pool model for mRNA homeostasis. mRNA molecules are synthesized by RNA pol II from NTP precursors at a variable TR. mRNAs are transported to the cytoplasm (the pool) where they are degraded. Each specific mRNA is degraded by a characteristic DR that is proportional to the [mRNA] and to a specific degradation constant (k_d). This concentration can be sensed by a meter of a still unknown nature. Cross-talk (double-headed arrows) among the three machineries—transcription, degradation, and concentration sensor—is needed to keep proper individual [mRNA] species and total [mRNA]. In many instances, most [mRNAs] remain constant over long periods. That is a steady-state condition in which the TR and DR should be equal. In cases where [mRNA] changes due to environmental signals, the TR, the DR, or both can be altered. The figure represents the case of a decrease in [mRNA], but the opposite case would be represented reciprocally. In the transient state, [mRNA] changes as a function of time because the TR and DR are temporarily different. This situation can last minutes or hours but it necessarily ends in a new steady state.

between the two sides of the equilibrium that has also been used by evolution to establish specific regulatory strategies for every gene type: some genes requiring fast changes in expression, such as histones, have very short HLs, and those genes with a constitutive expression, such as glycolytic

enzymes, have stable mRNAs.[2,14] Clearly, should the steady state change (e.g., in response to the environment), it is not possible to predict whether it was due to changes in TR, DR, or both, without determining at least one parameter directly. In some cases, [mRNA] changes are transient (e.g., stress responses). In such cases, there is a return to the same or to a similar [mRNA] value. This can be achieved by the peak-shaped response in TR and DR[15–18] or by changing mRNA synthesis and decay simultaneously[19] (see later).

The pool model (Fig. 1) represents the situation for individual mRNA species. However, it can also be useful for the total [mRNA] of a given cell. There are, nevertheless, differences: for a specific mRNA, the [mRNA] per cell can be zero or can reach quite high values. Nevertheless, it has been suggested that an abnormally high [mRNA] can be toxic because it can affect chromatin structure, translation, mRNA turnover, and replication and cause the sequestration of RBPs. Surplus RNA can be rapidly degraded to avoid these toxic effects.[20] That is to say, a homeostatic control for total cytoplasmic [mRNA] should exist (represented by a meter in Fig. 1). All the current available data support this hypothesis. In the yeast *S. cerevisiae*, the study of total [mRNA], TR, and DR by comparing wild type and mutants indicated that [mRNA] tends to remain quite constant, although TR and HL vary compensatorily.[11] Our own studies have shown that over a wide range of growth conditions and mutants, the individual cell [mRNA] always falls within a narrow range (F. Carrasco *et al.*, unpublished results). It also seems that physiological [mRNA] is similar for different yeast species. Sun *et al.*[11] found that *Schizosaccharomyces pombe* has a very similar [mRNA] to *S. cerevisiae* despite its threefold larger size. In an *S. cerevisiae* cell, the estimated number of mRNA molecules varies between 15,000[21,22] and 60,000.[22] In mammalian cells, this number has been evaluated to be around 300,000 mRNAs/cell.[23] Mammalian cells vary vastly in size but are, in general, about 50–100 times bigger than a yeast cell. Thus, it seems that [mRNA] is, at least, 2.5 times lower. Bearing in mind that mammalian mRNAs are, on average, much more stable than the yeast counterparts and that the complexity of their transcriptome is greater because of the larger gene number (Table 1) and the extended complexity in mRNA processing, the DRs (and the TRs) in mammals are much lower than those in yeast cells. Since, as explained before, rates are measured as variations in [mRNA] and the size of higher cells is much bigger, a lower TR or DR does not mean, however, that the number of synthesized or degraded mRNA molecules over a given time is lower in mammals.

In this sense, it has been suggested by several authors that the mRNA HL increases with cell cycle length.[34,40] Indeed, mRNA HLs in prokaryotic organisms are very short (see Table 1). Since many model prokaryotes (e.g., *Escherichia coli*, *Bacillus subtilis*) have quite short generation times, several authors have proposed that mRNA stabilities

Table 1. Relation between HL and cellular parameters

	Cell cycle	HL average or median	HL limits	References	Different mRNAs[a]	Cell volume
Prochlorococcus	12–24 h	2.5 min	0.5–18 min	24	~2000	1 fL
E. coli	20 min	5/6.8 min	1–16 min	25,26	~4200	0.5 fL
B. subtilis	30 min	4 min	1 to >15 min	27	~4100	1 fL
Halobacterium salinarum	1.9 h	10 min	5 to 18 min	28	~2900	0.5 fL
Sulfolobus	6 h	4.5 min	2 to >20 min	29	~2300	
S. cerevisiae	90 min	12/21 min	~3/~200 min	11,14	~5600	50 fL
S. pombe	90 min	59 min	~10/~250 min	11,30,31	~4970	150 fL
Plasmodium falciparum	Complex life cycle	9.5–65[b] min	[c]	32	~5300	
T. brucei	Complex life cycle	13 min		33	~10,500	
Arabidopsis thaliana cultured cells	19 h	3.8 h	Minutes to days	34	~20,000	
A. thaliana whole plants	5.9 h		Minutes to days	35	~20,000	
Mouse dendritic cells (*in vivo* labeling)	26 h	26 min		36	~20,000	
Mouse dendritic cells (actinomycin D)	26 h	80 min		36	~20,000	
Mouse ES cells		7.1 h		37	~20,000	1200 fL
Mouse myoblasts		2.9 h	0.5 to 10 h	38	~20,000	
Mouse fibroblasts	30–60 h	4.6 h	3.8 to 5.4 h	39	~20,000	
Mouse NIH3T3 fibroblasts	20 h	7.6 h	2 to 30 h	1	~20,000	
Human Hep2/Bud8	50 h	5–10 h	Minutes to days	40(recalculated by Sharova *et al.*[37])	~19,000	
Human B cells		5.2 h	4 to 6.4 h	39	~19,000	
Human HeLa	16–24 h				~19,000	4000–5000 fL
Human ES cells	7 h				~19,000	

[a] Number of predicted protein-coding genes.
[b] Depending on the development stage.
[c] Empty places are due to unknown data.

increase from prokaryotes through free-living eukaryotes (yeast), which have intermediate HLs and generation times, to higher eukaryotic cells with very long cell cycles and HLs (see Table 1 for references). However, the discovery of many examples of prokaryotes whose generation times are comparable to mammalian cells, such as *Prochlorococcus* or *Sulfolobus* (Table 1), indicates that the very short mRNA HL in some prokaryotes can be merely the result of their different cell physiology.[29] The comparison made between free-living and multicellular eukaryotes, nevertheless, suggests that as single-cell organisms, in general, live much faster, the turnover of their molecules should also be faster. In this sense, it is interesting to point out that if an mRNA species had a much longer HL than the cell's generation time, many of those molecules would be inherited through several successive generations, which would limit the response to environmental changes, a key component of any free-living cell survival strategy.

Single and Omics Methods to Measure mRNA Stabilities

In order to develop theories on the functions of mRNA stability in gene regulation, it is important to know not only the chemical kinetics determinants, as explained before, but also the actual mRNA HLs and their changes when cells cope with different situations.

Techniques for measuring mRNA HLs have been known for 40 years now.[10,41–46] They were based on three alternative strategies. The first (B in Fig. 2) is to block transcription either with drugs or using conditional mutants. Once transcription has been inhibited, only DR machineries act. The steady state

