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Chapter 6

Interplay between Transcription and RNA Degradation

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Abstract

Amount of mRNA depends on the both the rates of mRNA transcription in the nucleus and mRNA degradation in the cytoplasm. Although each of the processes was studied independently, recent studies demonstrated the interplay between transcription and mRNA degradation in various cellular processes, such as cell-cycle, cellular differentiation, and stress responses. In this review, we discuss the benefit of the interplay in the gene expressions and the mechanisms how these two processes are coupled. We also review recent genome-wide methods to measure the rates of transcription and degradation.

Keywords: RNA degradation, transcription factor, RNA binding proteins, synthegradase, RNA buffering, mRNA imprinting, NGS

1. Introduction

Gene expression involves multiple processes such as the transcription, translation, and degradation of messenger RNAs (mRNAs). Each of these processes was studied independently. In the nucleus, RNA polymerase II (RNAPII) and various transcription factors are recruited to the promoter of protein-coding genes to initiate transcription [1, 2]. Nascent mRNA is co-transcriptionally capped at 5′-end [3, 4], spliced [5], and matured at the 3′-end [6] (**Figure 1**). During these post-transcriptional modifications, every transcript is associated with various RNA-binding proteins (RBPs), forming large ribonucleoprotein complexes (mRNPs). This mRNP assembly process is subject to quality control by nuclear surveillance mechanisms [7, 8]. After the quality control, mRNPs are transported to cytoplasm.

In the cytoplasm, the translationally inactive mRNPs would accumulate in P bodies or stress granules where mRNPs are degraded [9, 10] (**Figure 2**). Degradation of the cytoplasmic

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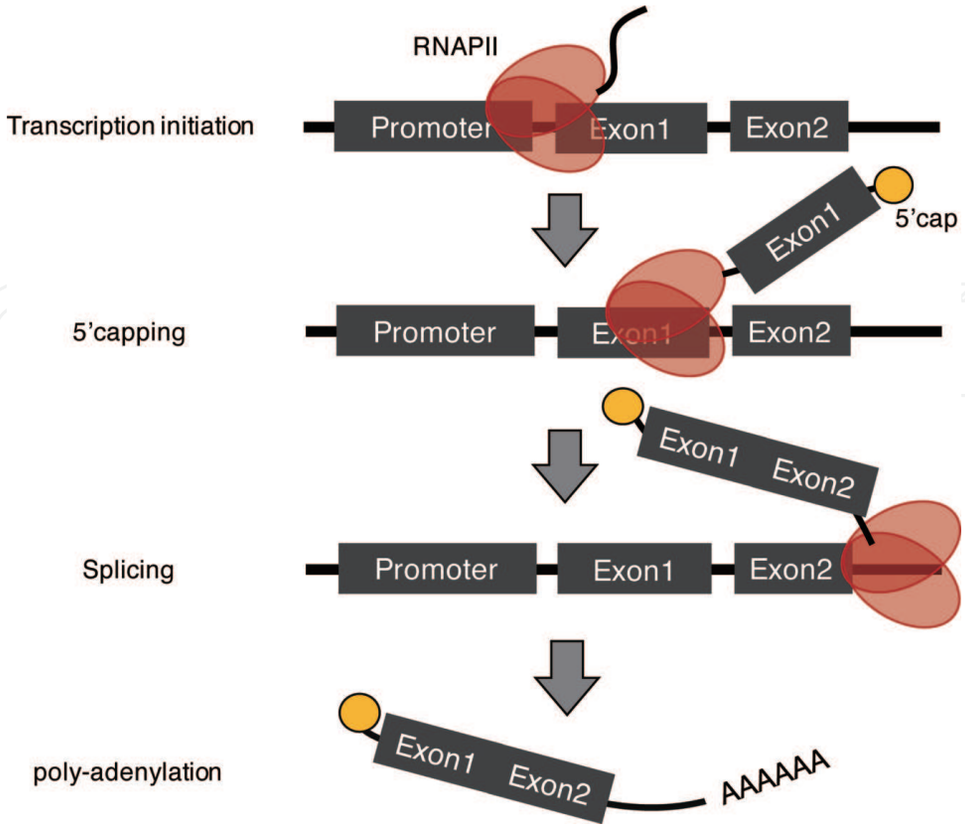


Figure 1. Scheme of co-transcriptional mRNA processing. An m^7G cap (a circle) is added co-transcriptionally to the 5′end of the nascent RNA. During the elongation, introns are removed by splicing machinery. Cleavage and polyadenylation are mediated after the transcription to form mature transcripts.

