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Review

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The Many Pathways of RNA Degradation

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From the earliest comparisons of RNA production with steady-state levels, it has been clear that cells transcribe more RNA than they accumulate, implying the existence of active RNA degradation systems. In general, RNA is degraded at the end of its useful life, which is long for a ribosomal RNA but very short for excised introns or spacer fragments, and is closely regulated for most mRNA species. RNA molecules with defects in processing, folding, or assembly with proteins are identified and rapidly degraded by the surveillance machinery. Because RNA degradation is ubiquitous in all cells, it is clear that it must be carefully controlled to accurately recognize target RNAs. How this is achieved is perhaps the most pressing question in the field.

RNA Degradation—Conserved Basic Features

In all organisms tested from all kingdoms of life, RNA degradation is a prevalent activity. Overall, the emerging picture is that despite the immense complexity of specific RNA degradation pathways, there are substantial similarities in the basics of RNA degradation between bacteria, archaea, and eukaryotes, underlining its major, and long-standing, importance.

There are three major classes of intracellular RNA-degrading enzymes (ribonucleases or RNases): endonucleases that cut RNA internally, 5′ exonucleases that hydrolyze RNA from the 5′ end, and 3′ exonucleases that degrade RNA from the 3′ end. Endo and 3′ exonucleases have long been characterized in all domains of life, whereas 5′ exonucleases were, until recently, believed to be absent from bacteria (de la Sierra-Gallay et al., 2008; Mathy et al., 2007).

Most genomes encode a plethora of RNases, often with overlapping activities, making redundancy a general feature of RNA degradation systems. With some important exceptions, mutation of a single RNA degradation enzyme does not generally result in a complete block to RNA degradation in either eukaryotes or bacteria. This indicates that multiple enzymes are able to recognize the same target RNAs. This redundancy presumably enhances the overall efficiency and robustness of degradation pathways.

Many of the enzymes and cofactors involved in RNA processing and degradation are multifunctional. In yeast, for example, both the 5′ exonuclease Rat1 and the 3′ exonucleases of the exosome complex not only target and degrade RNAs transcribed by RNA polymerases I, II, and III but also function in RNA-processing reactions that generate the mature termini of stable RNA species. Similarly, in bacteria the same factors participate in RNA maturation and in the degradation of both stable RNAs and messenger RNAs (mRNAs) (Deutscher, 2006). Such dual functions require that a single enzyme can precisely process some RNA species to generate defined ends while retaining the capacity to degrade other RNAs entirely—even the same RNAs under different circumstances.

This multiplicity of function that characterizes ribonucleases in both bacteria and eukaryotes underlines the key importance of mechanisms that specifically identify and target aberrant RNAs and RNA-protein complexes. This specificity is frequently conferred by cofactors, of which many have been identified.

Cofactors for RNA Degradation

Helicases

The ATP-dependent RNA helicases are a large protein family that participates in almost all pathways of RNA processing and degradation. The eukaryotic exosome complex and the bacterial degradosome each exhibit both 3′ exonuclease and endonuclease activity and function together with helicase family members: Mtr4 and Ski2 with the exosome, and RhlB with the degradosome (see Bernstein et al., 2008; Chandran et al., 2007; Lebreton et al., 2008; Schaeffer et al., 2008; Schneider et al., 2009; Taghbalout and Rothfield, 2008). RNA helicases can undergo large-scale movement upon ATP binding and hydrolysis and can translocate along nucleic acids, potentially unwinding secondary structure or displacing bound proteins and/or RNA. Alternatively they might act as "place markers" remaining temporarily fixed in position while signaling to, or directly recruiting, the degradation machinery (reviewed in Cordin et al., 2006; Rajkowitsch et al., 2008).

Polymerases

All exonucleases have problems initiating degradation close to stable stem structures (see Deutscher, 2006 and references therein), and the use of polymerases to add a single-stranded "landing pad" for 3′ exonucleases is therefore likely to be an ancient mechanism. In eukaryotes the TRAMP polyadenylation complexes act as major cofactors for the exosome complex in both the budding yeast *Saccharomyces cerevisiae* (reviewed in Houseley et al., 2006) and the fission yeast *Schizosaccharomyces pombe* (Buhler et al., 2008). The TRAMP complexes contain a poly(A) polymerase (Trf4 or Trf5 in budding yeast—Cid14 in *S. pombe*), a zinc-knuckle putative RNA-binding protein (Air1 or Air2 in budding yeast), and an RNA helicase (Mtr4 in budding yeast). Defective nuclear RNAs are tagged with a short poly(A) tail by TRAMP, which also recruits the exosome. In human cells, homologs of all TRAMP components are present (see Table 1 for human homologs of yeast RNA degradation factors) and RNA