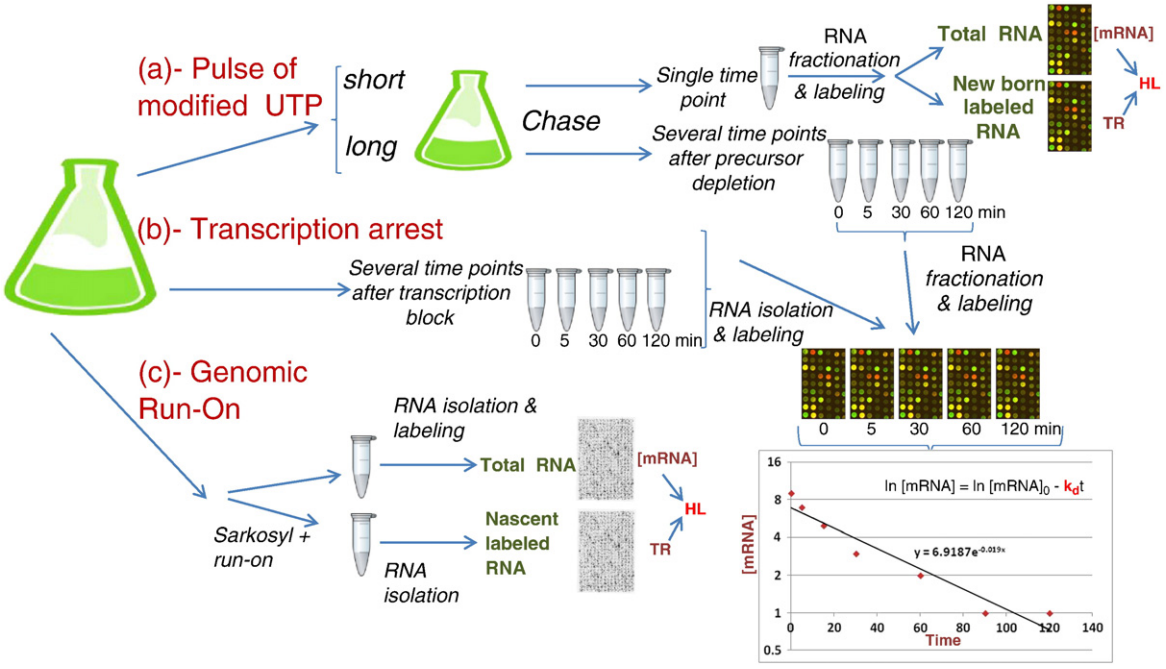

Fig. 2. Methods to study mRNA stability on a genomic scale. (A) *In vivo* labeling with labeled UTP precursors. Two variants of the method are possible. A short pulse with the precursor (4sU or 4tU) is enough to label all newborn mRNAs. Thiolated RNAs are biotinylated and purified. Both total (or non-labeled RNA) and newborn RNA are used to hybridize microarrays (or for RNA-seq). This allows to determine the [mRNA], TR, and HL for every RNA (mRNAs and ncRNAs) that can be confidently quantified in both fractions.[11,31,36,47] Alternatively, a longer pulse is used to label most of the RNAs present in the cell, followed by a chase of unmodified UTP.[48] Decay curves are then used to determine k_d and HL [see Eq. (3) in the main text]. (B) mRNA stability can be measured directly by impeding transcription using inhibitors or thermosensitive mutants and then by following the disappearance of mRNAs over time by reusing sampling at different time points. This technique is the most classical one and is commonly used for single-mRNA analyses combined with northern or qPCR. For the genomic analysis, the method is scaled up by microarray hybridization.[14] (C) The GRO protocol[49] uses a cell sample for the run-on labeling of elongating RNA polymerases in the presence of ^{33}P-UTP. Radioactive isolated RNA is then used for microarray hybridization and allows the calculation of RNA pol II density in every gene. This density is proportional to the nascent TR. The [mRNA] is calculated from another cell aliquot that is converted into cDNA using ^{33}P-dCTP. By assuming a steady state for mRNA production and degradation, or by applying kinetic laws for non-steady-state conditions (see the main text), mRNA HL can be determined.

is lost and the [mRNA] reduces progressively at a rate that directly depends on k_d [Eq. (6)]. As it may be assumed that cells do not grow after the transcription shutoff (in fact, this unphysiological condition is one of the caveats of the technique), $\mu = 0$ and a simple integration of Eq. (2) provides the following:

$$[\text{mRNA}] = [\text{mRNA}]_0 \times e^{-kd\ t} \quad (7)$$

and by using natural logarithms, we obtain:

$$\ln[\text{mRNA}] = \ln[\text{mRNA}]_0 - k_d t \quad (8)$$

A graphical representation of the experimentally determined [mRNA] after the transcription shutoff allows measurements to be taken of the negative slope of the straight line.[10] Then, by using Eq. (3), the HL can be determined. The first problem here is that the supposedly straight line is always the tendency line of a limited number of the time point determinations of [mRNA] (see Table 2 for a list of advantages and disadvantages). Moreover, it is assumed that mRNA decay follows simple first-order kinetics but is not necessarily true given the multiple step processes involved (see below).[10,43,51] Moreover, the experimental error associated with any determination is enlarged by fitting the tendency line. On the other hand, the transcription shutoff may not be complete[52] and may affect cell physiology; thus, it probably affects the measured HL (discussed in Refs. 6 and 50). In spite of all these caveats and others mentioned in other publications,[6,46,50] this technique is widely used because it is very easy and straightforward and does not require complex protocols. Most existing results were obtained by using a suitable drug to shut off transcription (see Table 2). RNA pol II conditional mutants can be used in some cases, such as *rpb1*-1 thermosensitive in *S. cerevisiae*. In this case, the mutant is already physiologically compromised before the temperature change, as recently demonstrated.[11] Change in the culture medium to one that represses transcription is another alternative, but it is suitable only for specific genes.[42,43,53] Another possibility is to change the natural promoter with a regulable one; for instance, the Tet-off regulatory system.[53] This promoter is shut off by the presence of tetracycline (or doxycycline), which does not affect cell physiology. The disadvantage is the need to build a chimerical gene construct and the non-natural regulation of it and, additionally, induction under some stress conditions is observed.[54] However, it has the advantage of being a non-perturbing protocol for single mRNA HL determinations in any culture circumstances.[16,43]

The second HL determination technique is based on the *in vivo* labeling of the mRNA pool by using a modified nucleotide (A in Fig. 2). Originally, this technique used a radioactive nucleotide precursor.[10,42] The large amount of radioactivity required and the low sensitivity for most mRNAs precluded its general use (Table 2). Nevertheless, it is not affected by most of the problems observed in the transcription shutoff method and it has been recently adapted for genome-wide measurements (see below) by

Table 2. Survey of methods for mRNA stability determination

Method	Subtype	Advantages		Limitations			
Transcription shutoff	Conditional allele RNA pol II inhibitors	Simple method	Genome-wide upgradable	Involves heat shock to cells Involves toxic shock to cells	Difficult to use in dynamic situations	Requires a time lapse (for multiple sampling) Bias on specific gene groups	Requires a mutant strain Inhibitors can affect cell physiology
	Regulable promoters		No perturbation to cells	Not genome-wide upgradable	Promoter induction after stress		Requires an engineered strain
Estimation from RA and TR	Assuming steady state	True *in vivo* natural growth conditions No stress	Genome-wide upgradable	Assumes steady-state conditions for mRNAs	Error increased by mathematical calculations	Relies on calculations from other experimental values	Mathematically linked to the TR and [mRNA]
	Not in steady state	True *in vivo* natural growth conditions	Single sampling	Valid only for time series			
Pulse and chase with labeled UTP precursors	Short pulse and single sampling	No stress Useful in dynamic situations		Assumes steady-state conditions for mRNAs	Long and demanding protocol		Requires a short time lapse for incubation
	Long pulse and chase	True *in vivo* natural growth conditions No stress	Genome-wide upgradable			Requires a time lapse (for multiple sampling)	Difficult to use in dynamic situations

Modified from Ref. 50.

substituting radioactivity for other modified nucleotide precursors (A in Fig. 2).

Since the establishment of functional genomics, these two techniques have been upgraded to the genomic level. The classical shutoff technique has been adapted to the genomic scale by merely changing the northern (or qPCR) single gene analysis to a whole transcriptome analysis by DNA microarray hybridization (see B in Fig. 2). This extension not only allows to determine the mRNA HL data set for a given organism but also has provided novel results with both good and bad news. The good news is that functionally related genes tend to have similar HLs,[14] which supports the idea of posttranscriptional (PT) regulons[55] (see below). The bad news is that the previously detected caveats of the technique are now more evident: stress-response genes are induced or repressed during shutoff[56] and the required time lapse (especially) for long-lived mRNAs differentially affects several gene groups. For instance, ribosomal protein (RP) genes' HLs in yeast have been undervalued because the inhibition of transcription may lead to the destabilization of these mRNAs.[48,50] In fact, an extensive bias of HL due to stress shock has been demonstrated.[11] In any case, most of the current HL genomic data sets were obtained by using any of the versions of this technique.[14,19,30,34,37,56,57] Hence, it is important to note that most genomic data sets can be heavily biased toward overestimated HL because most of the used RNA pol II inhibitory drugs (actinomycin D, thiolutin, DRB, phenantroline, cordycepin, and α-amanitin), or temperature-sensitive RNA pol II alleles, do not produce an immediate shutoff.[11,36,52] Moreover, some gene groups could be specially biased, as described before for RP genes and stress-induced genes.

The *in vivo* labeling of mRNA with a pulse of UTP precursors has been especially improved for genomic studies. Many years ago, it was shown that mammalian cells can use 4-thiouridine (4sU) and 4-thiouracil (4tU) as precursors of UTP.[58–60] 4sU can be incorporated into cells either directly (as in mammals[1,36]) or by the ectopic expression of a nucleoside transporter (as in yeasts[11,12]). 4tU can be rapidly metabolized by *S. cerevisiae* cells[36] but requires the ectopic expression of uracil phosphoribosyltransferase in mammalian cells.[47] In both cases, these sulfur-substituted nucleotide precursors are incorporated instead of U into newly synthesized RNA. This is more advantageous than the previously used ^{32}P-labeling given the possibility of purifying labeled RNA, even when it is scarce. If labeling is done over long times, most cellular mRNA can be labeled and the chase along different time points after changing to a U medium (instead of 4tU) can be used to determine HL as in shutoff techniques (A in Fig. 2, long). This possibility has only been used once to date in *S. cerevisiae* cells.[48] It appears to be quite a reliable technique, although the possibility of reusing the 4tU from old labeled RNA by the salvage nucleotide pathway is a putative concern. The caveat would once again be the overestimation of HL. The other alternative is a short pulse (6 min in yeasts, 10–120 min in mammalian cells). In this case, only the newly synthesized mRNA is labeled. Analyzed labeled mRNA is the mature one that recently appeared in the cytoplasm (newborn), although it is conceivable that a small part of the signal comes from nascent mRNA. If the TR is considered constant during the labeling period, the [mRNA] obtained during the labeling time can be used to calculate TR [integration of Eq. (1)], provided that several technical corrections are made.[1,11,12,36] As the steady state is assumed, the simultaneous determination of total [mRNA] from a non-labeled RNA fraction allows the calculation of k_d in Eq. (6), if the culture-specific growth rate (μ) is known. The obvious advantage of this technique is that there is no need to take several time points, which reduces the complexity of the protocol and allows it to be used in dynamic situations, such as stress response in yeast[12] or a response of mouse dendritic cells to lipopolysaccharide.[36] In these dynamic cases, more complex mathematical data processing is required to evaluate mRNA HLs. Protocols based on *in vivo* labeling, however, require purification steps for labeled RNA that should be repeated for all the samples with identical efficiency if real absolute TR and DR are needed. In this sense, the recent development of a protocol variant for the yeast *S. cerevisiae*, called comparative dynamic transcriptome analysis (cDTA), which uses an internal control of *in vivo* labeled *S. pombe* RNA, is an interesting improvement.[11]