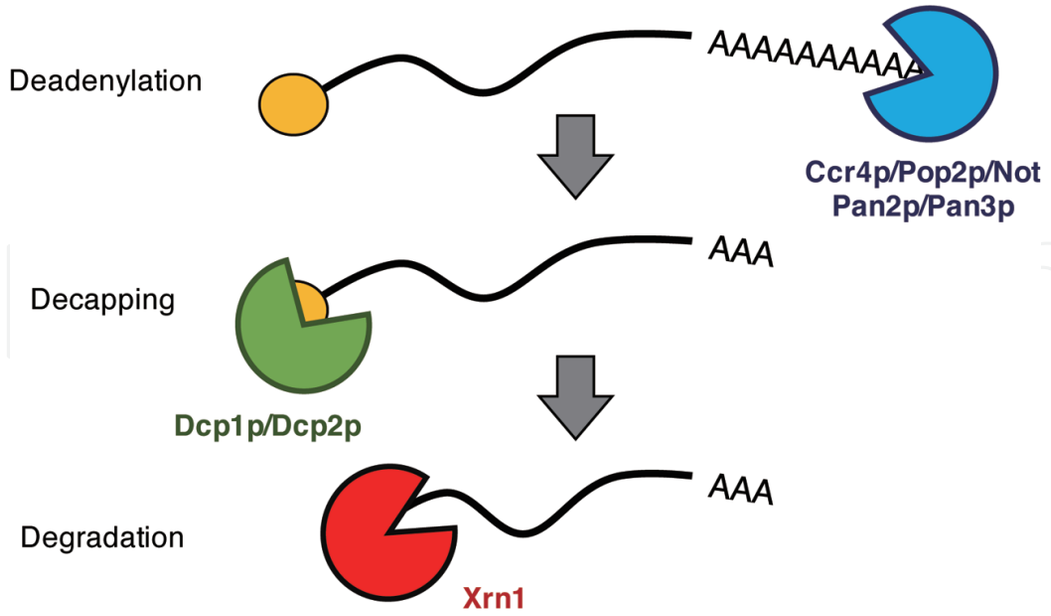


Figure 2. The 5′ → 3′ degradation pathway exonuclease-mediated decay begins with shortening of Pan2/Pan3 or CCR4-not complexes. After the decapping of 5′cap structure (a circle), the body of mRNA is degraded with 5′-to-3′polarity by XRN1.

Names in yeast	Human homologs	Function
Ccr4p	hCCR4	Carbon catabolite repressor 4. Catalytic subunits of the complex
Pop2p	CNOT7/CNOT8	Also known as Caf1 (Ccr4 associated factor 1). Related to RNase D family
Not	CNOT1	Negative on TATA. A large scaffolding protein
Pan2p	PAN2	PolyA nuclease2. Contains a nuclease domain of the RNase D
Pan3p	PAN3	PolyA nuclease3. Co-factor of Pan2
Dcp1p	DCP1A/DCP1B	Decapping protein1, Co-activator
Dcp2p	DCP2	Decapping protein2. Catalytically active decapping enzyme
Pat1p	PAT1A/PAT1B	Recruit Lsm1-7 to P-bodies to trigger decapping
Lsm1-7p	LSM 1-7	Seven Sm-like proteins. Deadenylation-dependent mRNA decapping factors
Dhh1p	RCX/p54	DEAD box helicase. ATP-dependent RNA helicase in mRNA decapping
Xrn1p	XRN1/XRN2	Major 5′-3′ Exoribonuclease1, requiring 5′ monophosphate

Table 1. Yeast RNA degradation factors and its human homologs.

mRNA is initiated by shortening of the poly(A) tail, which is called deadenylation. In yeast, this deadenylation is catalyzed either by Ccr4p/Pop2p/Not complex or by the Pan2p/Pan3p complex [11, 12]. After the deadenylation, the 5′-cap structure was removed by the concerted action of the decapping complex, Dcp1p/Dcp2p, which is stimulated by Pat1p, the Lsm1-7p, and Dhh1p [13, 14]. The decapping reaction exposes the 5′-monophosphate of the terminal residue, promoting the 5′ → 3′ degradation pathway by the major cytoplasmic exoribonuclease Xrn1p [15] (**Table 1**).

The life of mRNA seems to be straightforward. However, recent studies have shown evidence of the interplay between transcription and degradation: transcription rate is regulated by decay factor; degradation rate is regulated by transcription factor and even by some promoters. This complex network enables cells to shape appropriate gene expression profiles during cell cycle processes, cellular differentiation, stress and immune responses [16–18].

2. Biological processes coupling transcription and decay

The functional connection between the transcription and degradation of mRNA shapes the characteristic patterns of gene expression. In this section, we introduce several examples of the coordination between transcription and degradation in various biological processes.