Table 1. Human Homologs of Yeast RNA Degradation Factors

Complex	Protein Names in Yeast	Human Homologs (% Identity)	Information
5′-End Processing Enzymes			
	Dcp1	DCP1B (34%), DCP1A (33%)	Member of decapping complex with Dcp2
	Dcp2	DCP2 (37%)	Catalytic pyrophosphatase subunit of decapping complex
	Rat1	XRN2 (41%)	Nuclear 5′ exonuclease
	Xrn1 (Kem1)	XRN2 (36%), XRN1 (35%)	Cytoplasmic 5′ exonuclease
Core Exosome			Highly conserved RNA decay complex
	Rrp44 (Dis3)	Rrp44 (44%)	Only catalytic component of the core exosome; 3′ hydrolytic exonuclease and endonuclease activity
	Csl4	EXOSC1 (48%)	Member of core exosome complex
	Rrp4	EXOSC2 (41.6%)	Member of core exosome complex
	Rrp40	EXOSC3 (35.1%)	Member of core exosome complex
	Rrp41	EXOSC4 (35.4%)	Member of core exosome complex
	Rrp42	EXOSC7 (25.1%)	Member of core exosome complex
	Rrp43	EXOSC8 (29%)	Member of core exosome complex
	Rrp45	EXOSC9 (34.8%)	Member of core exosome complex
	Rrp46	EXOSC5 (29.1%)	Member of core exosome complex
	Mtr3	EXOSC6 (27%)	Member of core exosome complex
Exosome-Associated Factors			
	Rrp6	EXOSC10 (32%)	Nuclear-specific exosome component; 3′ hydrolytic exonuclease
	Rrp47 (Lrp1)	C1D (32%)	Nuclear exosome cofactor
	Mpp6	MPP6 (distantly related)	Nuclear exosome cofactor
	Ski7	unclear	Cytoplasmic exosome cofactor, connects exosome to Ski complex
TRAMP Complexes			Nuclear RNA degradation factors involved in RNA quality control and cryptic RNA degradation
	Trf4 (Pap2)	POLS (37%)	Nuclear poly(A) polymerase, TRAMP4 complex component
	Trf5	PAPD5 (36%)	Nuclear poly(A) polymerase, TRAMP5 complex component
	Air1, Air2	ZCCHC3 (37%, 39%)	TRAMP complex components
	Mtr4	SKIV2L2 (52%)	Helicase, TRAMP complex component, has TRAMP-independent functions
Ski Complex			Cytoplasmic mRNA degradation complex, also involved in viral suppression
	Ski2	SUPV3L1 (40%), SKIV2L (38%)	Cytoplasmic helicase, member of exosome cofactor Ski complex
	Ski3	unclear	Member of exosome cofactor Ski complex
	Ski8	unclear	Member of exosome cofactor Ski complex
Sen1-Nrd1-Nab3 Complex			Involved in Pol II termination and processing/degradation of snoRNA, snRNA, and crytpic ncRNAs
	Sen1	LOC91431 (36%)	Helicase
	Nrd1	unclear	RNA-binding protein
	Nab3	unclear	RNA-binding protein
Lsm Complexes			7 member ring-shaped RNA chaperone complexes
	Lsm1	LSM1 (45%)	Member of cytoplasmic Lsm1-7 complex
	Lsm2	LSM2 (63%)	Member of both Lsm complexes
	Lsm3	LSM3 (41%)	Member of both Lsm complexes
	Lsm4	LSM4 (31%)	Member of both Lsm complexes
	Lsm5	LSM5 (51%)	Member of both Lsm complexes

Table 1. *Continued*

Complex	Protein Names in Yeast	Human Homologs (% Identity)	Information
	Lsm6	LSM1 (45%)	Member of both Lsm complexes
	Lsm7	LSM6 (41%)	Member of both Lsm complexes
	Lsm8	LSM7 (48%)	Member of nuclear Lsm2-8 complex
Ccr4-NOT Complex			Cytoplasmic deadenylation complex
	Ccr4	hCCR4 (18%)	Member of Ccr4-NOT complex
	Pop2	CNOT7 (39%), CNOT8 (37%)	Member of Ccr4-NOT complex
	Not1	CNOT1 (27%)	Member of Ccr4-NOT complex
	Caf40	unclear	Member of Ccr4-NOT complex
	Mot2	CNOT4 (37%)	Member of Ccr4-NOT complex
	Caf130	unclear	Member of Ccr4-NOT complex
	Not5	CNOT3 (33%)	Member of Ccr4-NOT complex
	Cdc36	CNOT3 (32%)	Member of Ccr4-NOT complex
	Not3	unclear	Member of Ccr4-NOT complex
	Caf120	unclear	Member of Ccr4-NOT complex
	Caf16	CNOT3 (26%)	Member of Ccr4-NOT complex
Miscellaneous			
	Dbr1	DBR1 (38%)	Debranches intron lariat structures
	Rnt1	RNASEN (32%)	Endonuclease involved in Pol I termination and Pol II termination on some small RNAs
	Swt1	unclear	Endonuclease involved in perinuclear mRNP surveillance

degradation intermediates have been identified that carry either poly(A) or tails that are predominantly A (Slomovic et al., 2006; West et al., 2006). However, functional analysis of a human TRAMP complex has yet to be reported.

The TRAMP-exosome combination constitutes a potent system that is responsible for nuclear surveillance of many different RNAs and RNA-protein complexes. The role of polyadenylation as a marker for nuclear RNA degradation by the exosome is conceptually akin to the role of polyubiquitylation in targeting proteins for degradation by the proteasome (Lorentzen and Conti, 2006).

Some 3′ exonucleases can function "in reverse" as RNA polymerases, including bacterial polynucleotide phosphorylase (PNPase) or the archaeal exosome (Mohanty and Kushner, 2000; Portnoy et al., 2005). The tails added are predominantly adenosine, presumably because this is the most abundant nucleotide in all cells. A plausible evolutionary model is that the ancestral RNA degradation activity was stimulated by the addition of such heteropolymeric tails. A dedicated RNA poly(A) polymerase subsequently arose, and in *E. coli* this still functions only in RNA degradation, acting together with PNPase and RNase R (see Deutscher, 2006), both of which are homologous to components of the eukaryotic exosome (Allmang et al., 1999; Symmons et al., 2002). The role of nuclear polyadenylation in promoting RNA degradation by the exosome therefore closely resembles the situation in *E. coli*. This leads to the suggestion that an ancestral role of polyadenylation in the stimulation of RNA degradation was retained in the eukaryotic nucleus, whereas a distinctly different role for poly(A) tails in mRNA stability and translation emerged in the eukaryotic cytoplasm (LaCava et al., 2005). Notably, all eukaryotes tested, with the exception of budding yeast, have cytoplasmic poly(A) polymerases (Kwak et al., 2008; Rouhana et al., 2005; Stevenson and Norbury, 2006), suggesting that the mRNA polyadenylation system may have developed in the cytoplasm before being transferred into the nucleus.

In human cells the addition of polyuracil tails can also stimulate RNA degradation (Mullen and Marzluff, 2008). Poly(U) polymerase activities are present in many eukaryotes (Kwak et al., 2008; Rissland and Norbury, 2008), suggesting that this pathway may also be widespread.

Chaperones

The closely related, ring-shaped complexes termed Lsm1-7 and Lsm2-8 in eukaryotes and Hfq in bacteria represent another family of cofactors present in most organisms analyzed (reviewed in Beggs, 2005). These act as chaperones promoting RNA-RNA and RNA-protein interactions and regulate the degradation of many RNAs. Poly(U) tails stimulate degradation of human histone mRNAs via recruitment of the Lsm1-7 complex (Mullen and Marzluff, 2008), which also plays an important role in general mRNA turnover. In bacteria, the Hfq complex is required for the function of numerous small regulatory RNAs (see below).