A third genome-wide alternative to determine mRNA stabilities is also based on the steady-state equilibrium. With the genomic run-on (GRO) method,[49] it is possible to calculate the TR and [mRNA] of a given yeast cell sample very easily (C in Fig. 2). Cells are instantaneously depleted of NTPs by permeabilization with sarkosyl detergent, which stops all transcription elongation complexes but does not disaggregate them. New initiations, however, are not allowed.[61] Then, in the presence of labeled nucleotides, nascent transcripts are extended by those elongating RNA polymerases. Afterwards, RNA is extracted and nascent labeled transcripts are hybridized to a single filter containing multiple gene probes. Given the assumption that RNA polymerases elongate at a constant rate, the quantification of their density provides a TR measure at the time of RNA labeling.[61] Since an aliquot of cells from the same culture is used to determine [mRNA], Eq. (6) can once again be used to calculate k_d.[49] In this case, as the values obtained for the TR and [mRNA] are in arbitrary units, they should be

converted into real units by comparing with the external TR and [mRNA] data sets. The μ growth factor can also be subtracted.[62] GRO may be used in whole yeast cells, which allows very rapid and accurate measurements. Although a similar GRO protocol has been developed in mammalian cells,[63] it has not been used for HL calculation. The GRO protocol offers an interesting advantage in that it is adaptable even to situations to which no steady-state conditions apply. Under such conditions, such as stress responses, Eq. (6) is not valid, but a differential equation can be used (see Refs. 13 and 46 for details). Some of the results obtained by this strategy are discussed in the next section. Recently, a fourth protocol useful for long time series under non-steady-state conditions has been published.[64]

An interesting point is to compare the different HL data sets obtained by distinct techniques. The deepest analysis has been done in *S. cerevisiae* data sets because many experiments have been done in this organism for a similar strain and under similar growth conditions using all the protocols described herein. A positive, but not very strong, correlation is seen for the comparisons between similar techniques (see Table 2 in Ref. 50). However, very poor or even no correlation has been observed when comparing the data sets obtained from different techniques.[11,48,50,64] There are many reasons for this. As explained before, each protocol has particular biases (Table 2). Noteworthy, they all require the mathematical processing of the experimental data (see Fig. 2). This processing is known to increase errors. In this way, experimental genome-wide data that correlate well become much less correlated after the required mathematical processing.[48] This can be the reason for such poor correlations in HL, whereas the original data on the TR and [mRNA] correlate much better.[12,50]

In the case of mammals, the data sets obtained by transcription shutoff with actinomycin D or by *in vivo* labeling also differ.[36] In general, the average HL for mRNAs is much longer than that obtained for yeast mRNAs, but the vast variation between experiments remains unexplained (from 26 min to 10 h; see Table 1), and the correlations between the different experimental protocols have not undergone in-depth research. Despite the poor overall statistical correlations, the classification of mRNAs to having short, medium, or long HLs generally coincides well in each studied species. This allows us to classify genes according to gene expression strategies using their mRNA HLs and other parameters[1,2] and to study the different strategies by which cells use mRNA stability to properly respond to environmental signals.

The Various Pathways of mRNA Degradation: An Overview

As discussed before, decay can be used to regulate the concentration of each specific mature mRNA. Additionally, however, as the transcription and maturation of mRNAs are processes with relatively high error rates, defective mRNAs are identified and eliminated through quality control pathways in order to minimize the synthesis of non-active or deleterious proteins. In some cases, these quality control pathways (Fig. 3) have been shown to also control non-defective mRNA levels.[65] In general, the substrate for decay pathways is not a naked mRNA, but an mRNA ribonucleoprotein particle (mRNP) assembled during or after transcription (Fig. 3), which can be remodeled in response to external stimuli or intracellular signaling.

All mRNA degradation pathways culminate by the activity of a few nucleases that function together with a plethora of factors modulating their function. 5′–3′ and 3′–5′ exonucleases and endonucleolytic cleavage activities exist in all organisms, while decapping and deadenylation activities are specific for eukaryotic mRNAs, which have protective structures at the mRNA ends [5′ N7-methyl guanosine cap and 3′-end poly(A)]. mRNA decay enzymes are mostly conserved from yeast to humans and, in general, single degradation enzymes are not essential. mRNA degradation is a highly efficient process, and this feature explains why a portion of RNA pol II products are hardly detected, such as spliced introns or many ncRNAs.

Nuclear mRNA degradation pathways

Synthesis of pre-mRNAs by RNA pol II is coupled to the addition of 5′-end capping, which confers protection against 5′-exonucleases. In addition, and

Fig. 3. mRNP metabolism from transcription to decay. mRNA associates with RBPs during its synthesis by RNA Pol II. These RBPs can be recruited to the transcriptional arena by either promoter binding factors (1) or the RNA Pol II C-terminal domain (2) or RNA Pol II itself (e.g., Rpb4/7) (3). mRNP follows remodeling during nuclear mRNA processing (4) and export (5) and upon arrival at the cytoplasm (6). Binding factors can recognize *cis* elements (e.g., ARE, GU-rich recognition element) or are charged onto mRNAs without sequence recognition. Cytoplasmic mRNP may engage in translation (7) or be directed to RNA foci (e.g., P-bodies and SGs) (8). mRNP can cycle among polysomes (active translation), P-bodies, and SGs (9). mRNA degradation may occur co-translationally (10) in P-bodies (11) or outside these complexes (12). Erroneous mRNPs are detected and degraded by quality control pathways acting at the nucleus (13) and the cytoplasm (14). RNA-associated factors can be proteins (RBP) or ncRNAs (6). They can be reused after mRNA degradation (15). In principle, released RBPs can be imported into the nucleus to act as a transcription regulator (16).

co-transcriptionally, pre-mRNAs are spliced to remove introns. At the end of transcription, pre-mRNAs are 3′-cut at specific sites and a poly(A) tail is added to protect RNA from 3′-exonucleases. From the beginning of its synthesis, mRNA associates with different proteins, and possibly also with ncRNAs, to

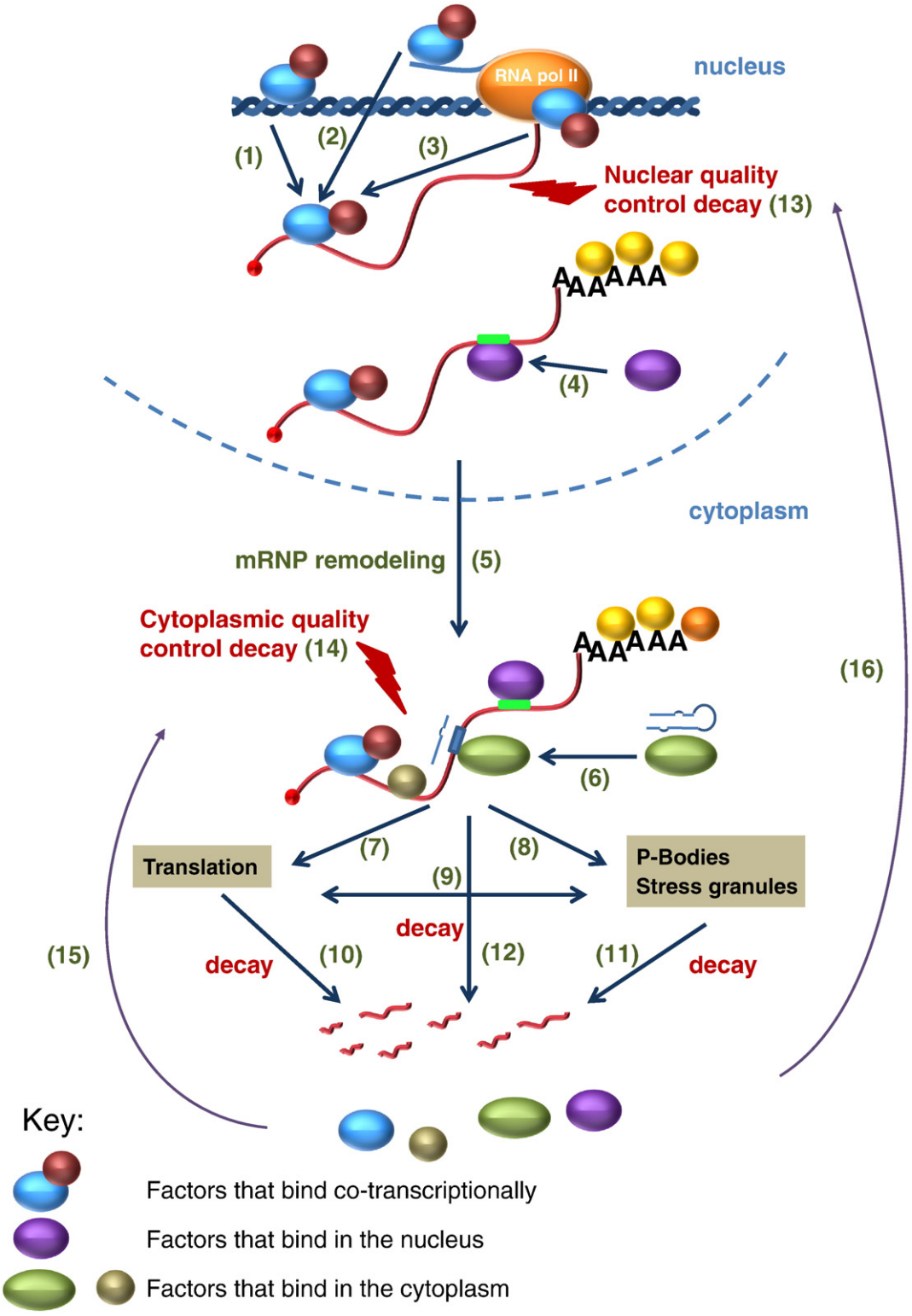

Fig. 3 (*legend on previous page*)

form mRNPs (Fig. 3). Defects in nuclear mRNA processing and in the formation of an export-competent mRNP can lead to the degradation of nuclear mRNA by surveillance pathways. Another way of avoiding the export of defective mRNPs is active nuclear retention, which will give time to complete the processing of mRNA or to degrade the erroneous transcript.[66,67]