To respond to environmental cues, cells must switch their steady level of gene expression in a rapid and transient mode. This sharp rise of mRNAs can be efficiently achieved if the

stabilization of transcripts enhances their transcription rates. An example for such functional coupling is observed in osmotic stress in *S. cerevisiae*. With mild osmotic stress (0.4 M NaCl), 121 mRNAs belonging to the functional groups "stress response" and "trehalose production" increase both transcription rates and stability [19]. The study of oxidative stress (0.5 mM H_2O_2) in fission yeast revealed a major role of transcriptional up-regulation in the stress, but also showed the first minutes after stress induction as a critical time for mRNA degradation to support the control rapid gene regulation by transcription [20]. In contrast to oxidative stress, a moderate heat shock induced a global trend for mRNA stabilization, whereas transcription rate contributed only a transient increase immediately upon stress [21]. The difference observed in these studies suggested the interplay between transcription and degradation is carefully regulated in the cells. Indeed, Shalem et al. demonstrated that alternative modes of such interplay determine the kinetics of the transcriptome in response to stress. They subjected yeast to two stresses; oxidative stress and DNA damage. In oxidative stress, many genes show fast response followed by relaxation, resulting in a quick and transient response, whereas in the DNA damage experiment, the response is slow and long enduring. Measurement of the genome-wide decay profile showed condition-specific changes in decay rates. In the transient response, most induced genes were destabilized, exhibiting counteraction between transcription and degradation. This interplay profile can reconcile a high steady-state level with short response time among induced genes. In contrast, slow repression response was achieved by destabilization of the transcripts [22].

As abnormal gene expression is deleterious to living cells, it is critical to maintain steady levels of mRNA; hence, mRNA levels are said to be "buffered". When genome-wide transcription was attenuated by mutating RNAPII of *S. cerevisiae*, the cells maintain a steady level of the transcripts by decreasing their decay rates [23]. This study also revealed that buffering of mRNA levels required the RNA exonuclease Xrn1. Conversely, impairing mRNA degradation by deleting deadenylase subunits of the Ccr4-Not complex caused the decrease in both degradation and synthesis rates [24]. This mutual feedback maintains the steady levels of mRNAs and establishes a cellular mRNA surveillance network. It is mysterious that the synthesis-decay feedback exists despite the separation of mRNA synthesis and degradation into nuclear and cytoplasmic compartments. One possible model was proposed by Haimovich et al. [25]. They showed that the components related to mRNA degradations shuttle between cytoplasm and the nucleus, in a manner dependent on proper mRNA degradation. In the nucleus, they associated with chromatin and regulated transcription rate.

Cross talk between mRNA synthesis and decay can also be gene specific. In budding yeast, stability of core histone mRNAs is temporally co-regulated with their transcription during the cell cycle. Entry into S phase showed rapid increase in their transcription, followed by a prompt decrease in their abundance right after exiting the S phase [26–28]. Similar to histone mRNAs, there should be numerous genes of which expression levels are regulated in a cell-cycle-dependent manner. By using DNA microarrays, Spellman et al. found that about 800 genes are cell cycle regulated, which correspond to 10% of all protein-coding genes in yeast genome [29]. The mechanism for how cells coordinate the characteristic and integrated expression pattern during cell-cycle is not fully understood.

Interestingly, a functional coupling between the transcription and degradation was exploited by herpes virus [30]. Gamma-herpesviruses encode a cytoplasmic endonuclease, SOX, which cleaves cellular mRNAs. These cleaved fragments are subsequently degraded by the cellular exonuclease Xrn1. This accelerated decay triggered the repression of RNAPII transcription rate. The findings suggest that mammalian cells can sense broad alternation in RNA degradation. It is not the initial cleavages by SOX that are detected, but rather the increased activity of cellular Xrn1 that generates a transcriptional response. Furthermore, the viral mRNAs escaped the degradation induced transcriptional repression, and this escape requires Xrn1. The opposing roles for Xrn1 in the host and viral transcriptional response may indicate that herpesviruses have evolved to benefit from this intrinsic feedback mechanism.

3. Mechanism underlying coupling transcription and decay

The mechanism underlying transcription in the nucleus affects mRNA decay in the cytoplasm and vice versa is intensively studied in *S. cerevisiae*. The regulation of mRNA decay mediated by the transcription is categorized into *cis*-acting elements and *trans*-activating factors. *Cis*-acting elements directly regulate the mRNA decay by interacting with RNA binding proteins and/or decay factors [31, 32]. *trans*-activating factors are recruited onto the mRNA during its transcription. This interaction is maintained in cytoplasm, regulating the stability of the mRNA. In contrast, there are only a few examples for regulation of the transcription by mRNA decay, and this is still under intense investigation.

3.1. *cis*-acting elements

mRNA contains 5′ untranslated region (UTR) and 3′UTR outside the coding region. These two UTR regulate the fate of mRNAs. Here we discuss how the transcription of 3′UTR regulates its length, and thus causes the modification of mRNA stability.