In addition, numerous other RNA-binding proteins function in many RNA degradation pathways, with a greater or lesser degree of sequence specificity (reviewed in Glisovic et al., 2008).

Roles of Small RNAs

In both bacteria and eukaryotes, large numbers of small regulatory RNAs have been characterized. Most characterized

bacterial small RNAs (sRNAs) act to alter the translation of separate target RNAs, either positively or negatively (see, for example, Huang et al., 2008; Sittka et al., 2008). Different sRNAs can functionally interact, and they can be regulated by poly(A)-stimulated degradation (Urban and Vogel, 2008). The bacterial sRNAs therefore show functional similarities to the endogenous, eukaryotic microRNAs (miRNAs) that also largely act by regulating mRNA translation (see below).

Many bacteria also encode other small RNAs that are transcribed from CRISPR (clustered regularly interspaced short palindromic repeat) loci and termed prokaryotic silencing RNAs (psiRNAs) or crRNAs. The CRISPR loci incorporate multiple short regions that match the sequences of invading viruses and are transcribed into long pre-crRNA transcripts that are processed to yield the individual crRNA/psiRNAs. These provide antiviral defense by guiding endonuclease cleavage of homologous viral RNAs by the Cascade complex (CRISPR-associated complex for antiviral defense) (Barrangou et al., 2007; Brouns et al., 2008; Hale et al., 2008). There is apparent functional similarity between bacterial crRNA/psiRNAs and eukaryotic small-interfering RNAs (siRNAs) that direct site-specific cleavage by the RNA-induced silencing complex (RISC).

Similarities and Differences in 5′ and 3′ Degradation Pathways

Most cellular RNAs are modified to protect them from 5′ exonucleases. In *Bacillus subtilis*, 5′ degradation of mRNAs by RNase J is stimulated by hydrolysis of the 5′ triphosphate (de la Sierra-Gallay et al., 2008; Mathy et al., 2007). In *E. coli*, primary transcripts that retain a 5′ triphosphate are partially resistant to degradation because the major endonuclease RNase E is sensitive to 5′ structure, strongly favoring degradation of processed RNAs with a 5′-monophosphate (see Celesnik et al., 2007). Degradation is stimulated by conversion of the protective triphosphate 5′-end structure to a monophosphate by the pyrophosphate hydrolyase RppH (Deana et al., 2008). Eukaryotic mRNAs carry protective 5′-cap structures that must be removed prior to 5′ exonuclease degradation. Notably, the decapping enzyme Dcp2 is related to bacterial RppH (Deshmukh et al., 2008; She et al., 2008).

In eukaryotes a major 3′ degradation activity is provided by the exosome complex, the core of which is structurally related to bacterial PNPase (Liu et al., 2006; Lorentzen et al., 2005; Symmons et al., 2002). Both PNPase and the eukaryotic exosome can also associate with endonucleases: In *E. coli*, PNPase binds to the endonuclease RNase E in the degradosome, whereas in yeast the N-terminal PIN domain of Rrp44/Dis3 provides the exosome with endonuclease activity (Lebreton et al., 2008; Schaeffer et al., 2008; Schneider et al., 2009). Both bacteria and eukaryotes also harbor multiple other 3′ exonucleases, which frequently show partially overlapping sets of substrates.

Degradation of Different Types of RNA in Eukaryotes

Several different classes of RNA degradation can potentially be discriminated.

- Processing: Essentially all RNA species are synthesized as larger precursors and must undergo 3′, and in many cases 5′, processing by nuclease activities. In addition, many excised spacer fragments must be degraded, as must introns excised from mRNA precursors. Such maturation pathways are integral to the processing of all classes of RNA and removal of the discarded material probably dominates total RNA degradation.
- mRNAs and non-protein-coding RNAs (ncRNAs): The regulated turnover of mRNAs is a key factor in the control of gene expression and an apparently universal feature of mRNA metabolism. Similarly, most characterized examples from the seemingly large numbers of unstable ncRNAs undergo rapid and continuous degradation. This "constitutive" degradation makes these classes of RNA distinct from the many stable RNA species.
- Quality control: Surveillance pathways appear to be active on all classes of eukaryotic RNA, constantly identifying and degrading defective RNAs and RNA-protein complexes. The in vivo activity of most surveillance pathways is hard to assess because, in contrast to RNA processing and mRNA turnover, most RNA surveillance occurs only on defective RNAs or ribonucleoprotein particles (RNPs), which are presumably quite rare. Functional analyses have therefore largely relied on artificially induced defects—for example mutations in the RNAs or processing factors—that may not fully reflect the naturally occurring spectrum of substrates. Exceptions include mRNAs with premature translation termination codons (PTCs). These are generated by alternative splicing, or by programmed genome rearrangements in some specific cell types, and are targets for nonsense mediated decay (NMD) pathways.

The three different RNA polymerases present in eukaryotic cells have very different products—but surveillance systems apparently see them all. Indeed, a notable feature of the major yeast RNA degradation activities is their universality—transcripts generated by RNA polymerases I, II, and III can all be targets for 3′ degradation by the exosome and its TRAMP cofactor, or for the Rat1 5′ exonuclease (Xrn2 in humans). The reasons for RNA degradation are broadly similar among the different classes of polymerase products, but there are no obvious structural features in common between the diverse substrates that are targeted by the TRAMP/exosome system or Rat1.

RNA Polymerase I

RNA polymerase I produces a single transcript, the polycistronic RNA encoding three of the four eukaryotic ribosomal RNAs (rRNA). The mature rRNAs are generated by a complex mix of endonuclease cleavages and exonuclease trimming. During rRNA processing, the external and internal transcribed spacer regions (ETS and ITS) are removed and degraded. Due to the high production of ribosomes (approximately 2000 min^{-1} in budding yeast), degradation of the pre-rRNA spacers ($\sim 3 \times 10^6$ nt min^{-1}) probably accounts for a substantial fraction of total cellular RNA degradation.

Surveillance of ribosomal precursors (pre-ribosomes) is highly active in yeast. Defective pre-ribosomes are largely degraded by the TRAMP and exosome complexes, but it remains unclear how

aberrant ribosomes are specifically identified and targeted. In the section "Kinetic Proofreading in RNA Processing" below we present a model for kinetic proofreading, in which activated, high-energy intermediates are reiteratively tested to provide very high overall fidelity.