A key player in nuclear retention and surveillance is the nuclear exosome, which contains a number of subunits including the evolutionary conserved 3′–5′ exonuclease Rrp6. The activity of the nuclear exosome is assisted by the TRAMP complex, which harbors unconventional poly(A) polymerase Trf4p.[68] Rrp6 executes a quality control of 3′-end formation. Thus, mRNAs with defects in the poly(A) tail are retained at the transcription sites[66,67,69] and this nuclear retention requires Rrp6.[70,71] Mutations in nuclear export factors can result in premature transcriptional termination and polyadenylation defects, which also induce mRNA retention and degradation by the exosome (reviewed in Ref. 72). Another mRNA quality control identifies unspliced pre-mRNAs, which exonucleolytically degrades the RNA from 3′ to 5′ by the nuclear exosome or from 5′ to 3′ by Rat1.[73] However, unspliced pre-mRNAs can be exported and degraded in the cytoplasm where they are targets of cytoplasmic quality control pathways (see below).[74,75] Interestingly, the nuclear exosome and the RNA polyadenylation activity of TRAMP seem to be involved in the regulation of the levels of small subsets of mRNAs in yeast and humans.[76–78] Moreover, it has been suggested that all mRNAs are subjected to a certain nuclear DR, whose relative importance in total mRNA degradation is determined by the degree of the nuclear retention of each mRNA.[79]

In summary, nuclear RNA degradation is mainly devoted to the scrutiny of erroneous molecules. Most valid mRNA molecules, and some types of wrong ones, reach the cytoplasm where they are subjected to new scrutiny to destroy potentially harmful ones. Moreover, in the cytoplasm, several mechanisms control the effective [mRNA] that is to be subjected to Eq. (2) and translation by ribosomes.

Cytoplasmic mRNA degradation pathways

Degradation of mature mRNA occurs mainly in the cytoplasm (Fig. 3). Cytoplasmic decay of eukaryotic mRNAs can occur through different pathways (extensively revised recently in Refs. 5, 6, 80, and 81). mRNA decay usually starts with the deadenylation of the 3′ poly(A) tail. The poly(A) tail can be shortened, depending on the mRNP, by one of three different deadenylation complexes: Pan2 and Pan3, the Ccr4–NOT complex, and PARN.[82] The poly(A) shortening rate is specific for each mRNA and is a key process that defines the mRNA HL.[51] Deadenylated mRNA can be degraded exonucleolytically from 3′to 5′ by the cytoplasmic exosome.[83] More often, the shortening of poly(A) tails is followed by the removal of the 5′-cap by the decapping complex Dcp2/Dcp1 and by subsequent 5′–3′ degradation by exoribonuclease Xrn1.[84,85] For some mRNAs, decay starts with internal endonucleolytic cleavage that is followed by degradation by the exosome and Xrn1 of the upstream and downstream products, respectively.[86]

Deadenylation of mRNAs is often a rate-limiting step and poly(A) tail length plays a role in gene expression control by regulating mRNA decay and translation.[5,87,88] Poly(A) tail shortening depends on the dynamic association with poly(A) binding protein Pab1 (PABP in mammals) and is closely related to the translational status of mRNA. Deadenylation could be a reversible process, as documented for some mRNAs in oocytes. For example, in fully grown primary mouse oocytes, the translation of the tissue-type plasminogen activator mRNA is transiently silenced by deadenylation, and upon meiotic maturation, the poly(A) tail is elongated and tissue-type plasminogen activator mRNA is actively translated.[89] Similarly in arrested *Xenopus* oocytes, maternal mRNAs remain underadenylated and these mRNAs become readenylated and translationally active during oocyte maturation.[90–92] Studies into *S. cerevisiae* indicate that functionally related mRNAs have similar tail lengths, suggesting the use of poly(A) tail length to control the expression of the yeast transcriptome.[88]

mRNA decapping has long been considered to be an irreversible step in mRNA degradation. However, re-capping of previously cleaved RNAs has been described in mammalian cells.[80] This raises the possibility that every stage of the RNA decay, even after Xrn1 degrades a large portion of the RNA, can be blocked and stamped by re-capping. Decapping usually takes place after mRNA poly(A) shortening[87] and the poly(A) tail and poly(A) binding protein Pab1 are negative regulators of decapping.[93] However, deadenylation-independent decapping in some transcripts has also been reported.[94,95] Decapping requires the replacement of the cytoplasmic cap-binding complex eIF4F at the 5′-cap with the decapping complex Dcp2/Dcp1. Therefore, decapping competes with translation initiation (reviewed in Refs. 5 and 96). The important role of decapping in gene expression control is suggested by the large number of factors that modulate decapping activity. Many of these factors act positively on decapping by stimulating the formation of a decapping complex or by enhancing decapping activity (e.g., Edc1, Edc2, Edc3, and the Lsm1–7 complex). Some of the factors promote decapping by directly inhibiting translation initiation, such as Scd6[97] and Stm1.[98] Another decapping enhancer, DEAD-box helicase Dhh1, seems to inhibit translation in a step after

initiation, yielding the accumulation of ribosomes on the transcript.[99] Likewise Pat1 directly enhances decapping by interacting with the Lsm1–7 complex and Dcp2, and directly represses translation.[100,101] Human Pat1b has been found to be associated with the Ccr4–NOT deadenylation complex and the Dcp1–Dcp2 decapping complex, thus probably linking these two processes.[102] All these physical and functional interactions of Pat1 have led to propose that Pat1 is a key player in silencing gene expression across eukaryotes by acting as a scaffold protein for the sequential binding of translational repression and decay factors (decaysome) onto mRNPs.[103]

5′–3′ exonuclease Xrn1 acts in mRNA degradation after decapping and is highly conserved in all eukaryotes. Xrn1 is the key degradation enzyme of translatable mRNAs and is also involved in several mRNA quality control pathways and in the degradation of mRNAs targeted by small interfering and microRNAs (miRNAs). Despite these relevant functions, *xrn1* mutants can survive and exhibit specific phenotypes. For example, the mutations in Xrn1 in *Drosophila* show defects during the development of certain tissues, suggesting that it controls the degradation of subsets of mRNAs.[104]

The cytoplasmic exosome consists of a multiprotein complex with 3′–5′ exonuclease activity that degrades mRNAs after deadenylation or endonucleolytic cleavage. The exosome contains nine core subunits and other associated subunits, some of which display enzymatic activity, which modulate the catalytic activity and substrate specificity of the complex and can differ between species (reviewed in Ref. 105).

All the enzymes and modulating factors involved in the general pathways of mRNA degradation are putative targets for regulating gene expression. In *S. cerevisiae*, the mutations in many of these factors show pleiotropic phenotypes and proteomic analyses reveal that some of them are phosphorylated *in vivo*. As discussed later in this review, decay factors may also function as coordinators of gene expression by connecting mRNA decay to other processes such as translation, transcription, or mRNA nuclear export (Fig. 3).

Cytoplasmic mRNA quality control degradation pathways

Cytoplasmic mRNAs harboring defects that impede effective translation are targeted by mRNA quality control pathways (Fig. 3) to ensure that energy and translation machinery resources are not utilized in the translation of aberrant mRNAs, which can synthesize abnormal proteins.

The best well-known surveillance pathway is non-sense-mediated mRNA decay (NMD) (reviewed in Refs. 5, 65, and 80). NMD targets mRNAs in which translation terminates prematurely: for example, mRNAs with premature translation stop codons, mRNAs with alternative translation start sites out of frame, and pre-mRNAs with introns containing premature translation stop codons or mRNAs with upstream open reading frames (ORFs). NMD is conserved in all eukaryotes and directs mRNAs to degradation through the action of three core universal factors: Upf1, Upf2, and Upf3. In yeast, the mRNAs targeted by Upf factors generally follow decapping and 5′–3′ mRNA degradation by exonuclease Xrn1. In mammals, checking newly synthesized mRNAs through a “pioneer-round” of translation sends NMD targets to decapping and/or deadenylation, and subsequently to exonucleolytic decay, or to endonucleolytic decay. Additionally to a role in the elimination of aberrant transcripts, it has been proposed that mammalian and yeast cells routinely utilize NMD to achieve proper levels of gene expression of “normal” mRNAs.[65] For example, in HeLa cells, the downregulation of Upf1 yields the upregulation of around 5% of correct transcripts.[106] Recently, it has been shown that NMD participates in the production of antigenic peptides for the major histocompatibility class I pathway.[107] In *S. cerevisiae*, NMD controls the steady-state level of the transcripts involved in the chromosome structure and cell surface dynamics[108] and that of transcripts encoding RP (Garre *et al.*, submitted).