3.1.1. 3′UTR

The turnover of an mRNA is mostly regulated by *cis*-acting elements located in the 3′UTR [33], such as AU-rich elements (AREs) [34, 35], GU-rich elements [36], PUF response elements [37], miRNA binding sites [38, 39], and the poly(A) tail [40]. In principle, the length of 3′UTR affect the stability of mRNA because longer 3′UTR would contain more *cis*-acting elements compared with short 3′UTR (**Figure 3**). Eukaryotic cells control the length of 3′UTR with alternative polyadenylation [41, 42]. Genome-wide polyadenylation maps were established by several RNA-seq studies. Direct RNA sequencing (DRS) technology provided a comprehensive view of global polyadenylation events in human and yeast, and estimated that 72% of yeast genes and more than half of human genes show alternative polyadenylation patterns [43]. Moreover, 3′ region extraction and deep sequencing (3′READS) was used to comprehensively map polyadenylation sites in the mouse genome [44]. 3′READS revealed that about 80% of mRNA and 66% of long noncoding RNA undergo alternative polyadenylation. Importantly, 3′READS found a global trend of up-regulation

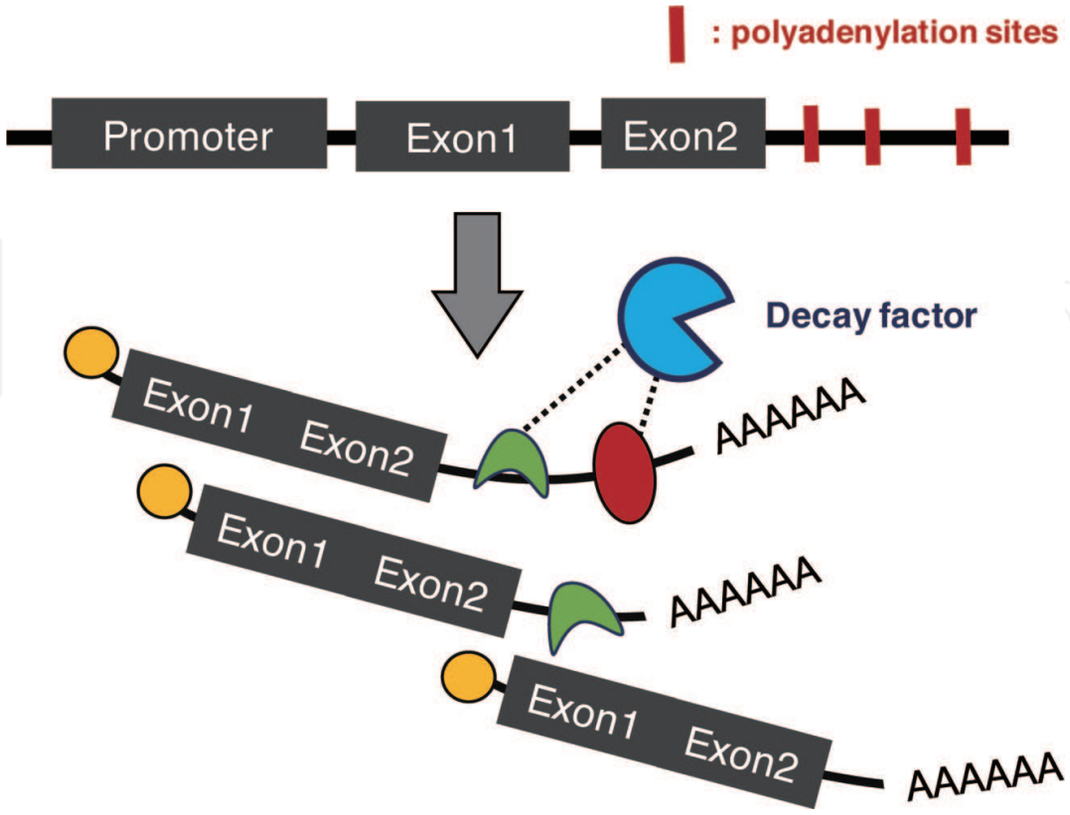


Figure 3. Alternative polyadenylation affects 3'UTR lengths. Longer UTRs allow more RBPs to associate with the mRNA (indicated by arrowhead and ellipse, respectively). The RBPs regulate the mRNA stability by recruiting decay factor.

of isoforms using promoter-distal polyadenylation sites in development and differentiation, suggesting that the RNA degradation pathway will be reconstructed globally through the development. These two studies, however, lack quantitative analysis of mRNA stability and 3'UTR length modification by alternative polyadenylation. Geisberg et al. developed a method to measure mRNA half-lives of mRNA isoform in yeast [45]. Based on clusters of isoforms with different half-lives, they identified hundreds of sequences responsible for mRNA stabilization. Specifically, the poly(U) sequence was found to be the stabilizing element.

3.1.2. Promoter regulates mRNA stability

Surprisingly, several reports showed that promoter regions also affect mRNA degradation after the mRNA leaves the nucleus. The first report of promoter-regulated mRNA stability was published in 1993. This study showed that swapping of the β-globin promoter in HeLa cells to that of the Herpes simplex virus 1 thymidine kinase (HSV1-TK) stabilizes a nonsense mutation in the mRNA, while this effect was not observed with the replacement for the CMV promoter [46]. A problem in this study was that the authors cannot rule out the possibility that different amounts of mature β-globin mRNAs may be caused by the different efficiencies of the splicing. This problem can be avoided by targeting genes without introns.

