Coupling mRNA Synthesis and Decay

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What has been will be again, what has been done will be done again; there is nothing new under the sun.

—Ecclesiastes 1:9 (New International Version)

Posttranscriptional regulation of gene expression has an important role in defining the phenotypic characteristics of an organism. Well-defined steps in mRNA metabolism that occur in the nucleus—capping, splicing, and polyadenylation—are mechanistically linked to the process of transcription. Recent evidence suggests another link between RNA polymerase II (Pol II) and a posttranscriptional process that occurs in the cytoplasm—mRNA decay. This conclusion appears to represent a conundrum. How could mRNA synthesis in the nucleus and mRNA decay in the cytoplasm be mechanistically linked? After a brief overview of mRNA processing, we will review the recent evidence for transcription-coupled mRNA decay and the possible involvement of Snf1, the *Saccharomyces cerevisiae* ortholog of AMP-activated protein kinase, in this process.

THE BEGINNING—mRNA PROCESSING IN THE NUCLEUS IS COUPLED TO TRANSCRIPTION

The primary RNA transcript in the nucleus of a eukaryotic cell undergoes multiple covalent modifications before it reaches the cytoplasmic compartment (Fig. 1). Several excellent in-depth reviews of mRNA processing and related topics have appeared recently, and the interested reader is referred to these reviews for detailed information (1–6). RNA polymerase II (Pol II) transcripts are modified at their 5' ends by the addition of a 7-methylguanosine (7-MeG) cap, at their 3' ends by polyadenylation, and internally by the removal of intronic sequences and in some cases by editing. What was not anticipated but is now well accepted is the fact that all of these processes occur cotranscriptionally. If they are completed successfully, nuclear export occurs (7–9), which is also linked to transcription (8).

The enzymes that cap the mRNA are recruited to the nascent transcript shortly after the 5' end emerges from the exit channel. Recruitment of the capping enzyme complex, containing RNA triphosphatase, guanylyltransferase, and the 7-methyltransferase activity, requires phosphorylation of Ser5 on the carboxyl-terminal domain (CTD) of Pol II by the transcription factor IIH (TFIIH)-associated kinase, Cdk7 (Kin28 in *Saccharomyces cerevisiae*), linking 5' capping to Pol II and transcription. Ser5 phosphorylation and the capping complex are lost as Pol II progresses down the gene (1, 10, 11).

mRNA splicing is coupled both to transcription and to nuclear export via recruitment of complexes involved in each process by elongating Pol II. Intron-associated sequences and the Ser2-phosphorylated form of the CTD are involved in elongating the transcript and recruiting the splicing machinery (reviewed in references 12 and 13). Because accurate and efficient splicing can be achieved *in vitro*, it was unexpected that the promoter can influence where splicing occurs in the nascent transcript (reviewed in reference 14). The simplest manner in which a promoter can influence splicing is for an alternative promoter to change the 5'-most exon and thus alter the pre-mRNA sequence and structure. However, alternative promoters of the same gene may influence where splicing occurs by recruiting transcription factors that have different activities, either by themselves recruiting different splic-

ing complexes or by influencing the rate of elongation, which can influence the location of alternative splice sites in the primary transcripts. Many of the components of the splicing and nuclear export machinery are conserved between the two evolutionarily divergent yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* and mammals (8, 15, 16), a testament to their importance even in *S. cerevisiae* where only about 5% of the genes contain introns (17). Despite the paucity of intron-containing genes in *S. cerevisiae*, they account for a disproportionate amount of total message and protein synthesis. Intron deletion from three essential genes in *S. cerevisiae* reduced transcript levels consistent with a mechanistic link between transcription and splicing that acts in both directions: transcription influences splicing, and splicing influences transcription (17). Thus, as in higher eukaryotes, there is an important coupling between splicing and transcription.

Cleavage downstream of the poly(A) addition site and poly(A) addition occurs by the concerted action of cleavage factors and the poly(A) polymerase and is mechanistically coupled to transcription termination (reviewed in reference 18). Cleavage and termination factor recruitment require Ser2 phosphorylation of the CTD (19, 20). A current model combines two former ideas of termination, invoking both a “torpedo”-like function of the nuclear exoribonuclease (Rat1 in the yeast *S. cerevisiae* and Xrn2 in mammals) and an allosteric change in the elongation complex that together cause transcription termination (21, 22). However, termination at genes transcribed by Pol II is complex, and the mechanism differs depending on several factors, including the presence or absence of a poly(A) tail, introns, and gene length (18).