Two other mRNA quality control pathways have been described across eukaryotes: no-go decay and non-stop decay. Both pathways initiate at the stalled ribosome. The no-go decay pathway targets mRNAs with elongation stalls via endonucleolytic cleavage close to the stalled ribosome. The resultant mRNA fragments are degraded by Xrn1 and the exosome (reviewed in Ref. 109). Non-stop decay induces the rapid degradation of mRNAs without stop codons. In both cases, the peptides released by the action of the decay pathway are rapidly degraded by the proteasome.[5,110]

Translation, Degradation, or Storage: Three Alternative Options for Cytoplasmic mRNA

After its export to the cytoplasm, mRNA can be translated, degraded, or stored as an untranslated mRNP (Fig. 3). The untranslated mRNP can be assembled in discrete foci. Several types of microscopically visible foci have been described. Although the function of these mRNA foci is not completely understood, it is proposed that they play an important role in the regulation of translation and mRNA decay and, therefore, in gene expression control. Two of the best characterized macromolecular RNA aggregates are processing bodies

(P-bodies or PBs) and stress granules (SGs), and both are highly conserved from unicellular organisms to human neurons (reviewed in Refs.111–116). It is quite possible that, in addition to microscopically visible foci, the cytoplasm contains smaller complexes that cannot be detected by fluorescent microscope.

One of the hallmark constituents of PBs are many mRNA decay factors, such as Dcp1/Dcp2; activators of decapping such as Dhh1, Pat1, Scd6, Edc3, and the Lsm1–7 complex; exonuclease Xrn1; proteins of the NMD pathway; and factors of the miRNAs repression pathway (described later in this review).[112,117,118] Indeed, mRNA degradation can occur in PBs[117] and this is possibly one of their functions. However, mRNA degradation can occur outside these complexes. Depletion of visible PBs in *S. cerevisiae*, *Drosophila*, or human cells does not perturb several mRNA decay pathways.[119–121] Moreover, the decapping enzyme has been associated with polysomes.[122] Recent data in *S. cerevisiae* reveal that 5′–3′ degradation can act on polysome-associated mRNAs and that NMD also degrades mRNAs associated with polysomes.[123,124] PBs do not contain ribosomal subunits, so the mRNAs sent to PBs appear to be those from which the translation machinery has been removed and are complexed with decapping/decay machinery.[112,125] PBs can be observed under normal conditions, but their number and size increase under cellular conditions that raise the pool of translationally repressed mRNAs, for example, during stress or when mRNA decay rates lower.[125,126] Interestingly, when cells return to favorable conditions, mRNAs can exit PBs and return to translation.[127–129]

Translationally repressed mRNAs can also localize in SGs, which contain components of the small ribosomal subunit, translation initiation factors, and the poly(A) binding protein (Pab1/PABP). Cell stress or treatments with drugs that stop translation in the initiation step induce the assembly of mRNP into SGs. However, the mechanism underlying the assembly of SGs is still unclear (reviewed in Refs. 111, 113, and 114).

The current view of mRNA dynamics in the cytoplasm indicates that mRNAs can move between active translation sites (polysomes), PBs, and SGs. Competition between translation activators and repressors would determine whether mRNA is engaged with polysomes or recruited into PBs (Fig. 3). It has been suggested that mRNP can shuttle back and forth between polysomes, SGs, and PBs and that the balance between these complexes regulates mRNA translatability.[127] This balance can be regulated by the availability of translation initiation factors.[112,113] We are still far from understanding all the functions that PBs and SGs perform. Nevertheless as these RNA foci are conserved, it is quite feasible that they play some key roles in regulating gene expression. It has been proposed that PBs concentrate decay enzymes locally to enhance the kinetics of mRNA decay pathways, to deplete decay enzymes from the cytosol, or to deplete some mRNAs from competing for translation machinery with other needed mRNAs (reviewed in Refs. 5, 111, and 114). In short, cytoplasmic mRNP complexes seem to represent a higher level of mRNP organization, which is critical for the proper regulation of mRNA translation and degradation in the cytoplasm.

What Are the Determinants of mRNA Stability?

Under optimal proliferation conditions, each specific mRNA has a characteristic HL that varies from a few minutes to some hours (Table 1). Significantly, functionally related mRNAs usually have similar HLs, which raises the concept of “decay regulon”.[55,130] When cells deviate from optimal conditions, the HLs of many mRNAs change and the changes of functionally related mRNAs seem to be coordinated to allow survival and/or adaptation.[16,130] All these observations imply that mRNA degradation is a controlled process. As with many biological processes involving nucleic acids, mRNA stability is controlled by the interplay between RNA sequence elements (*cis*) and diffusible *trans* factors. However, the degradation of some mRNAs by a stochastic uncontrolled mechanism is also a formal possibility that has not been examined thoroughly.

cis Elements

Traditionally, many studies have focused on *cis*-acting sequences and secondary structures within mRNA as stability regulatory elements. These elements can be recognized by specific RBP and/or complementary RNAs.[131,132] Many analyses have focused on the 5′ or 3′ untranslated regions (UTRs) of mRNAs, which also play important roles in the regulation of mRNA export from the nucleus, translation efficiency, and subcellular localization (reviewed in Refs. 133 and 134). One of the best described UTR regulatory sequences is the family of AU-rich recognition elements (AREs) located at the 3′UTRs of some mRNAs,[10] which promote mRNA decay in response to several intra- and extracellular signals (recently reviewed in Refs. 80 and 81). ARE sequences vary widely and contain a core AUUUA pentamer that can be found in 9% of cellular mRNAs.[135] Specific mRNAs, whose stability is regulated by AREs, include those of human proto-oncogenes c-FOS and c-MYC, several inflammatory factors such as tumor necrosis factor (TNF) or interleukines, and *S. cerevisiae* mRNAs responsive to iron availability.[81,136,137] ARE sequences are

recognized by RBPs, which generally drive the transcript to degradation by stimulating poly(A) shortening followed by exonucleolytic degradation from either 5′–3′ and/or 3′–5′. Examples of ARE binding proteins are discussed in the next section. Other examples of *cis*-acting elements in 3′UTRs include CPE,[138] and the GU-rich recognition element, which has been identified in human transcripts that exhibit rapid mRNA turnover.[139] Additionally, many mammalian miRNAs act through the binding of 3′UTR sequences.[134,140]

cis Elements have also been found in the 5′UTR of mRNAs, which include upstream initiation codons and upstream ORFs that can mediate mRNA decay through the NMD or other decay pathways. This is the case for *S. cerevisiae* mRNA encoding transcription factor (TF) Yap2, which contains two upstream ORFs that inhibit ribosomal scanning and promote mRNA decay.[141] Interaction elements for miRNAs have also been reported for 5′UTR.[142] In addition to 5′ and 3′-UTRs, mRNA ORFs can also contain *cis* elements that regulate mRNA stability (reviewed in Ref. 143). For example, c-Myc ORF contains a *cis* element capable of preventing its endonucleolytic specific cleavage, thus modulating mRNA stability.[144,145]

Interestingly, two recent studies into *S. cerevisiae* have shown that gene promoters contain *cis*-acting DNA elements that can regulate mRNA stability independently of any *cis*-acting elements within mRNA sequences.[146,147] This is discussed later.

trans Factors: RBPs

It has been estimated that 3% to 11% of the proteome of bacteria, archaea, and eukaryotes represents RBPs.[148] In *S. cerevisiae*, more than 500 proteins have been predicted to be RBPs.[149] At least 40 RNA binding motifs have been described for RBPs and RBPs, which frequently contain catalytic domains or protein–protein interaction activities (reviewed in Ref. 132). The best characterized family in animals is Hu/ELAV, which includes HuR, TTP (tristetraprolin), and AUF1 in mammals and ELAV and Smaug in *Drosophila* (reviewed in Ref. 134). Genomic approaches have shown that a typical RBP can bind a large number (sometimes hundreds) of different mRNAs. In some cases, RBPs also bind and regulate the stability of their own mRNAs (reviewed in Refs. 132 and 150). The mechanisms used by RBPs in mRNA stability control are discussed later.

trans Factors: small ncRNAs

The list of small RNA molecules that can act in *trans* over mRNAs becomes increasingly longer, including miRNAs, endogenous small interfering RNAs (endo-siRNAs), and Piwi-interacting RNAs (reviewed in Refs. 7, 151, and 152). Both miRNA and endo-siRNA are derived from long nuclear precursors that are processed, exported to the cytoplasm, and incorporated into miRNA-loaded and siRNA-loaded RNA-induced silencing complexes called miRISC and siRISC, respectively. miRNA directs the RISC complex to specific mRNA sequences by pairing the target mRNA at 3′UTR, or less frequently at coding regions. The nature of base pairing seems to be functionally relevant. Thus, the partial pairing of miRNA with its target yields inhibition of translation initiation, stall in translation elongation, or stimulation of nascent polypeptides proteolysis.[151,152] The repression of translation by miRNAs can be reversible. A well-studied example is CAT-1 mRNA, which encodes an amino acid transporter in human hepatoma cells. Under stress, the inhibition of CAT-1 translation—mediated by miRNA miR-122—is released, and CAT-1 mRNA exits P-bodies and engages with polysomes. Interestingly in this case, P-bodies serve as storage sites for miRNA-inhibited mRNAs.[128] Unlike partial pairing, a perfect or near-perfect pairing between miRNA and its target mRNA induces the degradation of the transcript by the action of argonaute-containing RISC-proteins, which produce an endoribonucleolytic cleavage of mRNA (reviewed in Refs. 81, 151, and 153). Additionally, miRNA can also induce target mRNA deadenylation by Pan2–Pan3 and Ccr4–NOT and the subsequent decapping by Dcp1–Dcp2.[154–156] In mammals, flies, and nematodes, endo-siRNAs are abundant and direct degradation of target mRNAs that pair perfectly, mediated by the catalytic argonaute.[151] It is interesting to note that many short-lived mRNAs in mammalian cells and *Arabidopsis* are targets of miRNAs,[34,36] which suggests the importance of these regulatory *trans*-acting factors in mRNA stability in higher eukaryotes. Thus, miRNAs can act analogously to RBPs in regulating mRNA stability (Fig. 3). During the dedifferentiation of human fibroblasts to induced pluripotent cells, some mRNAs are stabilized. It has been proposed that the stabilization mechanism involved, that is, the action of RBPs or the inhibition of specific miRNAs, is related to the function of the regulated mRNA.[157] Evidently, the co-regulation of mRNA stability by both RBPs and miRNAs is widespread.[158] For example, the mRNA encoding the TNF is a target of miR-16. The miR-16-RISC complex recruits the RBP TTP to TNF mRNA, and then TTP promotes its degradation.[159]