Most mutations that block pre-rRNA processing do not cause any substantial accumulation of pre-ribosomes, even though transcription continues. As a consequence, mutations causing defects in yeast ribosome synthesis are generally lethal due to loss of ribosome production. In contrast, inactivation of ribosome synthesis factors in *E. coli* generally leads to strong accumulation of pre-ribosomes. These are partially functional and hence the mutants are viable, if impaired in growth (see Kaczanowska and Ryden-Aulin, 2007). We would predict that the less active surveillance in bacteria reflects the use of default, equilibrium pathways (Deutscher, 2006). In these, simple competition between binding factors determines whether RNAs and RNPs mature or are degraded, and correct and aberrant assembly pathways are distinguished only by differences in binding energies.

The large number of ribosome synthesis factors that function during rRNA processing and ribosome assembly must ultimately be removed from the maturing pre-ribosomes. In many cases this is probably mediated by specific enzymes such as RNA helicases, GTPases, and AAA ATPases (reviewed in Henras et al., 2008). However, it seems likely that in other cases RNP disassembly might most readily be achieved by degradation of the RNA component. For example, several ribosome synthesis factors are released in association with the 5′ external transcribed spacer (5′ ETS) region of pre-rRNA, and the observed very rapid degradation of the 5′ ETS is predicted to promote efficient recycling. The 5′ ETS is degraded 3′-5′ by the exosome and TRAMP complexes, but the endonuclease activity of the exosome may contribute to rapid degradation (Lebreton et al., 2008; Schneider et al., 2009).

Mature, cytoplasmic ribosomal subunits are very stable and their degradation rate is generally undetectable, but there are some exceptions. There is evidence for surveillance and preferential degradation of translationally inactive ribosomal subunits via rRNA cleavage, at least in yeast (LaRiviere et al., 2006). The mechanism is not established but is possibly related to the "no-go decay" pathway that degrades mRNAs upon which ribosomes have stalled (Figure 1). In addition, recent analyses reveal that under starvation conditions, mature ribosomes can be engulfed by the vacuole and degraded in a process that has been termed "ribophagy" due to its apparent similarities to the degradation of other cytoplasmic components by the autophagy pathway (Beau et al., 2008; Kraft et al., 2008). In addition, a variety of different stresses can induce cleavage of the rRNA in budding yeast (Mroczek and Kufel, 2008; Thompson et al., 2008), presumably causing rapid translation inhibition.

RNA Polymerase II

Transcription by RNA Pol II produces many different classes of RNA, including messenger RNA precursors (pre-mRNA) and precursors to numerous stable RNAs including small nuclear RNAs (snRNAs) that function in pre-mRNA splicing, small nucleolar RNAs (snoRNAs) that function in ribosome synthesis, and miRNAs that regulate mRNA translation and stability, as well as many other ncRNA transcripts. Transcription by RNA Pol II is accompanied by cotranscriptional 5′-end capping, which confers protection against 5′ exonucleases. 5′ degradation therefore requires an initial decapping step to render the end accessible. Resident nuclear transcripts such as snRNAs and snoRNAs generally have a modified, hypermethylated cap structure, perhaps as an additional protection against decapping and 5′ degradation.

We can recognize the same steps in mRNA and ribosome biogenesis, with the pre-mRNA undergoing multiple processing events (particularly splicing) linked to the activity of multiple ATP-dependent helicases (see Staley and Guthrie, 1998). Intron removal and degradation release large nucleotide pools, especially in metazoans where introns frequently exceed 100 kb. Intron degradation requires a specialized debranching activity as the intron lariat released by the splicing machinery is circularized by an exonuclease resistant 2′, 5′-phosphodiester bond. The lack of generalized endonuclease activity in the nucleus is clear from the huge accumulation of intronic RNA observed in yeast mutants deficient in debranching. Further exonucleolytic processing of debranched introns leads to complete degradation in most cases or snoRNA biogenesis for intron-encoded snoRNAs (Figure 1).

The rate and timing of transcription of mRNAs are controlled by a set of highly complex and intricate mechanisms, but these are of limited use without equally precise control over the rate and timing of mRNA turnover, given that this determines the amount of mRNA that is actually available to direct protein production. Turnover of mRNAs therefore plays a key role in the overall regulation of gene expression. This topic has been expertly reviewed (see for example Doma and Parker, 2007; Isken and Maquat, 2008; Rougemaille et al., 2008b; Shyu et al., 2008) and will be discussed here only briefly. Pre-mRNAs, but not most other classes of Pol II transcripts, undergo site-specific 3′ cleavage that is coupled to the addition of a long poly(A) tail by the poly(A) polymerase PAP/Pap1. Unlike the poly(A) tails added by TRAMP, this does not lead to degradation, possibly because mRNA polyadenylation is highly processive and closely accompanied by loading of the poly(A) binding protein PABP/Pab1. In consequence the released mRNA has a tail of ~70–90A residues in yeast and ~250A in humans, which is covered by PABP.

The mRNA is then packaged for export in a process that is tightly linked to transcription (reviewed in Rougemaille et al., 2008b). Defects in mRNP assembly lead to exosome-dependent accumulation of the mRNA in association with the site of transcription, followed by mRNA degradation (Figure 1). This linkage of the defective mRNP to the transcription site can lead to the movement of the entire genetic locus to the nuclear pore complexes (Rougemaille et al., 2008a).