In 2011, two studies in the *S. cerevisiae* demonstrated clearly that promoters and associated *cis*-acting elements coordinate their transcription and decay (**Figure 4**). A conventional yeast promoter consists of a core element and an upstream activating sequence (UAS). Promoter swapping of native UAS of the *RPL30* gene with that of the *ACT1* gene increased the stability of *RPL30* mRNA significantly [47]. A *cis*-element, comprising two Rap1p-binding sites, and Rap1p itself are necessary and sufficient to induce stabilization of the transcript. Moreover, Rap1p stimulates both synthesis and decay of endogenous transcripts. Thus, this study proposed that interaction of Rap1p with the target promoter affects the composition of mRNP, resulting in modification of the mRNA degradation rate. Considering that Rap1p has an effect in coupling transcription with mRNA decay, this study also introduced a concept called "synthegradase". They also estimated at least 150 yeast genes would be regulated by synthegradases during optimal proliferation conditions. Notably, this number is likely to increase with different environmental conditions.

A second example is the study about cell cycle-regulated decay in yeast cells using single molecule fluorescence *in situ* hybridization (FISH) [48]. Promoter swapping of *SWI5* and *CLB2* genes with *ACT1* made their stability close to *ACT1*. This study also showed that the mitotic exit network protein Dbf2p accounts for the coordinated decay of the transcripts. Chromatin immunoprecipitation and RNA immunoprecipitation of Dbf2p showed that Dbf2p interacts with both the transcript promoter and mRNA, suggesting that this protein is recruited to the promoter and then subsequently stalled on the mRNA. As Dbf2 can interact with the Ccr4-Not complex [49], this promoter-regulated decay may manifest through the regulation of deadenylation.

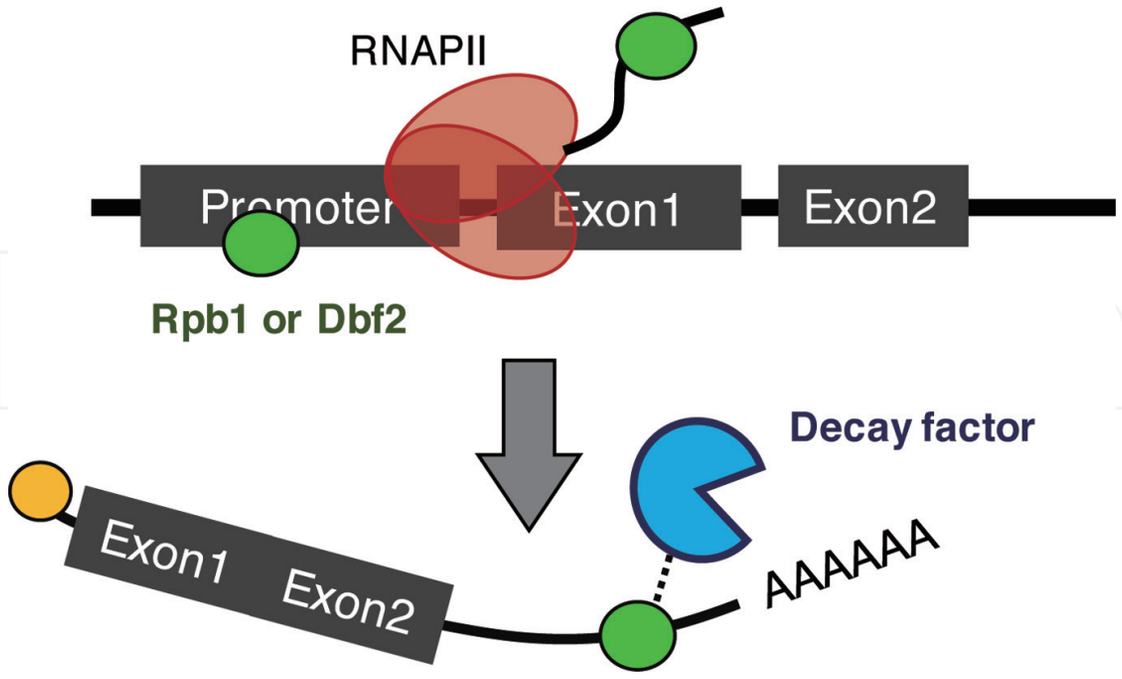


Figure 4. Promoter-regulating degradation. Transcription factor such as Rpb1 or Dbf2 (a circle on the promoter) binds to transcripts. After the export into the cytoplasm, the transcription factors in cytoplasm recruit decay factor to promote RNA degradation.