THE END—mRNA DECAY IN THE CYTOPLASM

The major pathways of mRNA decay in the cytoplasm are illustrated in Fig. 2. Not shown is a surveillance pathway that ensures

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FIG 1 Cotranscriptional RNA processing in the nucleus. The capping enzymes are recruited once Pol II is phosphorylated at serine 5 in the carboxyl-terminal domain (CTD) during early elongation. The capping enzymes consist of RNA triphosphatase (RT), guanylyltransferase (GT), and 7-methyltransferase (MT) activities. As Pol II switches to productive elongation, the serine 5 phosphorylation disappears and serine 2 phosphorylation becomes dominant. The elongating Pol II complex recruits splicing enzymes, and the spliceosome removes the introns while transcription proceeds. The serine 2-phosphorylated CTD recruits the polyadenylation factors, including the RNA cleavage components and the poly(A) polymerase (PAP) to cleave the mRNA following the polyadenylation signal(s) (AAUAA) and add the poly(A) tail.

that only correctly processed mRNAs escape degradation (23, 24). Correctly processed mRNAs bearing a 7-MeG cap at their 5' end are recognized by eukaryotic initiation factor 4E (eIF4E), which recruits the mRNA to initiation factor eIF4F, forms a complex with the small 40S ribosome, initiator Met-tRNA and initiation factors to begin translation (reviewed in references 25 to 27). mRNAs not associated with polysomes may be associated with processing bodies (P-bodies) and stress granules, sites of degradation and storage (28). However, there is evidence that for some mRNAs, association with P-bodies is a consequence, not a cause of removal from the translation cycle (reviewed in reference 28). Degradation of the cytoplasmic mRNA is usually initiated by shortening of the poly(A) tail. In yeast, deadenylation is catalyzed primarily by either the Ccr4-Not or Pan2-Pan3 complex (reviewed in references 6 and 29). Following deadenylation, the mRNA can be degraded processively from the 3' end by the exosome (2), resulting in an oligonucleotide whose cap is removed by a salvage pathway catalyzed by Dcs1 in yeast (30). Poly(A) tail shortening also promotes decapping by the Dcp complex (1), aided by auxiliary factors in particular the DEAD box helicase Dhh1 (1, 31). This leads to mRNA decay by the major pathway in the cytoplasm that requires Xrn1, a 5'-3' exoribonuclease that contains an RNA binding and RNase catalytic domain in its N-terminal region (4). There are also deadenylation-independent pathways of mRNA degradation that occur as part of quality control mechanisms, and these pathways do not require deadenylation (23, 32).

mRNA decay may be tightly coupled to a transcriptional program that is regulated by nutritional or stress conditions (9, 33, 34). Together, the transcriptional and posttranscriptional re-

FIG 2 mRNA decay pathways in the cytoplasm. There are two major cytoplasmic pathways to degrade mRNA that both start with the shortening of the poly(A) tail by one of the deadenylase complexes (Ccr4-Not or Pan2/3). The exosome can degrade the deadenylated mRNA in the 3'-to-5' direction, and the scavenger decapping enzyme, Dcs1, degrades the remaining cap. The most prominent pathway involves decapping by Dcp1 and Dcp2 and subsequent degradation by the 5'-to-3' exonuclease, Xrn1.

sponses allow a more rapid adaptation to new environmental conditions than would either mechanism acting alone. Regulation of mRNA decay is frequently mediated by sequence-specific RNA binding proteins (RBPs). Advances in recognizing RNA binding motifs in proteins, together with identification of specific RNA binding proteins has led to the understanding that mRNA decay could be a specific, highly regulated process (35–39). The current mRNA decay paradigm involves gene- or class-specific RBPs that bind sequences in either the 5' untranslated region (5' UTR), open reading frame (ORF), or more commonly the 3' UTR. The multitude of RBPs identified in the *S. cerevisiae* genome (38, 39) suggests that posttranscriptional regulation by RBPs is as ubiquitous as transcriptional control by DNA binding transcription factors.

Technical advances, both molecular and bioinformatic, have led to a dramatic increase in our knowledge of the nature of RBPs and the extent of their utilization to modulate gene expression at the posttranscriptional level. Several recent studies have identified RBP binding sites on a transcriptome-wide scale in *S. cerevisiae* (39, 40). The take-home message is that most yeast messages contain binding sites for RBPs, many RBPs bind multiple, functionally related mRNAs, and their binding can be dependent on conditions. These results suggest that posttranscriptional control of gene expression utilizes complex combinatorial inputs.

How important is this mode of regulation in yeast? A prominent family of RNA binding proteins in *S. cerevisiae* is comprised of five “Puf” proteins (*Drosophila* Pumilio (Pum) and *C. elegans* EBF). A deletion mutant lacking all five Puf family proteins (Puf1, Puf2, Puf3, Puf4, and Puf5) is viable (41) and showed differential regulation of 7% to 8% of all mRNAs. Mutants lacking individual PUF genes showed condition-dependent effects, and the targets of several Puf proteins were shown to belong to related gene families (37). Puf3, for example, has a role in regulating the synthesis of proteins destined for the mitochondrion. The observation that most mRNAs contain binding sites for more than one RBP sug-

gests that functional redundancy might explain why some RBP deletion mutants have subtle phenotypes.