In many eukaryotes, sites of successful splicing are marked in the mRNAs that are exported to the cytoplasm, thus maintaining a record of the nuclear history of the RNA. The splicing machinery deposits an "exon junction complex" (EJC) close to the splice site, whose distribution is assessed by the NMD machinery in the cytoplasm (Figure 1). This allows the rapid degradation by the NMD pathway of incorrectly spliced and

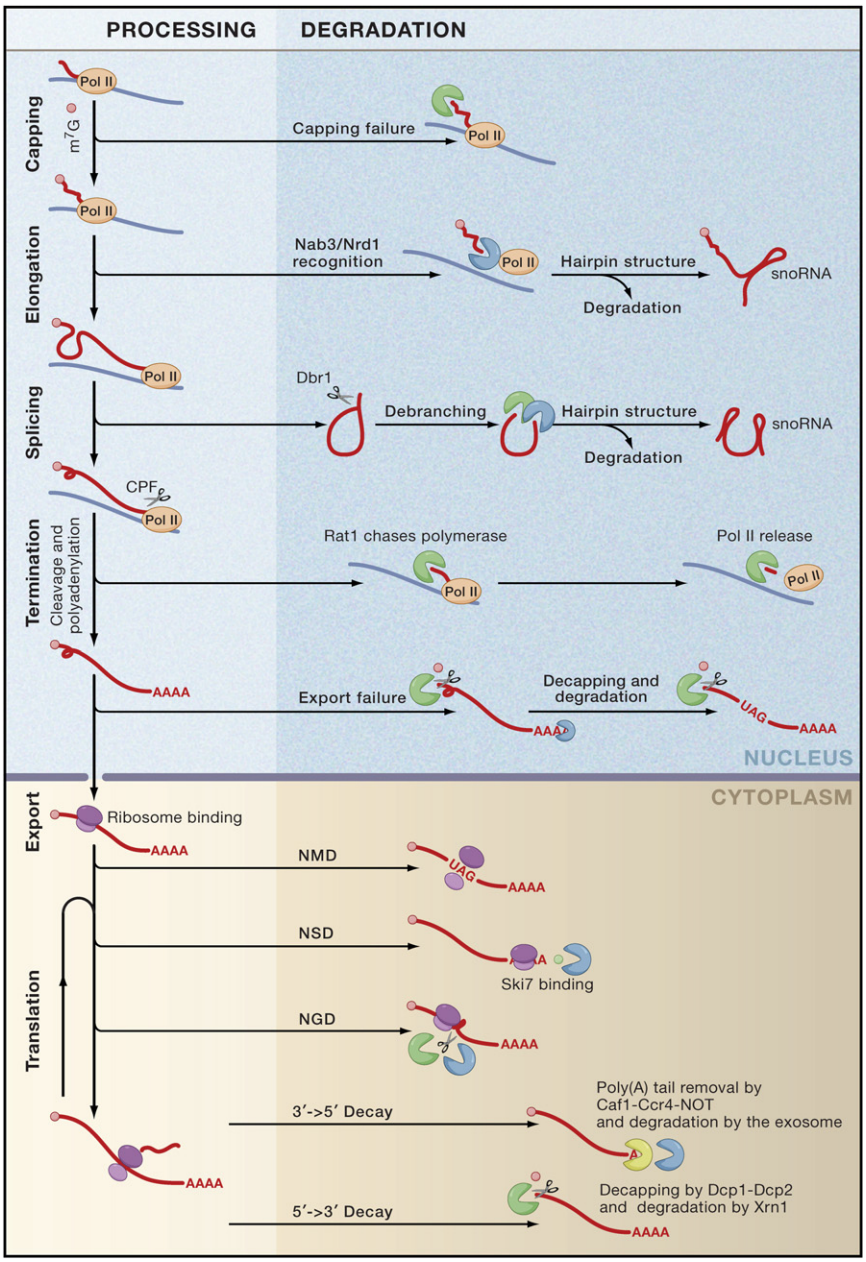

Figure 1. Processing and Degradation of RNA Polymerase II Transcripts

Processing of RNA Pol II transcripts can be divided into six phases.

Capping: An m^7G cap is added cotranscriptionally to the 5′ end of the nascent RNA. Failure of this step is predicted to lead to degradation by 5′ exonuclease Rat1 and transcription termination (see West et al., 2008).

Elongation: During elongation, modification of the C-terminal domain of RNA Pol II changes from serine 5 phosphorylation to serine 2 phosphorylation. If clusters of Nrd1-Nab3 sites are encountered during the Ser5P period, transcription is terminated, followed by 3′ exonucleolytic degradation (Gudipati et al., 2008). This leads to complete degradation or sn(o)RNA 3′-end formation depending on the nature of transcript.

Splicing: Introns are removed by splicing machinery, leaving the 5′-2′ linked intron lariat. This is debranched by Dbr1 (Chapman and Boeke, 1991) and degraded by exonucleases from both ends, leading, in most cases, to complete degradation or to maturation of intron-encoded snoRNAs.

Termination: Cleavage and polyadenylation are mediated by a large protein complex. The 3′ end of the RNA remains on the elongating polymerase but is no longer capped. This allows the 5′ exonuclease Rat1 to chase and "torpedo" the transcribing polymerase (see West et al., 2008).

Export: mRNA is packaged and exported to the cytoplasm. This can fail for multiple reasons, leading to degradation of the mRNA by 5′ and/or 3′ exonucleases and retention at the transcription site (Rougemaille et al., 2008b). Either coupled to nuclear export or occurring soon thereafter, the transcript undergoes a primary round of translation, followed by degradation if premature stop codons are detected. This occurs by different mechanisms in different organisms (Isken and Maquat, 2008).

Translation: mRNA undergoes multiple rounds of translation, during which time the poly(A) tail is progressively shortened. The major deadenylase activity in yeast and humans is probably a complex including Ccr4 and Caf1, both of which have deadenylase activity (see Schwede et al., 2008). Another deadenylase PARN may play more important roles in regulated deadenylation, particularly during development (see Kim and Richter, 2006).

Degradation can occur via surveillance pathways, including non-stop decay (NSD) and no-go decay (NGD), that deal with stalled ribosomes (Isken and Maquat, 2008). Following poly(A) tail removal the mRNA body can be decapped by the Dcp1-Dcp2 complex and then subject to either 5′ exonuclease degradation by Xrn1 or 3′ degradation by the exosome.

other defective mRNAs that potentially encode truncated proteins (reviewed in Isken and Maquat, 2008; Stalder and Muhlemann, 2008).

Once in the cytoplasm mRNAs undergo progressive deadenylation at rates that are specific for each mRNA species. This provides a timing mechanism that confers a defined lifetime, rather than a stochastic half-life, on each mRNA species. The mRNP therefore contains information that defines its own life span, although the nature of most determinants remains poorly understood. The best characterized stability/instability elements are the AU-rich elements (AREs), which are bound by a number of proteins to either extend or shorten mRNA life span, often in response to extracellular signals (see, for example, Vasudevan and Steitz, 2007).