Apart from all the mentioned ncRNAs being involved in RNA interference, recent data have demonstrated that almost the entire yeast and human genomes are transcribed, mostly by RNA pol II. Thus, a huge number of RNA molecules, whose functions are largely unknown, is synthesized. In addition to “spurious” transcripts (see below), which are quickly degraded by the exosome,

relatively stable and functional ncRNAs have been found in all the eukaryotes studied. For instance, a small ncRNA population produced by pervasive transcription in yeast is the so-called stable unannotated transcripts (SUTs).[160] Small ncRNAs are present in almost all eukaryotic cells and participate in widespread and essential regulatory mechanisms. It is conceivable that some function of these ncRNA on the regulation of target mRNAs will be discovered in the near future. The number of long non-coding RNAs (lncRNAs) is also continuously growing.[161–166] Some of them could also have roles in mRNA stability control.

Degradation of ncRNA

Like any RNA, and regardless of their roles in regulating mRNA stability, ncRNAs have a limited life span. The mechanism underlying ncRNA stability is currently poorly understood. miRNAs are degraded by Xrn1 and by the nuclear 5′–3′ exonuclease Xrn2/Rat1 both *in vitro*[167] and *in vivo*.[168] Furthermore, this degradation is antagonized by pairing miRNAs to their mRNA targets, allowing the establishment of networks of mutual regulation between miRNAs and their target mRNAs.[168] In some cases, extensive pairing of miRNAs to their target sequences can also promote miRNA degradation. In this case, 3′–5′ exonucleases are involved and degradation requires previous 3′ poly-U tailing and subsequent 3′–5′ trimming.[169]

Many small ncRNAs are associated with promoters (e.g., promoter-associated small RNAs), terminal regions (termini-associated small RNAs), and transcription start sites, or they are related to transcription initiation.[170–172] ncRNAs longer than 200 bp (lncRNAs) have also been associated with canonical genes in the yeast *S. cerevisiae*.[160,173] Most of these lncRNAs are divergently transcribed from protein-encoding genes,[160,173,174] but others arise from the promoters located in coding regions.[174] The vast majority of this pervasive transcription generates cryptic unstable transcripts (CUTs), which are difficult to detect in wild-type cells. CUTs are transcribed by RNA pol II and their synthesis involves termination directed by Nrd1 and Nab3 RBPs,[76] the same factors that are responsible for transcription termination of snoRNAs.

Degradation of CUTs requires their polyadenylation by the poly-A polymerase component of the TRAMP complex, as well as the 3′–5′ exonuclease activity of the exosome.[175] This poly-A tail-dependent decay is similar to that operating in prokaryotes and is probably more primitive than the cytoplasmic degradation that is stimulated by deadenylation. Thus, detection of CUTs is easier in yeast strains expressing defective exosome. In contrast to CUTs, SUTs are only partially sensitive to the nuclear exosome and they are degraded by NMD machinery and cytoplasmic 5′–3′ exonucleases.[176] Both CUTs and SUTs produce 3′ extended species that are subjected to cytoplasmic degradation,[176] situating these RNA molecules closer to metazoan long ncRNAs than to the small ncRNAs described above.[161]

An additional population of yeast lncRNAs (longer than 200 bp) has been recently defined by its strong sensitivity to the presence of cytoplasmic 5′–3′ exonuclease Xrn1. These Xrn1-sensitive unstable non-coding transcripts are predominantly antisense to protein-coding genes and contribute to their regulation.[162] Similarly, the lncRNAs involved in controlling galactose-activated genes and other yeast-inducible genes are degraded by decapping enzyme Dcp2.[163] Mammalian lncRNAs include thousands of intergenic, intronic, and antisense RNA species.[7,164–166] The stability of these molecules has been recently analyzed genome-wide.[177] Most mouse lncRNAs turned out to be stable, at least in the neural cell line utilized in the study, showing a range of half-lives that is comparable to mRNAs.[177] Interestingly, intergenic and antisense lncRNAs are more stable than those derived from introns.[177]

ncRNAs are, in general, less abundant than most mRNAs but the ncRNA landscape is at least as diverse as mRNAs in terms of stability.[160,162] This diversity may reflect a regulatory reciprocal coupling between the HLs of mRNAs and those ncRNAs transcribed nearby. Poly(A) tail and Pab1/PABP play important roles in the equilibrium between degradation and translation. Since many ncRNAs also contain the 5′-cap and 3′-poly(A) tails, one intriguing question is whether translation initiation factors and Pab1/PABP also play a role in the regulation of ncRNA stability.

Regulation of mRNA Decay: The Importance of Kinetics

In principle, cells can change either the TR or DR in order to replenish or deplete the mRNA pool. Nevertheless, systematic whole-genome studies have revealed that cells change the TR more frequently than the DR in response to environmental stimuli.[17,19,36] In optimally growing yeast, a clear positive correlation has been found between the steady-state mRNA levels and the TR, but not HLs.[2,30] Similar results have been reported in mammalian cells.[1] We have previously explained this as a consequence of the asymmetry in Eq. (6). In any case, it seems that cells do not use decay machinery as the main mechanism to maintain proper [mRNA] (Fig. 1). The advantage of stimulating transcription in the case of *de novo* gene activation or blocking transcription for gene silencing

is obvious. However, choosing the TR to modify the levels of expressed genes is not as obvious. Indeed, there are many cases in which the changes of specific mRNAs are governed mainly by their DRs (see below). However, other cases involve changes in both TR and DR. For instance, the HL of *S. cerevisiae* mRNAs encoding RP decreases (concomitantly with a decrease in the TR) when changing the culture from glucose to galactose,[48,49,178] or upon heat shock.[56,179] A change in the HL is probably used as a rapid means to decrease these mRNAs and to downregulate ribosome production and cell growth. Thus, it seems that when rapid changes are required, cells target DRs, often together with TRs, to do the job. Interestingly, it has been noticed that yeast mRNAs encoding regulatory proteins (e.g., TFs, cell cycle regulators) have abnormally short HLs (in relation to the average HL), whereas "housekeeping" mRNAs have long HLs.[11,30,122] Similar observations have been made in *Arabidopsis*,[34] *Trypanosoma*,[33] mouse,[1,36,37] and human.[40] Note, however, that average HLs vary substantially from one organism to another. It has been proposed that having stable housekeeping mRNAs saves energy.[1,2] It is interesting to note that the mRNAs encoding RPs are very stable in higher eukaryotes in both animals and plants,[1,34,47] whereas the HLs of these mRNAs are intermediate in yeast.[11,50] This suggests that lifestyle affects gene expression strategies, including mRNA HLs. Currently, most HLs have derived from experiments done in cell samples under steady-state conditions. Yet what happens during responses to intra- or extracellular stimuli? As a general rule, it seems that in response to environmental changes that do not arrest cell proliferation, TR changes determine quantitative changes in [mRNA], but HL values are more important to sharpen the response.[19,36] In this sense, it is interesting to note that the changes in TRs are good predictors of the changes in [mRNA]; peaks observed during transcriptional responses are normally similar to those in [mRNA], but they always precede them by a variable time[13,16–18,36] (which depends on k_d, as predicted by chemical laws[14,40,134]). In fact, this dependence has been used as a technique to calculate k_d in time series experiments in both *S. cerevisiae* and in *Plasmodium*.[64] Given the differences in mRNA HLs between single-cell organisms and mammalian cells, this delay is about 5–10 min in yeast, and from 15–30 min[36] to some hours[40,180] in mammalian cells. As stated before, changes in the TR are usually transient; that is, peak shaped.[13,16–18,36] Hence, in the case of mRNAs with very long HLs, the increase in the TR may never provoke a respective increase in [mRNA] given that the time required for it exceeds the obligatory delay caused by kinetic law (see, for instance, Fig. 3 in Ref. 40 for a theoretical discussion). This explains why mRNAs whose levels change rapidly (see above) must have short HLs. The importance of HL values and their changes during development has been extensively reviewed recently.[134]

Changes in mRNA HL in response to the environment have been observed in both yeast and mammalian cells.[12,13,16–18,36,130,134] There are many cases in which the response is homodirectional: increase [mRNA] by raising the TR and by lowering the DR (increase in mRNA stability). For instance, in response to osmotic stress, the TR of induced genes increases, while the DR of their mRNAs decreases.[18,130] Likewise, for downregulated genes, the most common behavior is the homodirectional strategy, a decrease in the TR and an increase in the DR, as with RP genes in response to osmotic stress,[18,130] or heat shock.[179] In this way, energy consumption diminishes and the response speed accelerates. However, cases in which both the TR and DR decrease in response to changes in the environment have also been shown for some genes in yeast.[19]