Another example of an RBP with a defined physiological role in modulating gene expression is Meu5, an RBP that is involved in meiosis and sporulation in *Schizosaccharomyces pombe* (42). Meu5 binds to and stabilizes one class of sporulation-specific transcripts. Sporulation-specific transcription occurs normally in the absence of Meu5, but reduced transcript levels, due to enhanced decay, lead to reduced protein levels and defective spore formation. This process is an elegant example of a feed-forward regulatory loop involving activation of the *MEU5* gene by the sporulation-specific transcription factor Mei4, which leads to binding and stabilization of about 80 sporulation-specific transcripts by Meu5. There are numerous examples of mammalian RBPs with a role in regulating mRNA decay (43–47), implicating this process as a conserved mechanism of posttranscriptional gene regulation.

Surprisingly, RBPs can also influence transcription. TAT (*trans* activator of transcription) is a small RNA binding protein encoded by HIV. TAT is produced after HIV infection and activates transcription of the HIV genome by binding to a site (*trans*-activation response element [TAR]) in the 5' region of viral transcripts. TAT helps recruit the positive transcription elongation factor b (P-TEFb), a cyclin-dependent kinase that stimulates transcription elongation by phosphorylating the Pol II CTD. P-TEFb inhibits two negative elongation factors, NELF (negative elongation factor) and DSIF (DRB sensitivity-inducing factor [DRB stands for 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole]), that cause pausing. Phosphorylation of DSIF by P-TEFb converts it into a positive elongation factor that stimulates productive transcription from the viral genome (reviewed in reference 48). Thus, TAT is an antipausing factor. Whether cellular RBPs can play a similar role is unknown.

LINKING THE BEGINNING AND THE END—COUPLING TRANSCRIPTION TO mRNA DECAY

Promoter-regulated mRNA stability. The nuclear events in RNA processing are accomplished by proteins that bind the nascent mRNA in concert with transcription. Recent studies suggest that mRNA decay in the cytoplasm may also be coupled to transcription (reviewed in references 3 and 49). Recent observations using budding yeast provide strong evidence that promoter sequences, and even the act of transcription itself, can be coupled to mRNA decay. When transcription factor (TF) binding sites in the regulatory region of a yeast gene were replaced by those from another gene, mRNA decay kinetics were altered in a manner reflecting the gene from which the TF binding sites had been obtained, suggesting that promoter sequences could influence mRNA decay (50). An extension of these studies involved promoter swapping of multiple genes from two related yeast species (51). Analysis of the hybrid genes indicated that both transcription and mRNA decay were affected. Analysis of mRNA decay rates between different cell types and between different mammalian species suggested that a similar relationship between transcription and mRNA decay could occur in humans (51). In conclusion, gene sequences outside the body of the transcript have been shown in several studies to influence the rate of decay of mRNA in the cytoplasm.

A second example is the cell cycle-regulated decay of transcripts of the *CLB2* and *SWI5* in *S. cerevisiae*. The coordinated decay of these transcripts is dependent on their promoter sequences and is regulated by two paralogous mitotic exit network

protein kinases, Dbf2 and Dbf20 (52). Using chromatin immunoprecipitation (ChIP) and RNA immunoprecipitation (RIP), Dbf2 was found to interact with both the promoter and message for *CLB2* and *SWI5*, suggesting that it is recruited to the promoter and then subsequently deposited on the mRNA as it is transcribed. Dbf2 is known to interact with the multifunctional Ccr4-Not complex, linking it to both transcription and mRNA decay (53). Whether Dbf2 is actively recruited to the *CLB2* promoter by a transcription factor or is present by some other mechanism is unknown.

A third example is suggested by observations made in the *GAL* system in yeast. The addition of galactose to cells growing on raffinose or a nonfermentable carbon source induces a rapid transcriptional response that enables metabolism of galactose. Readition of glucose causes a rapid shutdown of transcription that is accompanied by rapid degradation of the induced transcripts, an example of glucose-induced mRNA decay (54–57). However, if the promoter of the *GAL7* gene is replaced by the constitutive *ADH1* promoter, the *GAL7* transcripts escape glucose-induced decay (33). The authors attributed the enhanced stability of the *ADH1*-promoted transcripts to the absence of a transition from one carbon source to another. Another interpretation would attribute the enhanced stability of the *GAL7* transcripts synthesized in glucose-containing medium to the *ADH1* promoter. The *ADH1* promoter contains binding sites for Rap1, a TF that was shown to enhance stability of transcripts made under its control (50). Thus, the promoter, and by implication, the TF, not the altered nutritional conditions, might be the causal factor for enhanced mRNA stability.