It is widely assumed that mRNAs that have been deadenylated, or have been translationally repressed by miRNA binding, are released from polysomes prior to 5′ degradation in cytoplasmic processing bodies (P bodies) (see Franks and Lykke-Andersen, 2008; Shyu et al., 2008). However, recent analyses have called these conclusions into question by showing that,

at least in yeast, the major steps in mRNA 5′ degradation, deadenylation, decapping, and 5′ exonuclease digestion can all occur on polysomes (J. Coller, personal communication).

Until recently, analyses of eukaryotic mRNA and pre-mRNA degradation have concentrated on exonuclease activities; however, a regulated human mRNA turnover pathway that utilizes the endonuclease Pmr1 has long been known (see Peng et al., 2008), and recent analyses suggest that this is more prevalent than anticipated. In *Drosophila*, NMD was found to be initiated by cleavage of the defective RNA (Gatfield and Izaurralde, 2004), and a metal-ion binding, PIN domain in the NMD factor Smg6 is required for this activity (Huntzinger et al., 2008). Endonuclease activities were recently identified in other PIN-domain proteins: Swt1, which is implicated in perinuclear mRNP surveillance (Skruzny et al., 2008), and the exosome component Rrp44 (Lebreton et al., 2008; Schaeffer et al., 2008; Schneider et al., 2009).

Cleavage of the nascent mRNA at the polyadenylation site provides an entry point for the 5′ exonuclease Rat1, which then chases the transcribing polymerase and causes termination—the "Torpedo" mechanism (see West et al., 2008). This is important to reduce readthrough of elongating polymerase II into adjacent genes and associated downregulation of promoter elements. However, termination of Pol II transcripts can be achieved in ways other than polyadenylation-linked cleavage. Cotranscriptional endonuclease cleavage of nascent human β-globin RNA is important for transcription termination (see West et al., 2008), although the mechanism is not established. Termination on yeast snRNA genes and many snoRNAs is triggered by a complex between the RNA helicase Sen1 and the Nrd1-Nab3 heterodimer. Nrd1 interacts with the C-terminal domain of the large subunit of RNA polymerase II and both Nrd1 and Nab3 can bind to the RNA transcript (Carroll et al., 2007; Gudipati et al., 2008; Vasiljeva et al., 2008). This leads to release of a nonpolyadenylated transcript that undergoes exonucleolytic 3′ processing, involving the exosome complex, to the mature 3′ end of the RNA. Nrd1-Nab3 acts as an exosome cofactor, indicating a dual role in terminating transcription and recruiting the exosome to these RNAs. It has, however, been difficult to determine which activities are actually responsible for 3′-end maturation of snRNAs and snoRNAs. Mutations in the exosome or other 3′ exonucleases lead to accumulation of extended precursors but not to loss of the mature RNA. Presumably this reflects redundancy in the processing pathways.

3′-end processing on other yeast snoRNAs is initiated via endonucleolytic cleavage by the RNase III-like double-strand-specific endonuclease Rnt1. However, this apparently does not provide an entry for Rat1, and termination still requires the Nrd1-Nab3-Sen1 complex (Kim et al., 2006). In human cells, snRNA 3′-end maturation is substantially different and requires a specific modification, phosphorylation of Ser7, in the 7 amino acid *C-t*erminal repeat *d*omain (CTD) of the largest subunit of RNA Pol II (Egloff et al., 2007). This surprising finding revealed that Pol II termination can be defined at transcriptional initiation and is not just dependent on sequences encountered during elongation.

RNA polymerase II also generates the precursors to the very small RNA species, miRNAs, siRNAs, and piRNAs, which in some cases target mRNAs and other RNAs for degradation. These pathways are described in detail elsewhere in this issue and will be discussed only briefly here. The effectors of miRNA and siRNA function are two related RNP complexes, the cytoplasmic RISC and the nuclear RNA-induced transcriptional silencing complex (RITS). Each complex can direct site-specific cleavage of target RNAs that show extensive complementarity to the miRNA or siRNA, which is mediated by the "slicer" activity of an Argonaute protein (Figure 2). In the cytoplasm, the RISC complex also targets mRNAs that show only partial complementarity to the miRNA, generally binding to sequences in the 3′-untranslated region. This results in decreased translation and increased 5′ and 3′ degradation (see Figure 2 and Eulalio et al., 2008; Wu and Belasco, 2008).

The siRNAs and miRNAs are themselves subject to active degradation by the 3′-exoribonuclease Eri1, which acts to negatively regulate the activity of miRNA/siRNA-mediated gene repression by degrading siRNA-containing duplexes (Figure 2). Eri1 also functions in rRNA processing (Ansel et al., 2008; Gabel and Ruvkun, 2008), and this is also the case for two DEAD-box helicases that function together with Drosha in pri-miRNA processing (Fukuda et al., 2007), suggesting the evolution of the miRNA-processing system from pre-existing RNA-processing factors. In plants a related but distinct family of single-strand-specific *s*mall RNA *d*egrading *n*ucleases (SDNs) limits miRNA levels and is important for normal development (Ramachandran and Chen, 2008) (Figure 2). Both the Eri1 and SDN families are widely conserved among eukaryotes, and it seems likely that these regulatory pathways will be of widespread importance.

Non-Protein-Coding RNAs

Recent analyses have demonstrated that yeast and human cells transcribe almost their entire genomes, implying the existence of a huge mass of hidden, or cryptic, ncRNAs, which are believed to be generally transcribed by RNA polymerase II (reviewed in Amaral et al., 2008). In fact, evidence for the existence of a complex population of nuclear RNAs in mammalian cells is not new; metabolic labeling performed 30 years ago indicated that the majority of newly synthesized transcripts are retained and degraded within the mouse nucleus, forming the heterogeneous nuclear RNA (hnRNA) population (see, for example, Brandhorst and McConkey, 1974). However, over time these observations came to be largely discounted, until supported by deep sequence analyses (Birney et al., 2007; Han et al., 2007; Maeda et al., 2006).