Although these two works are focused on specific genes, Dori-Bachash et al. extended to the genome-wide scale [50]. They demonstrated that swapping UAS between two yeast species affected both transcription and degradation. Adjacent yeast genes sharing a common promoter displayed similar mRNA decay rates, which also indicated that promoters couple transcription and degradation. Notably, similar coordination between transcription and degradation were found in mouse and human models. Because the diverse genes and regulatory elements were associated with promoter-regulated coordination, this phenomenon could be generated by genome-wide mechanisms of gene regulation.

3.2. *trans*-acting proteins

trans-acting proteins are recruited onto the mRNA during transcription, and affect post-transcriptional regulation after mRNA is exported to nucleoplasm. This process is termed "mRNA imprinting", which confers classical genetic information flexibility [51]. This mRNA imprinting lasts throughout the mRNA lifetime and is required for proper post-transcriptional regulation. Here, we focus how mRNA imprinting regulates the degradation rate.

3.2.1. Rpb4 and Rpb7

To date, the best characterized *trans*-acting proteins are two subunits of the core RNAPII, Rpb4p and Rpb7p. Rpb4p and Rpb7p associate with the core polymerase as a heterodimer. Two studies provided evidence that the nascent pre-mRNA emerging from the active site of RNAPII interacts with Rpb7p [52, 53]. Moreover, Rpb4/7p shuttle between the nucleus and the cytoplasm [54], suggesting that this heterodimer influences mRNA physiology in the cytoplasm. These facts suggest that Rpb4/7p would be imprinted on the mRNA. Several pieces of experimental results revealed that Rpb4/7p promotes the mRNA decay [55, 56]: both Rpb4p and Rpb7p affected the deadenylation step; both Rpb4p and Rpb7p interact with the mRNA decapping components of the Pat1p-Lsm1-7p complex; and Rpb4p and Rpb7p localized to cytoplasmic P-bodies where mRNA is degraded. In this manner, Rpb4/7p would link the activity of the basal transcription apparatus with that of the mRNA degradation machinery [57].

3.2.2. Snf1

Snf1p is the yeast ortholog of human AMP-activated protein kinase (AMPK) involved in diverse stress environments [58–60]. Recent studies also revealed that Snf1p is related to post-transcriptional regulation. Culturing yeast in glucose-containing growth medium represses Snf1-dependent transcription of target genes and promotes mRNA degradation of the corresponding mRNAs, which is called glucose-induced decay of mRNA [61, 62]. In low glucose concentrations, Snf1 activates the transcription of glucose-induced genes required for energy metabolism. In contrast, when glucose concentration is high, termination of transcription and activation of the degradation of the glucose-induced transcripts occur, resulting in rapid reduction of mRNA levels. Braun et al. fused nonglucose-responsive genes *MAP2* and *IDP2* to the *ADH2* promoter. This promoter swapping caused a significant destabilization of these

mRNAs, indicating that the *ADH2* promoter alone is responsible for glucose-induced mRNA decay [63]. To understand the molecular mechanism of Snf1-dependent decay, quantitative mass spectrometry was used to identify proteins phosphorylated in a Snf1-dependent manner [64]. This phosphoproteomic analysis identified 210 Snf1-dependent phosphopeptides in 145 proteins. Notably, mRNA decay factors, such as Eap1p, Ccr4p, Dhh1p, and Xrn1p were the targets of Snf1p-dependent phosphorylation. As expected, mutation of three Snf1-dependent phosphorylation sites in Xrn1 reduced glucose-induced mRNA decay. Therefore, Snf1p-dependent transcription and decay of glucose-specific mRNAs could be activated by triggering the cytoplasmic decay factors.

3.3. mRNA decay factors modulating transcription

Currently, two mRNA decay factors are proposed to regulate the transcription: Ccr4p/Pop2p/Not complex (deadenylase) and Xrn1p (exoribonuclease). Ccr4p/Pop2p/Not complex is deadenylase, catalyzing the initial deadenylation step of polyadenylated mRNAs prior to their decapping. Historically, Ccr4p, the major catalytic subunit, was initially discovered as an activator of transcription [65, 66], rather than deadenylase [67]. Other studies showed that Not proteins repress the transcription of TATA-less promoter [68, 69]. Furthermore, the Ccr4/Not complex was involved in transcription elongation by interacting with RNAPII [25, 70]. Although numerous studies indicate the bifunctional aspect of Ccr4p/Pop2p/Not complex in posttranscriptional regulation, no study, to our knowledge, has focused on the cross-talk between mRNA synthesis and degradation. To reveal the whole picture of the complex, further investigations are necessary.