These three examples are analogous to swapping promoters of the globin gene reported in 1993 (58). These studies showed that the stability of the mRNAs made from the globin promoter were less stable than those synthesized when a viral promoter was fused to the globin genomic sequences. Because genomic sequences of the globin gene were used, production of the mature mRNA required splicing, and because splicing can be influenced by promoter sequences as described above, one could attribute the different amounts of mature globin mRNA to different efficiencies of splicing. However, the yeast genes studied do not contain introns, so coupling to the splicing machinery cannot be invoked to explain how sequences outside the body of the mature transcript could influence mRNA decay.

Transcription-regulated mRNA stability. There is increasing evidence that the transcriptional machinery can influence mRNA decay rates. The first evidence came from studies of two subunits of RNA Pol II, Rpb4 and Rpb7. The dissociable Rpb4/7 heterodimer forms the stalk of Pol II, just below the RNA exit channel. Several reports suggested that Rpb4/7 binds to nascent mRNAs as they emerge from the polymerase and accompanies them to the cytoplasm where it influences their decay rate (59–61). The effect is greatest for mRNAs involved in protein synthesis where deletion of *RBP4* leads to increased half-lives of these mRNAs. Both Rbp4 and Rbp7 appear to promote deadenylation and thus effect degradation by both the Xrn1- and exosome-dependent pathways (60, 61). Rbp4 and Rbp7 interact with two P-body factors that promote mRNA decay, Pat1 and the Lsm1 to Lsm7 complex, and loss of *RPB4* results in increased P-body formation similar to other decay factors (60, 61). There is conflicting evidence regarding the relative levels of Rbp4/7 compared to the other core subunits of Pol II (62, 63). Therefore, it is unclear

whether there is enough free Rpb4/7 for it to fulfill its essential function in transcription if it is also bound to a large number of nascent mRNAs. Although ChIP studies show that Rbp4/7 is bound to chromatin in the same regions as a core Pol II subunit, Rbp3 (64), there is some evidence that Rbp4/7 dissociates from the Pol II complex during elongation (63). A recent study investigated a possible cytoplasmic role of Rpb4 in a novel manner (65). These investigators created a yeast strain in which the only form of Rpb4 was expressed as a fusion protein with Rpb2, a core subunit of Pol II. The yeast strain expressing this fusion protein had a phenotype remarkably similar to that of a strain with wild-type *RPB4*, restoring mRNA synthesis and decay to normal levels, suggesting that the major role of Rpb4 was performed in the nucleus as a component of Pol II. Although it is possible that a small amount of Rpb4 was present in the cytoplasm and was sufficient to perform a cytoplasmic function in mRNA decay, these studies favor an indirect role of Rpb4 in cytoplasmic mRNA decay.

Two other components of the Pol II transcription machinery have also been implicated in regulating mRNA stability. One of these, yeast Cdk8 (also known as Srb10, Ssn3, Ume5, Nut7, Gig2, Rye5, and Ssx7), is a cyclin-dependent protein kinase (CDK) associated with Mediator, the coactivator recruited by transcription factors to recruit Pol II to promoters. Cdk8 was reported to decrease the stability of meiotic transcripts made in nutrient-rich conditions (66) and was necessary for glucose-induced mRNA decay (56, 67). Recent evidence links Cdk8-dependent phosphorylation of the CTD to a step of transcription elongation (68). The other Pol II-associated component implicated in mRNA decay is the TFIIF-associated Mat1, a component of the CDK that phosphorylates Ser5 of the CTD (39). Defective Pol II transcription due to homozygous deletion of Mat1 was not accompanied by a reduction in steady-state mRNA levels as expected, a result the authors attributed to widespread stabilization of mRNAs (69).

The apparent alterations in mRNA decay caused by loss of either Rpb4/7 or a CTD kinase could be due to indirect consequences of the mutations as suggested by recent studies of an Rpb4-Rpb2 fusion protein (65). The correlations observed between decreased transcription and corresponding changes in mRNA decay rates need to be confirmed by experiments that demonstrate causality. For example, in the case of Rpb4/7, by demonstrating that when binding of Rpb4 to a particular mRNA is eliminated, the influence of Rpb4/7 on its stability is also eliminated without affecting the decay of other mRNAs. In the case of the CTD kinases, an effect on mRNA stability could be an indirect consequence of defective capping or another nuclear mRNA processing step.