The ncRNAs probably fall into several different classes: some may be basic transcriptional noise, generated at low levels throughout the genome due the inability of the transcription machinery to identify true promoters. On thermodynamic principles, a plausible signal to noise for Pol II transcription initiation has been suggested at around 10^4 (Struhl, 2007), corresponding to a large number of nonspecific transcripts in any organism with a large genome. High levels of short, cryptic antisense transcripts that are targets for the exosome are generated from promoter regions in human cells (Core et al., 2008; He et al., 2008; Preker et al., 2008; Seila et al., 2008) and yeast (Davis and Ares, 2006) (Neil et al., 2009), and this may also be the

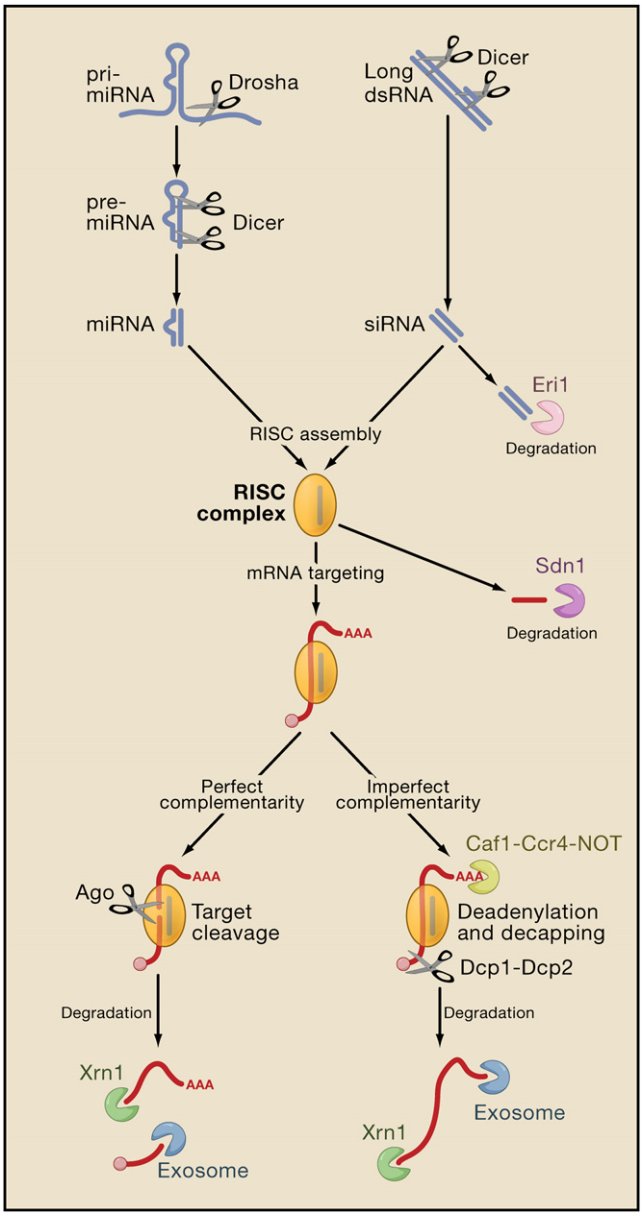

Figure 2. siRNA- and miRNA-Directed RNA Degradation

The primary transcripts for endogenous microRNAs (pri-miRNAs) generally undergo two-step processing. Cotranscriptional cleavage by a complex that includes the double-strand endonuclease Drosha generates a pre-miRNA (Morlando et al., 2008). This is exported to the cytoplasm, where cleavage by Dicer generates a mature-sized, double-stranded product. In contrast, exogenously added and endogenously synthesized long, perfectly complementary double-stranded RNAs are processed to small-interfering RNAs (siRNAs) by the dicer complex alone. In both cases, a short (approximately 22 nt) single-stranded RNA is finally integrated into the RNA-induced silencing complex (RISC), which always includes a member of the Argonaut (Ago) protein family—the key mediators of miRNA function (see Diederichs and Haber, 2007). The active RISC complex can target RNAs that show complete complementarity to the miRNA or siRNA, leading to site-specific endonuclease cleavage by Ago, followed by degradation of the resulting target RNA fragments by Xrn1 and the exosome. However, endogenous miRNAs can, and generally do, target mRNAs with only partial complementarity. Interactions with Ago result in decreased translation, together with increased decapping by Dcp1/Dcp2 and increased deadenylation by the Ccr4-Caf1-NOT complex (Eulalio et al., 2009). This is followed by 5′ and 3′ exonuclease degradation of the mRNA body by Xrn1 and the exosome. For more detailed information, see Eulalio et al. (2008) and Wu and Belasco (2008). The activity of miRNA and siRNA pathways is also modulated by RNA degradation. Double-stranded siRNAs can be degraded by Eri1-related proteins, while single-stranded miRNAs can be degraded by Sdn1-related proteins (Ansel et al., 2008; Gabel and Ruvkun, 2008; Ramachandran and Chen, 2008).

case for transcription terminator regions. These may be of functional importance or simply reflect the accessibility of the chromatin structure in promoter regions. In addition to these possibly "spurious" transcripts, both yeast and human cells also contain ncRNA transcription units that appear to fit most reasonable definitions of genes, being transcribed from apparently dedicated promoters. These ncRNA genes can be located either within intergenic regions or antisense to protein-coding genes.

Discussion of the function of these diverse ncRNAs is beyond the scope of this Review. However, a common feature that all seem to share is high instability, which probably explains why they largely escaped detection and analysis for so long. For example, an ncRNA transcribed through the yeast *GAL1-10* cluster was found to be present at the extremely low abundance of 1 molecule per 14 cells (Houseley et al., 2008). The degradation of ncRNAs is best understood in yeast, where several distinct features each contribute to rapid degradation. The first is redundancy; individual RNAs can be targeted for degradation by the exosome complex by several different cofactors (Milligan et al., 2008). The second is cotranscriptional association of the surveillance machinery. The exosome cofactors, Nrd1-Nab3, and the TRAMP complex associate with at least some nascent transcripts (Carroll et al., 2007; Houseley et al., 2007; Vasiljeva et al., 2008), in effect pretargeting RNAs for destruction as soon as their synthesis is completed. Recent data hint that a third feature promoting rapid ncRNA degradation might be endonuclease cleavage.