Xrn1 targets cytoplasmic RNA substrates marked by a decapped 5′ monophosphate for further 5′-to′3′ degradation [71–73]. In 2013, two studies revealed the functional role of Xrn1p in the crosstalk between transcription and degradation. Haimovich et al. performed serial experiments that suggest the direct role of Xrn1 in transcription [25]. First, Xrn1p shuttled between the cytoplasm and the nucleus in a manner dependent on mRNA degradation. Second, GRO-seq data demonstrated that the densities of active Pol II are affected by deleting Xrn1p or by mutating its active site. A similar result was also confirmed by single-cell FISH. Third, the whole-genome-binding feature of Xrn1p showed that Xrn1p binds to promoters of genes of which transcription is highly affected by Xrn1p disruption, suggesting that promoter binding is a transcriptional function. Fourth, inhibition of Xrn1p accumulated transcriptionally incompetent Pol II at the nascent mRNAs. This result suggested that Xrn1p functions in transcription elongation. Therefore, the researchers concluded that Xrn1 is an essential factor for mRNA synthesis-degradation coupling, and referred to Xrn1p as "synthegradosome." The report published by Sun et al. showed that depletion of Xrn1p caused a global activation of mRNA transcription monitored by comparative dynamic transcriptome analysis (cDTA) [23]. They also searched for nuclear factors, which repress mRNA transcription by Xrn1, and identified transcription repressor Nrg1 as the downstream of Xrn1. Increase in mRNA degradation rates are compensated by an increase in mRNA transcription, suggesting that overall mRNA levels are "buffered". This study showed that Xrn1p was required for the RNA buffering. As summarized above, the two studies reached different conclusions regarding the

consequences of deleting or inactivating Xrn1p. From these results, we may conclude that Xrn1p is related to coupling mRNA synthesis and degradation; however, the mechanism of this interplay is still unresolved.

4. Direct measurements for transcription and degradation rates at the genome-wide level

The difficulty in studying the interplay between transcription and degradation is in measuring the kinetics of the processes, especially at the genome-wide level. Recent advances in RNA-seq technologies enable us to determine the rate of transcription and/or degradation.

4.1. BRIC-seq

RNA stabilities are measured by the decrease in RNA after inhibiting transcription [74–76]. However, transcription affects degradation rates, as discussed previously, which obscure the native half-lives of transcripts. Tani et al. developed an inhibitor-free method termed 5′ Bromo-uridine (BrU) Immunoprecipitation Chase-deep sequencing analysis (BRIC-seq) [77, 78]. BRIC-seq applies BrU for metabolic labeling of endogenous transcripts. After removing BrU from the medium, total RNAs are then isolated from the cells at sequential time points. BrUs-labeled RNAs are purified through immunopurification by using BrU antibody. The half-life of each transcript is calculated from the decreasing amount of BrU-RNA measured by RNA-seq (**Figure 5**).

4.2. GRO-seq, PRO-seq, NET-seq

Global Run-On sequencing (GRO-seq) was developed to measure transcription rate. GRO-seq maps the genome-wide positions, amounts and orientation of transcriptionally engaged RNAP [79, 80]. In GRO-seq, transcription is inhibited in living cells, and then reinitiated in isolated nuclei under conditions that allow labeling of nascent transcripts (nuclear run-on) with BrU. Capturing nascent transcripts from active RNAP provides a direct synthesis rate of the transcription. Similar to GRO-seq, precision nuclear run-on sequencing (PRO-seq) maps the location of active RNAP at base pair resolution [81]. PRO-seq uses biotin-labeled NTP (biotin-NTP) during the nuclear run-on procedure. Addition of only one of the four biotin NTPs restricts RNAP to incorporating a single or a few identical bases, resulting in sequence reads that have the same 3′ end base within each library. Native elongating transcript sequencing (NET-seq) can also obtain a nascent transcription profile with single-nucleotide resolution [82–84]. In NET-seq, nascent RNA was detected in the active site of RNAP by immunoprecipitation of FLAG-tagged RNAP.

4.3. 4sU-seq and TT-seq

Here we would like to introduce two methods that can determine the kinetics of both transcription and degradation. These two technologies will advance the study of the