Despite these caveats, the evidence obtained by altering the transcriptional machinery, either promoters or Pol II-associated factors, strongly suggests that there is a mechanistic link that couples mRNA synthesis and decay. There is also evidence obtained using the converse approach, mutating the RNA decay machinery and observing an effect on transcription rates. The first such evidence was obtained in *Escherichia coli*. This report showed that inactivation of RNase III or RNase E reduced mRNA degradation rates but was not accompanied by increased mRNA levels as expected. This led the authors to conclude that reduced rates of decay were balanced by an unknown mechanism that led to reduced synthesis rates (70). This interesting observation was apparently not confirmed in prokaryotes, but recent studies performed in budding yeast suggest that mRNA decay mechanisms

can have an influence on mRNA synthesis rates as described below.

Xrn1 buffers mRNA levels. An interdependence of mRNA synthesis and decay rates in *S. cerevisiae* on a genome-wide scale was evident in two recent reports. Both reports studied the consequences of loss of components of the RNA degradation machinery (71, 72) and concluded that only one of them, Xrn1, was instrumental in linking mRNA synthesis and decay rates such that decreases in one could be balanced by appropriate changes in the other. The end result was a relatively constant amount of total cytoplasmic mRNA; hence, mRNA levels were said to be “buffered.”

Xrn1 of yeast is a large, nonessential, highly conserved cytoplasmic 5'→3' exoribonuclease whose well-defined biochemical function is to degrade mRNA containing a free 5' monophosphate end produced by either deadenylation and decapping or by endoribonucleolytic cleavage (reviewed in reference 4). *XRN1* mutants that were isolated in genetic screens in yeast have a wide array of phenotypes, including sensitivity to Li²⁺ ions (73), and defects in genetic recombination, meiosis, and telomere maintenance (reviewed in reference 74). Deletion of *XRN1* or mutation of conserved residues in its nuclease domain leads to stabilization of mRNAs on a global scale, consistent with its role in the major cytoplasmic mRNA decay pathway (71, 72, 75). Whether its role in other processes such as nuclear fusion and spindle pole body duplication (76) is a consequence of its role in mRNA degradation is unknown. In *S. cerevisiae*, Xrn1 has an essential paralog, Rat1, which functions in RNA degradation in the nucleus (77). Rat1 also functions in termination of transcription and in mRNA cap surveillance with a binding partner, Rai1 (21, 22, 76). Xrn1 and Rat1 are highly conserved in the N-terminal nuclease domain. Overexpression of the nonconserved C-terminal region of Xrn1 leads to a reduced growth rate, suggesting that it may serve a regulatory role (78). This region interacts with the decapping enzyme, Dcp1, in *Drosophila melanogaster* and the decapping activator, Edc4, in humans (4). The nonessential yeast RNA helicase Dhh1 is the major activator of the decapping enzyme (79) and is thus an indirect activator of Xrn1. The yeast scavenger decapping enzyme, Dcs1, is also an activator of Xrn1 (80). If Rat1 is directed to the cytoplasm by deletion of its nuclear localization signal (NLS), it can complement an *xrn1Δ* mutation for growth, mRNA decay, benomyl sensitivity, sporulation, and lethality with *ski2Δ* (an RNA helicase). Conversely, if Xrn1 is directed to the nucleus by appending an NLS, it can complement the lethality of a *rat1-1* temperature-sensitive mutation. Thus, Xrn1 and Rat1 are interchangeable in function when localized to the correct compartment of the cell (81).

Xrn1 also has a role in stabilizing antisense transcripts in *S. cerevisiae* (75). Deletion of *XRN1* or inactivating an *xrn1-ts* allele led to accumulation of a novel class of antisense, noncoding (ncRNA) transcripts called XUTs (Xrn1-sensitive unstable transcripts) primarily (66% in *xrn1Δ* strain) from within the coding region of genes. The enhanced level of XUTs was associated with reduced gene-associated Pol II levels and reduced SET1-dependent trimethylated histone H3 lysine 4 (H3K4) consistent with gene silencing in the ORF of the genomic region from which the XUT originated. Similarly, in the nucleus, Dcp2 and Rat1 are involved in degradation of large ncRNAs (82) that have been shown to regulate transcription and posttranscriptional processes.

The role of Xrn1 in mRNA synthesis is unresolved and controversial. The study by Haimovich et al. (71) attributed a stimula-

tory role of Xrn1 in transcription to a direct effect on gene activity. Using nuclear run-on assays, they demonstrated a genome-wide reduction in Pol II activity in a strain in which *XRN1* had been deleted or bore a mutation eliminating its nuclease activity. In addition, fluorescence *in situ* hybridization (FISH) analysis showed a reduced number of transcripts per affected gene as a result of the loss of *XRN1*, which is consistent with the reduced Pol II occupancy seen in the XUT analysis (71). Consistent with a direct effect on transcription, evidence was presented that Xrn1, as well as several other proteins involved in cytoplasmic mRNA degradation, was present upstream of the transcription start sites of the genes most affected by the loss of *XRN1*. This was unexpected because previous studies had shown that $\geq 90\%$ of Xrn1 was cytoplasmic (83).