In response to stress, the majority of stress-responsive genes show a single point change in the DR during the stress response, suggesting a single underlying regulatory event,[36,130] although in some cases, complex patterns of DR changes have been seen.[16,17] For the majority of expressed genes that are not affected much by moderate stress, the DR does not seem to change,[17,36] indicating that changes in the DR are probably not due to a general change in decay machinery, but to mRNA-specific actions. This specific regulation of mRNA stability is usually mediated by RBPs, which controls the so-called PT regulons,[55] as discussed later. Generally, after some minutes of stress response, most genes reverse the change in the DR in parallel to the TR by returning to the initial values. In other instances, the DR acts counterintuitively by opposing the action of TR. mRNA stability decreases while the TR increases. This strategy is energy-consuming, but it speeds up the response since the kinetics of transcriptional change, as previously explained, is proportional to k_d.[13,19,36,134]

Another strategy reported is variation in the DR without a change in the TR.[16,17] This is not a common phenomenon in yeast. However, it has been recently proposed that modulating decay rates of mRNAs and proteins can be used in mammals to alter gene regulatory networks without changing TRs.[181] A mathematical model demonstrates that a nonspecific control over DRs (change in global decay by a common factor) can lead to an altered expression pattern without the need to change the TR. This new expression mode is similar to those leading to cell differentiation in higher eukaryotes and explains why experimental observations have found correlations among cell types, expressed sets of TFs, and global DRs (discussed in Ref. 181). The

possible reason for this result is that the low mRNA and protein concentration of TFs and regulatory proteins can be profoundly affected by general changes in DRs, leading to a new expression program. An extensive review on the roles of mRNA decay in animal development has been recently published.[134] General changes in DRs have also been observed in *S. cerevisiae* under conditions that do not support growth, for example, after cells enter stationary phase,[178,182] or shortly after a change from glucose to galactose medium,[49,178] or after severe osmotic stress.[18,183] This global effect on DR is probably due to repression of some general mRNA decay factors. Indeed, global stabilization of mRNAs induced by severe hyperosmotic stress, as well as by severe heat shock and glucose deprivation, is mediated by the inhibition of two major deadenylases, Ccr4/Pop2/Not complex, and Pan2/Pan3.[183] This inhibition is independent of translation.[183] Poly(A) binding protein (PABP) and the deadenylation enzymes can also be targets for general modulation of DR. For example, miRISC interacts with PABP and the CAF1 and CCR4 deadenylases *in vitro*, thus stimulating deadenylation. Cumulative results from a number of laboratories suggest that deadenylases are the most common targets of various destabilization mechanisms, and it is also common to a variety of stresses in yeast and in mammalian cells (see, e.g., Refs. 140, 183, 184, and 185). However, how signaling pathways regulate these decay enzymes is still unresolved.

The parasitic protists provide an interesting example of differentiation programs that involve changes in DR. The mRNAs HL of *Plasmodium* increases during the asexual life cycle from an average of 9.5 min in the ring stage to 65 min in the schizont stage (Table 1). Although it is accompanied by a global change in the TR, the observed mRNA accumulation after schizogony seems to be mainly caused by a general increase in HLs.[32] In *Trypanosoma brucei*, the influence of DR is critical because regulation of transcription initiation seems to play little role. Control of gene expression is maintained by the combination of gene copy number and mRNA stability,[33] involving well-defined PT regulons.[57] Indeed, during the differentiation of trypanosomes, changes in the DR plays the main role in determining new mRNA levels. It is interesting to learn that also in mammalian cells, a change in the DR is important for differentiation. However in this case, changes are not general but are conducted by specific RBPs or miRNAs. For example, when human fibroblasts are dedifferentiated to form pluripotent stems (induced pluripotent cells), three independent regulatory mechanisms are induced to allow a coordinated turnover of specific groups of mRNAs, and some become more stable while others are destabilized.[157]

The Coordination of mRNA Decay: PT Regulons

A number of studies have revealed that the targets of the mRNA decay pathways are not single mRNAs. Instead, groups of mRNAs encoding functionally related proteins are coordinately regulated by one or more specific RBPs as PT regulons.[55,56,131,186] This coordinated regulation, which occurs also at levels other than mRNA decay, leads to proper expression and stoichiometry of cellular machineries, complexes, or pathways. The coordinated regulation of the regulon is achieved by RNA binding factors that might regulate RNA processing, nuclear export, subcellular localization, translation, and decay (Fig. 3). A single mRNA can be a member of more than one regulon, as determined by its interactions with a combination of RBP.[55] These RBPs control the fate of the mRNA, specify its DR, and determine how the mRNP fate changes in response to intra- and extracellular signals. The composition of mRNPs is dynamic (Fig. 3): mRNPs are formed during transcription and undergo remodeling during RNA maturation and nuclear export, upon arrival at the cytoplasm, and in response to signals.[187] The nuclear history of an mRNP can affect its cytoplasmic fate, and the cytoplasmic mRNP status can signal back to the nucleus. The cross-talk between mRNA degradation and transcription is discussed in the next section.

PT regulons have been found across eukaryotes through the use of massive techniques to identify RBP targets (reviewed in Refs. 131 and 186) or to find common HL profiles during physiological responses.[1,16–18,36,49] In some of them, PT regulation is mainly executed at the mRNA decay level. For example, the AREs containing mRNAs that encode the proteins involved in the inflammation response are co-regulated by RBP TTP in mammals. Pro-inflammatory factors induce the expression of TTP, which binds its ARE-containing targets and nucleates the assembly of various decay factors. TTP mRNA also contains an ARE and TTP is thus capable of negatively regulating its own expression. TTP promotes mRNA decay by recruiting the Ccr4–NOT deadenylation complex and by driving mRNAs to P-bodies for degradation. TTP can also nucleate the assembly of SGs under stress. The importance of TTP-regulated turnover of the pro-inflammatory regulon is demonstrated by the fact that mice lacking TTP developed various diseases related to systemic inflammatory syndrome (reviewed in Ref. 188). In *S. cerevisiae*, RBP Cth2, a homolog of mammalian TTP, co-regulates the decay of mRNAs responsive to iron (Fe^{+3}) depletion.[136] Cth2 binds to ARE elements and is able to interact with decapping activator factor Dhh1 to mediate mRNA turnover.[189] Interestingly, Cth2 shuttles between the nucleus and

the cytoplasm, and its export is dependent on transcription. This raises the possibility that it is loaded on target mRNAs in the nucleus and is exported together with mRNA; moreover, disruption of its shuttling capacity leads to defective mRNA decay in the cytoplasm and reduced localization in PBs.[190] Figure 3 depicts a model based on all these results.

The modulation of RBP activity by signaling pathways is another way of regulating mRNA decay. In mammals, the signaling MAPK p38 is activated by stress and inflammatory stimuli. Activated p38 promotes phosphorylation of TTP by MK2 kinase. Phosphorylated TTP interacts with 14-3-3 proteins, which inhibit the recruitment of deadenylases to TTP-target mRNAs. Conversely, under normal conditions, the function of TTP in the degradation of pro-inflammatory mRNAs is stimulated by its dephosphorylation by phosphatase PP2A (reviewed in Ref. 80).

Signaling pathways can also act at the level of general mRNA decay factors. For example, several stresses of *S. cerevisiae* cells cause the phosphorylation of Dcp2 by kinase Ste20. Ste20 acts as an MAPKKKK in several yeast MAPK pathways that are activated in response to external stimuli. In response to stress, the phosphorylation of Dcp2 is required for its accumulation in PBs and for the assembly of SG.[191] Another study in *S. cerevisiae* has shown that the cAMP-dependent protein kinase inhibits the formation of PBs by the phosphorylation of the decapping activator and translation repressor Pat1.[192]

In summary, although initial studies have begun addressing how signaling pathways control the mechanism of global and specific mRNA turnover, detailed understanding requires further work.

The Interplay between mRNA Synthesis and Decay

As we have extensively discussed above, changes in mRNA levels originate from any asymmetric variation in TRs and DRs. According to this view, any general transcriptional or mRNA decay perturbation should involve a significant change in global mRNA concentrations. Experimental evidence, however, contradicts this prediction. Impairing transcription with a point mutation in yeast RNA pol II decreases absolute mRNA synthesis rates, but its impact on mRNA levels is small.[193] This observation has been confirmed by cDTA.[11] Similarly, impairing yeast mRNA degradation by deleting the deadenylase subunits of Ccr4–NOT complexes lowers decay rates, as measured by cDTA, but its consequences on mRNA levels are less pronounced than expected.[11] Although the underlying mechanism is unknown, these results can globally be explained by somewhat compensatory effect of decay rates when transcription is compromised, and vice versa (see also Ref. 187).