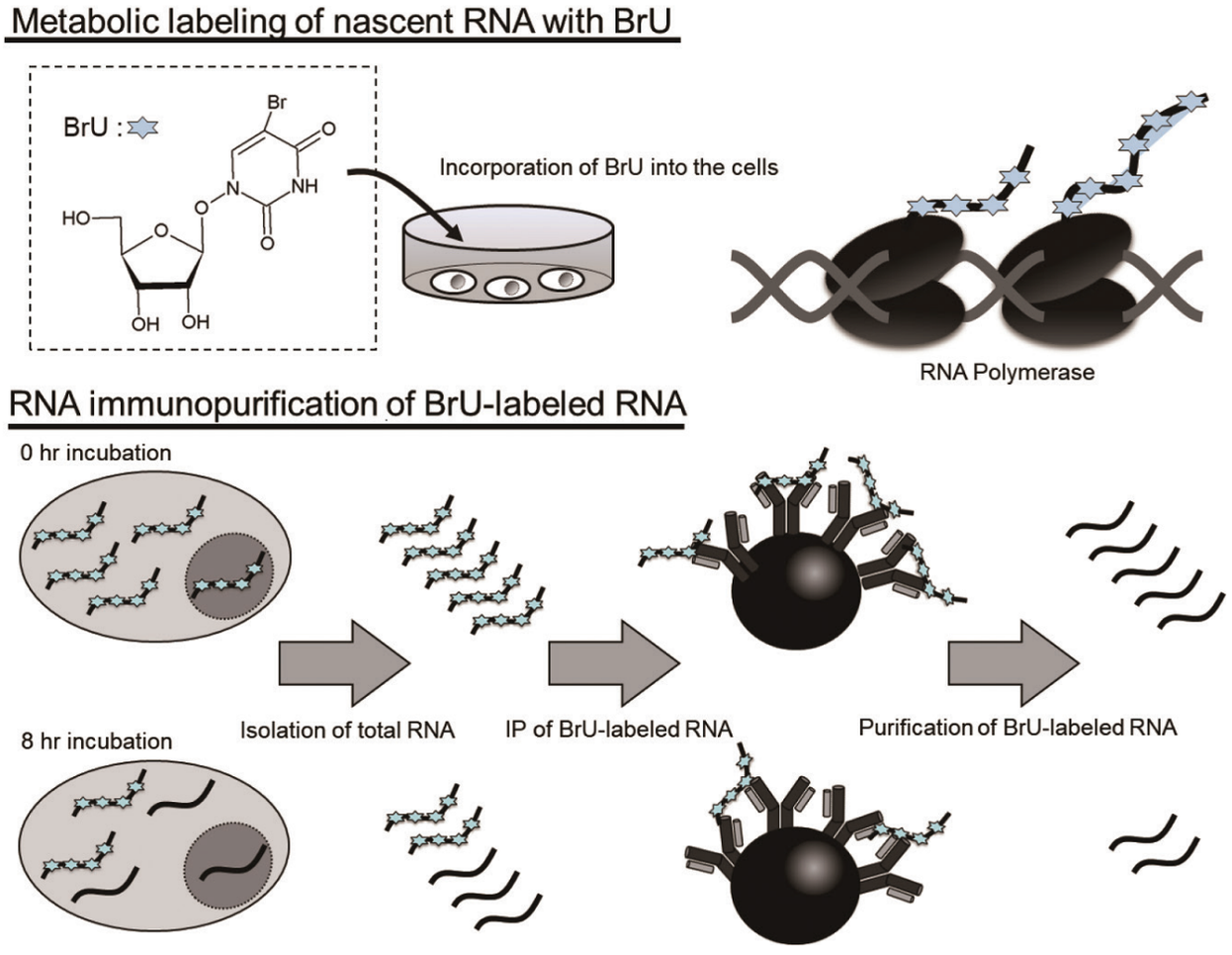


Figure 5. Overview of the BRIC-seq protocol.

interplay between transcription and degradation. Rabani et al. combined pulse labeling of mRNA with 4sU and computational modeling to estimate RNA transcription and degradation rates [85]. Newly transcribed RNA (4sU-labeled RNA) contains nascent RNA transcribed during the labeling pulse. When the labeling time is sufficiently short, the 4sU-labeled RNA is still in the nucleus, reflecting the average transcription rate. A computational model separates the RNA levels into transcription and degradation, and thus estimates the degradation rates from the experimental results of total RNA level and transcription rate.

The disadvantage of 4sU-seq is that it fails to map transcripts uniformly, because only a short 3′ region of nascent transcripts is labeled with 4sU, and long pre-existing 5′ regions dominate the RNA-seq data. To overcome this 5′ bias, transient transcriptome sequencing (TT-seq) fragments the 4sU-RNA before isolation. This fragmentation permits the immunoprecipitation of only newly transcribed 4sU-RNA fragments. Notably, TT-seq monitors RNA synthesis, whereas GRO-seq, PRO-seq, and NET-seq detect RNAs attached to RNAPs. Furthermore, TT-seq can determine transcription termination sites because TT-seq detected transient RNA downstream of the polyadenylation site.

5. Conclusion

The balance between mRNA transcription and decay determines the mRNA levels, which is a key aspect in the gene regulation. The study of interplay between transcription and decay is only the beginning. Our knowledge is still limited to the specific signaling pathway in yeast. As described in chapter 4, genome-wide analysis of transcription and decay will provide a comprehensive view of the interplay. Moreover, it will be critically important to verify the coupling of transcription and decay in mammalian system because mammalian cells contain numerous RBPs with defined roles in mRNA decay. It would be interesting to determine whether any of these RBPs also regulate transcription. It is a well-known fact that aberrant regulation of gene expression causes serious diseases. Therefore, studying the interplay between transcription and decay in mammalian cells will be beneficial for understanding diseases with defects in RNA expression levels.

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