The evidence that Xrn1 is also present in the nucleus is somewhat perplexing because Xrn1 and Rat1 are functionally interchangeable when directed to the correct compartment, yet Rat1 is essential. This suggests that Xrn1 is not present in high enough concentrations in the nucleus to complement the loss of Rat1. The second complication is that Rat1 is able to complement the loss of Xrn1 function when targeted to the cytoplasm, yet Rat1 is not able to modulate transcription in the nucleus in the absence of *XRN1*. One hypothesis to explain this dilemma is that a cytoplasmic 5'-3' exonuclease, either Xrn1 or Rat1 Δ NLS, is important for the nuclear import of other mRNA decay factors, and these other factors are required for transcriptional activation. In summary, these studies suggested that Xrn1 positively affects transcription on a genome-wide scale and confirmed that effect for two inducible systems, *GAL* and heat shock.

In contrast, the research by Sun et al. (72) described a negative effect of Xrn1 on global transcription rates. Deletion of *XRN1* led to a decreased rate of global mRNA decay and an increased global transcription rate resulting in a 3.2-fold increase in global mRNA abundance. Deletion of *XRN1* was unique among 40 components of mRNA processing pathways in leading to an imbalance between mRNA transcription and decay. Thus, they proposed that Xrn1 plays a unique and pivotal role in balancing mRNA synthesis and decay in *S. cerevisiae*. They attributed the negative transcriptional role of Xrn1 to an indirect effect acting through the transcriptional repressor encoded by *NRG1* whose transcript levels were Xrn1 dependent. Consistent with this idea, they demonstrated a small but significant increase in global transcription rates in an *nrg1* Δ strain. However, in the absence of *XRN1*, the synthesis rate of *NRG1* transcripts increased, an effect that should have led to a decrease in global transcription but instead resulted in an increase.

Although Sun et al. (72) did not localize Xrn1 to genomic regions and they were unable to demonstrate a defect on *in vitro* transcription using *xrn1* Δ nuclear extracts, they provided evidence that altered transcription of *xrn1* Δ required that it be nuclear by using the "anchor-away" technology (84). However, the Xrn1 exonuclease activity in the cytoplasm was increased in the experiment, an effect they attributed to its exclusion from the nucleus. The increased cytoplasmic Xrn1 nuclease activity could complicate interpretation of the experiment, as could the use of a *tor1* mutant strain, which was employed to avoid rapamycin-induced inhibition of Tor1 functions. In contrast to the *XRN1* deletion and catalytic mutant, the exclusion of Xrn1 fusion protein from the nucleus had no effect on the synthesis rate of *NRG1* transcripts, suggesting that Xrn1 does not have a direct role in modulating *NRG1* mRNA levels.

FIG 3 Circular regulation of transcription and mRNA decay. (A) The first model, the model of Haimovich et al. (71), links transcription and decay together by the mRNA decay factors (DF) that shuttle between the cytoplasm and nucleus to regulate both processes. (B) The second model, the model of Sun et al. (72), shows that increased synthesis rates (SR) lead to increased levels of *XRN1* mRNA, resulting in an increase in decay rates (DR). Increased levels of Xrn1 protein promote the higher synthesis rates inhibiting the induction of a global transcription repressor (Nrg1?). (C) The third model, the model of Braun et al. (94), shows Snf1 activating Xrn1, which leads to transcription of glucose-repressed genes and stabilization of the mRNA and subsequent glucose-induced decay.

Thus, the two studies arrived at different conclusions regarding the consequences of deleting or inactivating *XRN1* as summarized in Fig. 3. In the work by Haimovich et al. (71), deletion of *XRN1*, or inactivation of its exonuclease activity, led to reduced transcription rates as determined by nuclear run-on assays and by induction of gene expression. Sun et al. (72), in contrast, concluded from their studies that deletion of *XRN1* led to globally enhanced mRNA synthesis rates as measured by incorporation of 4-thiouracil. Despite these apparent contradictions, both reports concluded that mRNA levels could be "buffered" from changes in either synthesis or decay by compensatory alterations in the other pathway. Sun et al. (72) attributed the discrepancy between the effects of *XRN1* deletion in the two studies to different methods of measuring mRNA synthesis rates, 4-thiouracil incorporation versus ChIP of Pol II. However, this interpretation does not account for the reduced levels of induction of *GAL* and heat shock gene expression, the reduced number of transcripts per gene determined by FISH, or reduced rates of mRNA synthesis determined by nuclear run-on assays (71).