This parallel co-regulation of global mRNA synthesis and degradation might reflect the action of a hypothetical factor that modulates transcription and decay in response to any alteration in mRNA homeostasis.[11] The previously discussed antagonistic coordination between transcription and mRNA decay, varying in opposite directions in response to stress or developmental stimuli,[16,19,31,130] supports this idea. An alternative explanation stems from approaching gene expression as a single system in which all the stages are mechanistically coupled.[187,194] This view is based on the multiple examples of mechanistic coupling between transcription, RNA processing, and mRNA export,[195] and between translation and mRNA decay.[196] By inference, we can assume that mRNA decay also globally impacts transcription. The impact of mRNA decay machinery on mRNA synthesis remains to be determined; however, the effect of mRNA synthesis machinery on its decay has already been documented.[187]

Recently, it has been discovered that *cis*-acting elements in the upstream activating sequences of yeast promoters can determine the transcript's decay kinetics.[146,147] This effect of yeast promoters on mRNA decay depends on the TFs that bind the upstream activating sequence. For instance, as indicated earlier, the yeast TF Rap1, which regulates hundreds of yeast genes, and a short *cis*-acting element comprising two Rap1-binding sites, are necessary and sufficient to induce enhanced decay onto a reporter mRNA.[146] In a parallel study, Trcek *et al.*[147] found that the specificity and timing of the decay of *SWI5* and *CLB2* mRNAs, encoding cell cycle proteins, are controlled by their promoters. This control was executed through the RBP Dbf2, which seems to load onto mRNAs co-transcriptionally. What is the mechanism underlying the link between a promoter-binding TF and decay machinery? The simplest hypothesis suggests that after binding the promoter, the TF can affect the loading of specific factors on mRNP, termed mRNA imprinting,[8] which, in turn, regulates mRNA decay after mRNA is exported to the cytoplasm.[146,147,187] RNA polymerase subunits Rpb4 and Rpb7 represent the first prototype of this mechanism; they are co-transcriptionally loaded onto the mRNA (Fig. 3) and capable of enhancing mRNA decay.[179,193,197]

Yeast Rpb4 and Rpb7 form a heterodimer (Rpb4/7) that was originally identified as a subunit of RNA pol II.[198] Later, it was shown to play a more general role in gene expression. Rpb4/7 shuttles between nucleus and cytoplasm.[199] Remarkably, Rpb4/7 can stimulate mRNA export, translation, and decay.[129,179,197,200] Goler-Baron *et al.*[193] demonstrated that the co-transcriptional binding of Rpb4/7 with the emerging

transcript is dependent on the recruitment of Rpb4/7 to RNA pol II. They employed two Pol II mutant forms that poorly recruit Rpb4/7 due to the mutations in either Rpb1 or Rpb6. Significantly, interaction of Rpb4/7 with the transcripts of these Pol II mutants is compromised, despite the presence of Rpb4/7 in excess over the RNA pol II molecules in the nucleus. Furthermore, these mutant cells do not support efficient poly(A) shortening and mRNA decay[193] or translation.[129] The overexpression of both Rpb4p and Rpb7p, which increases the portion of mutant RNA pol II that manages to recruit Rpb4/7, partially restores mRNA decay in mutant cells. Thus, the authors proposed that the interaction of Rpb4/7 with mRNA occurs only in the context of RNA pol II and that it is required for Rpb4/7 to be capable of stimulating translation and mRNA decay.[129,193] The cells carrying this mutant RNA pol II are unable to properly regulate mRNA stability in response to stress and have lost the usual negative coordinated regulation between mRNA production and degradation.[201] Thus, by co-transcriptionally loading Rpb4/7 onto transcripts, RNA pol II can regulate the translation and decay rates of these transcripts. By doing so, Rpb4/7 defines a new class of master gene expression factors, "mRNA coordinators", which regulate the entire life of mRNAs, from synthesis to degradation.[129] We suspect that the mRNA coordinator consists of a multifactorial complex, larger than just Rpb4 and Rpb7. Based on their features, we suspect that a number of other factors are candidates of being mRNA coordinators or components of a larger coordinator complex. Among them are factors originally described to act in transcription elongation (the THO complex), mRNA export (Mex67), processing (CPEB), and translation (Sro9).[8]

The recent discoveries demonstrating the capacity of RNA to move from one cell to another,[202,203] combined with the mRNA imprinting concept, whereby the fate of the mRNA is predetermined in the nucleus, opens up the possibility of a new kind of epigenetics heredity. Accordingly, the donor cell can impact the phenotype of the recipient cell by exporting an imprinted mRNA. Thus, even should donor and the recipient cells synthesize the same repertoire of mRNAs, a different imprinting nature can impact the fate, localization, translation, and decay of the self- *versus* transported RNAs.[8]

The existence of mRNA coordinators helps explain communication from transcription to decay, and the robust homeostasis of mRNAs in response to transcriptional perturbations.[187] However an additional link may exist from decay to transcription to allow the preservation of mRNA homeostasis in response to fluctuations in mRNA stability. Such a feedback between mRNA decay and transcription may be exerted by the components of cytoplasmic mRNA degradation machinery, which also play transcriptional roles. Good candidates include the yeast Rpb4/7 and Ccr4–NOT complex. So far, the latter complex has been considered to play two independent roles: mRNA deadenylation during cytoplasmic degradation[204] and stimulation of transcription elongation.[205–207] If the two roles of Ccr4–NOT turned out to be coupled, Ccr4–NOT can be considered as a factor involved in the feedback mechanism between mRNA decay and transcription, like Rpb4/7.

Synthegradases: Factors that stimulate (or repress) both mRNA synthesis and decay

Similar to Rpb4/7, Rap1 and Ccr4–NOT belong to a new category of cellular elements that we call "synthegradases"[146] due to their dual capacity to stimulate (or repress) both mRNA synthesis and degradation. The pivotal role of synthegradases in gene expression would allow signaling pathways to modulate either of their two arms coordinately. Synthegradases might serve as a mechanistic basis for the characteristic "peaked" behavior of many genes whose expression responds to environmental changes in a manner that stimulates (or represses) both mRNA synthesis and decay (see, e.g., Ref. 201). Recent comparison between the mRNA decay kinetics of two related *Saccharomyces* species has revealed a significant difference in the HL of 11% of orthologous mRNAs.[208] In half of them, a change took place in both mRNA decay and transcription, and in most of these cases, the changes occurring in the two processes were in the same direction.[208] Moreover, some yeast factors seem to have evolved in a manner that either stimulates both mRNA synthesis and decay or represses both processes simultaneously. Among the most notable factors are Rpb4p and Ccr4p.[208] This work suggests that the levels of at least 5.5% of the yeast mRNAs are regulated by synthegradases under optimal proliferation conditions. The dual roles of promoters and synthegradases might have evolutionary implications; a single mutation in either a promoter or a synthegradase can affect both transcription and mRNA degradation, which would otherwise require at least two independent mutations (see also Ref. 209).

Future Perspective

A major long-term challenge in the field is to be able to predict the HL of a given mRNA based on the RNA sequence, the composition of its associated factors (e.g., proteins, ncRNAs), its current and previous cellular localization, as well as cell physiology and environmental conditions. To make progress toward this goal, we need to address more immediate

challenges. For example, while most of the components of the basal mRNA decay machineries are most probably known, a major challenge for future work is to obtain detailed mechanistic understanding of how specific mRNA decay modulators target the basal machinery to modify the process. The issue of decay regulon[55,131] is still far from being clear. In those cases that it is mediated by a common factor, how does the factor find all the mRNAs of this regulon? Is it based on random collisions, or there are mechanisms that bring the factor and the RNAs together (e.g., co-transcriptional imprinting)? Do the RNA members compete for binding the factor? It is commonly appreciated that performance of mRNA decay responds to external and internal signals. Another challenge is to decipher how the various signaling pathways impact mRNA decay. As discussed earlier, investigators have already begun to make interesting discoveries along this line. Internal signals that do not involve "classical" signaling pathways are even more interesting. Assuming that mRNA decay is a central process in gene expression, it is conceivable that it responds to internal signal such as changes in the metabolism (this issue was addressed by some investigators and discussed earlier in this review), including changes in reactive oxygen species, changes in cell size and cell cycle, reorganization of cellular matrix, changes in lipid, carbohydrate homeostasis, and so on. A particular and relatively more studied aspect is the impact of translation on mRNA decay. The inverse correlation between translation and mRNA degradation is well documented and discussed in this review. However, mechanistic understanding is far from being clear. One mechanism involves the role of the translation-releasing factor eRF3. It was proposed that the interplay between eRF3, PABP, and the deadenylases leads to translation-dependent activation of deadenylation.[210] However, this model cannot represent all cases. This is because introducing stem–loop structures in the 5′ UTRs of *MFA2* or *PGK1* mRNAs, in a manner that blocks translation initiation, does not affect deadenylation kinetics.[211,212]

The linkage between mRNA decay and (RNA pol II) transcription is one of the main focuses of this review. As this field is in its infancy, much remains to be studied. First, is there a clear mechanistic linkage between RNA pol I and pol III transcription and their transcript degradation? Is it also mediated by mRNA imprinting? Second, as we view it, the "raison d'être" of synthegradases is their capacity to couple between mRNA synthesis and decay. What is the scope of the synthegradases phenomenon? Is there a common denominator to their function? How many of them bind promoters, and/or associate with elongating transcription apparatus and/or polyadenylation complex? A more general issue is whether a tight linkage between synthesis and decay characterize also synthetic/degradation system of other biological polymers such as proteins, lipids, and carbohydrates. How many of the synthegradases function as mRNA coordinators? How many mRNA coordinators does the yeast organism contain, and what is the full repertory of their components? We believe that a key issue for future work is to decipher the mechanism underlying the coordination and the role played by the coordinators in enabling a cross-talk between mRNA decay and the other stages of gene expression.

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Abbreviations used:
TR, transcription rate; DR, degradation rate; HL, mRNA half-life; TF, transcription factor; 4sU, 4-thiouridine; 4tU, 4-thiouracil; ncRNA, non-coding RNA; lncRNA, long non-coding RNA; RNA pol II, RNA polymerase II; RBP, RNA binding protein; ARE, AU-rich recognition element; UTR, untranslated region; NMD, non-sense-mediated decay; mRNP, mRNA ribonucleoprotein particle; PB, processing body or P-body; miRNA, microRNA; endo-siRNA, endogenous small interfering RNA; PT, posttranscriptional; TTP, tristetraprolin; SG, stress granule; RISC, RNA-induced silencing complex; cDTA, comparative dynamic transcriptome analysis; RP, ribosomal protein; GRO, genomic run-on; ORF, open reading frame; TNF, tumor necrosis factor; SUT, stable unannotated transcript; CUT, cryptic unstable transcript.

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