Snf1 and transcription-coupled mRNA decay. If balancing mRNA synthesis and decay is an important function for the cell, one might expect that a strain lacking *XRN1* would lose viability or have a severe growth defect. Instead, lack of *XRN1* causes only a 2-fold reduction in growth rate in complete medium (85). How-

FIG 4 mRNA-associated pathways targeted by Snf1. The black shapes represent Snf1 targets identified in a phosphoproteomic study (94). UAS, upstream activating sequence; ADA, Ada2/Gcn5/Ada3 transcription activator complex; SAGA, Spt-Ada-Gcn5-acetyltransferase; SLIK, SAGA-like complex; mRNP, messenger ribonucleoprotein particle.

ever, Xrn1 gains in importance under conditions of stress, particularly glucose starvation or the presence of toxic Li^{2+} concentrations (75, 80, 86). Environmental stress leads to major alterations in mRNA levels as the genes that respond to the adverse conditions are up- or downregulated (87). The yeast ortholog of AMP-activated protein kinase, Snf1, plays an important role in multiple stress conditions, including carbon (88) and nitrogen limitation (73), toxic cations (89), heat stress (88), high salinity and alkaline pH (90), and hydroxyurea (87). Snf1 is regulated positively by three upstream kinases (88) and negatively by protein phosphatases that dephosphorylate T210 in the activation loop. The major protein phosphatase that inactivates Snf1 is Glc7 in combination with a regulatory subunit, Reg1 (91–93). Snf1 has both a direct role in stress response by substrate-level phosphorylation (94, 95) and an indirect role due to its importance in transcriptional control of gene expression (96). Snf1 also has a role in posttranscriptional regulation of gene expression. When an analog-sensitive allele of Snf1 is inhibited during glucose depletion, *SNF1*-dependent transcripts are as rapidly degraded as in glucose-induced mRNA decay (94). Conversely, in a *reg1* mutant, in which Snf1 is constitutively active, the same transcripts are protected from glucose-induced mRNA decay (56, 94).

To identify Snf1 targets that participate in posttranscriptional

gene regulation, a global phosphoproteomic study was undertaken (94). Snf1-dependent phosphorylation of about 150 proteins was observed, many of which were phosphorylated uniquely after glucose depletion, conditions in which Snf1 is maximally active. Proteins involved in mRNA metabolism constituted a prominent category of Snf1-dependent phosphorylated proteins (Ccr4, Cdc73, Dcs2, Dhh1, Leo1, Mpt5/Puf5, Nab6, Ngr1, Pbp1, Puf3, Scp160, Whi3, and Xrn1) (Fig. 4). Three Snf1 targets, Ccr4, Dhh1, and Xrn1, participate in glucose-induced mRNA decay as shown by the enhanced stability of Snf1-dependent transcripts when any one of these three genes was deleted. Two Puf proteins were targets, Puf3 as discussed above and Puf5/Mpt5, an RBP that is involved in mating type switching. Puf5/Mpt5 promotes mRNA degradation by recruiting the Ccr4-Not complex along with the helicase Dhh1 and the decapping enzyme Dcp1 to promote deadenylation, decapping, and decay (97–99). Puf5/Mpt5 also interacts with the translational repressor Caf20 (100). Several other Snf1 targets (Ngr1, Nab6, Scp160, and Whi3) have annotations suggesting that they may participate in gene-specific pathways. Thus, Snf1 may have a global role in posttranscriptional gene regulation.

There is both genetic and biochemical evidence for a role of *SNF1* in mRNA buffering through its interaction with *XRN1*. First, syn-

thetic lethality was observed between *XRN1* and *REG1* (94). The synthetic lethality of the *xrn1 reg1* double mutant required Snf1, suggesting that Xrn1 is necessary to counteract what would otherwise be a deleterious consequence of activating Snf1. Such a function might explain the enhanced importance of Xrn1 under conditions of nutrient- or cation-induced stress that activates Snf1 (101). Surprisingly, mRNA accumulation of Snf1-dependent transcripts was dramatically reduced when *XRN1* was deleted or was exonuclease deficient, whereas an increase of mRNA abundance would be expected because Snf1-dependent transcripts were stabilized by the absence of *XRN1*. These results suggest that mRNA synthesis is compromised by deletion or mutation of *XRN1*, in agreement with the data from the Choder lab (71) and in apparent disagreement with data from the Cramer lab (72). A direct causal link between Snf1 and Xrn1 activity was suggested by the phenotype of an *XRN1* mutant lacking the Snf1-dependent phosphorylation sites in its C-terminal region. Specifically, a strain in which three Snf1-dependent phosphorylation sites in Xrn1 were mutated to Ala showed reduced rates of decay of Snf1-dependent transcripts, whereas a strain in which the sites had been mutated to Asp behaved similarly to the wild type (94). Thus, Snf1-dependent phosphorylation of Xrn1 appears to be important for its exoribonuclease activity during glucose-induced mRNA decay. The importance of Snf1-dependent phosphorylation of Xrn1 on other transcripts and on its ability to balance mRNA synthesis and decay during vegetative growth need to be assessed.

Another connection between transcription-coupled mRNA decay, Xrn1, and Snf1 is the observation that in the absence of *XRN1*, the expression of the Snf1-interacting repressor, *NRG1*, is reduced 5-fold (72). Nrg1 and its paralog Nrg2 are zinc finger DNA binding proteins that function as transcriptional repressors by recruiting the Ssn6/Tup1 (also known as Cyc8/Tup1) corepressor complex to repress *SNF1*-dependent gene expression (102–105). Nrg1 was implicated in mRNA balancing by Sun and coworkers (72). Nrg1 and Nrg2 interact with Snf1 (106) and are phosphorylated by a specific isoform of casein kinase in a stress-dependent manner (107). A recent report suggests that Puf5/Mpt5 regulates the stability of *NRG1* mRNA by binding in its 3' UTR and recruiting Dhh1 and the Ccr4-Not complex to inhibit its translation and to induce its decay (108). Dhh1 and Puf5/Mpt5 were identified as the substrates or downstream effectors of Snf1, and Ccr4 was dephosphorylated in a Snf1-dependent manner (94). Thus, there are multiple connections between Snf1 and transcription-coupled mRNA decay. In summary, the results suggest that Snf1 might have an important influence on mRNA buffering when yeast cells are shifted from a fermentable to a respiratory, non-fermentable carbon source. For example, Snf1 might phosphorylate, directly or indirectly, an RBP and/or enzymes involved in coupling mRNA synthesis and decay (Fig. 3C). Xrn1 might represent an example of a Snf1-affected enzyme involved in mRNA synthesis and decay, but there is a need for Snf1 to influence the process in a specific manner. The binding or activity of one of the RBPs identified in the phosphoproteomic analysis might be regulated by *SNF1*-dependent phosphorylation to influence the synthesis and decay specifically of *SNF1*-dependent transcripts.

CONCLUSIONS

Does our current understanding of mRNA processing provide any insight that might explain mechanistic coupling of mRNA synthesis and decay? The steps in mRNA processing that occur in the nucleus—capping, splicing, polyadenylation, and nuclear degradation—have been shown to be coupled to transcription by re-

cruitment of protein complexes that are associated with a specific form of the CTD. The coupling achieves several important functions. It provides a mechanism that allows quality control so that incorrectly or unprocessed mRNAs do not enter the cytoplasm. In addition, coupling can provide a kinetic boost to a series of processes that can occur independently but would do so inefficiently or slowly if not linked in time and space. Coupling transcription to mRNA decay might confer similar advantages by balancing the two processes to maintain mRNA homeostasis.

Viewed as another step in mRNA processing, cytoplasmic mRNA decay might be linked to transcription using mechanisms analogous to those employed in nuclear pre-mRNA processing. As was suggested by work by Dahan and Choder (49), an RBP or complex that was recruited in a promoter-dependent manner could remain associated as an RNA-protein complex after the RNA was exported from the nucleus and could regulate its decay in the cytoplasm. Recruitment could occur in concert with a particular form of the CTD, a specific transcription factor, sequences in the mRNA, or by some combination of these processes. However, this model does not explain how an RBP could regulate mRNA synthesis as well as decay.

The HIV TAT protein provides a well-studied precedent for an RNA binding protein that associates with a nascent RNA and influences transcription. TAT has been reported to influence both initiation and elongation (reviewed in reference 48). TAT enhances transcription elongation by recruiting an antipassing factor, P-TEFb, in mammalian cells. Although there is no yeast homolog of P-TEFb, other factors involved in transcription elongation might be the target of a hypothetical TAT analog. To balance the rates of synthesis and decay, the activity of the hypothetical mRNA binding protein would have to be sensitive to the rate of transcription initiation or elongation and be able to influence both synthesis and decay. Thus far, no proteins or protein complexes that have both of these properties have been identified. Xrn1 has been proposed to play this role in *S. cerevisiae*, but the evidence regarding whether it does so by directly influencing transcription is controversial (71, 72). The role of Snf1 and the proposed role of Nrg1 in these processes need to be examined to understand how they participate in transcription-coupled mRNA decay.

Research in this area would benefit from the input of other investigators using model organisms in addition to budding yeast to test the generality of transcription-coupled mRNA decay. Mammalian systems in particular are well poised to contribute in a substantial fashion because numerous RBPs with defined roles in mRNA decay have been identified. It would be interesting to determine whether any of these RBPs also influence transcription. It is of paramount interest to determine whether the metazoan homologs of *S. cerevisiae* Xrn1 are involved in buffering mRNA levels by balancing rates of mRNA synthesis and decay.

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