RNA Polymerase III

RNA Pol III produces multiple small stable RNAs, including tRNAs, the 5S rRNA, the U6 snRNA, and the RNA component of signal recognition particle (SRP). Most of these have far simpler processing than ribosomal RNA as the mature 5′ ends are mostly at the transcription initiation site, with the 3′ ends produced by simple trimming. An exception is tRNA, which undergoes 5′ cleavage by RNase P and has a 3′-terminal CCA added by a dedicated polymerase. Less is known about the surveillance of Pol III transcripts than the products of other polymerases. However, RNA Pol III products do undergo nuclear surveillance. This can occur via poly(A) addition by the TRAMP complex and 3′ degradation by the exosome. Evidence for this exists for 5S rRNA, U6 snRNA, the RNA component of SRP, and pre-tRNAs (Copela et al., 2008; Kadaba et al., 2006). The most studied TRAMP-exosome substrate is initiator $tRNA^{imet}$ that is undermethylated (Schneider et al., 2007; Vanácová et al., 2005); however, the major surveillance pathway for most undermodified tRNAs involves 5′ degradation by the Rat1 and Xrn1 exonucleases (Chernyakov et al., 2008). Involvement of the nuclear 5′ exonuclease Rat1 implies the nuclear import of

defective tRNAs. Uncharged yeast tRNAs are imported by the adaptor Mtr10/Kap111 (reviewed in Hopper and Shaheen, 2008), and this pathway may also allow other defective tRNA species to be "inspected" by the nuclear RNA surveillance system. Surprisingly, in human cells, plants, yeast, and other fungi, tRNAs can undergo cleavage of the anticodon loop (Jochl et al., 2008; Thompson et al., 2008). This can be under developmental regulation or, like rRNA cleavage, can occur in response to oxidative stress, potentially acting to reduce translation.

RNA Polymerases IV and V

In plants, two additional RNA polymerases have been characterized (see Wierzbicki et al., 2008). Termed RNA Pol IV and V (or nuclear RNA polymerase D and E) these appear to function specifically in siRNA-mediated gene silencing, with RNA Pol IV generating the siRNA precursors and RNA Pol V generating ncRNA targets for the siRNAs.

Why Is RNA Degradation so Efficient?

The most notable feature of most characterized eukaryotic RNA degradation pathways is their striking efficiency. In yeast mutants with ribosome synthesis defects, the pre-rRNAs are generally degraded with almost undetectable intermediates—kilobases of RNA with dozens of associated proteins apparently just vanish—and this despite their very high rates of synthesis. Degradation of ncRNAs is also so efficient that their widespread existence is only now becoming apparent. So—why has this dramatically high efficiency been selected by evolution?

One possible explanation for the extreme rapidity of RNA degradation, and perhaps also for the apparently greater prevalence of exonuclease activities rather than endonucleases, is the finding that a variety of different types of small RNA (such as miRNAs, siRNAs, and piRNAs) exert potent effects on gene expression at multiple steps from chromatin structure to translation and mRNA turnover. There may therefore have been strong and long-standing selection against the accumulation of random RNA fragments, particularly from highly expressed RNAs. Indeed, loss of TRAMP-mediated RNA degradation is known to allow inappropriate entry of rRNA and tRNA fragments into the RNAi pathway in fission yeast (Buhler et al., 2008).

Bacterial PNPase and the archaeal exosome each include three active sites for phosphorolytic exonuclease activity, but this phosphorolytic activity has apparently been lost in the eukaryotic exosome (Dziembowski et al., 2007). Instead, the eukaryotic exosome core can associate with the hydrolytic exonucleases Rrp44/Dis3 (which is related to *E. coli* RNase R) and Rrp6/PM-Scl100 (which is related to *E. coli* RNase D). A notable difference between phosphorolytic and hydrolytic exonuclease activities is that hydrolysis is strongly thermodynamically favored, whereas phosphorolysis is energetically neutral. This might make the eukaryotic exosome much better than PNPase or the archaeal exosome at degrading through stable RNA-protein (RNP) structures—without generating degradation intermediates that can enter the RNAi pathway.

It is also possible that RNAs accumulating in the absence of active degradation could disrupt DNA replication and other activities more directly by forming RNA-DNA hybrids, as has been proposed for telomeric ncRNAs in yeast strains defective for the 5′ exonuclease Rat1 (Luke et al., 2008).

In addition, the accumulation of RNA species is predicted to cause sequestration of RNA-binding proteins, both cognate and noncognate. In some cases the abundance of specific proteins may become limiting. For example, a rapidly growing budding yeast cell synthesizes some 2000 ribosomes per minute—a number close to the estimated abundance of several ribosome synthesis factors (Ghaemmaghami et al., 2003). Thus a delay of just 1 min in recycling these factors will lead to their effective depletion. In some cases the ribosome synthesis factors are released from the pre-ribosomes in association with excised spacer fragments, underlining the importance of the observed rapid spacer degradation. Moreover, the relative abundance of different RNA-binding factors can have a substantial impact, for example in the selection of alternative sites of pre-mRNA splicing. Alterations in the relative levels of constitutive RNA-binding proteins can lead to tissue-specific splicing patterns (see, for example, Kashima et al., 2007; Venables et al., 2008). Some factors involved, such as hnRNP A1, show limited sequence specificity and could well be bound nonspecifically by accumulated RNA degradation intermediates.

A further important predicted function for pervasive RNA degradation is in viral suppression; the RNA surveillance machinery would be capable of compromising, even if not completely degrading, the genomes of single-stranded RNA viruses. It is therefore unsurprising that single-stranded RNA viruses show a wide range of end adaptations expected to thwart surveillance including capping, polyadenylation, tRNA mimicry, terminal hairpins, and 5′-3′ panhandle structures (see Hong et al., 1998). These features are mostly multifunctional making their contribution to viral RNA stability hard to analyze; however, evidence exists for polyadenylation directly impacting the stability of hepatitis B virus (Lee et al., 2008) and for capping being required to protect brome mosaic virus from 5′ exonucleases (Ahola et al., 2000). Viruses are also known to utilize cellular mRNA stabilizing factors; the Sindbis virus 3′ UTR has a number of stabilizing elements, including poly(U) tracts, that interact with currently unidentified host proteins (Garneau et al., 2008). The evolution of viruses immune to this innate defense has, of course, driven the emergence of much more advanced systems for viral RNA degradation such as the siRNA system in plants and invertebrates. Viruses have also hijacked RNA degradation components to aid their replication. Brome mosaic virus uses the Lsm complex to turn off translation (Noueiry et al., 2003) and move to P bodies where replication can occur (Beckham et al., 2007). Given that an RNA cannot be replicated and translated simultaneously, this switching event is required after the translation of sufficient replication factor to allow unimpeded viral replication.

Finally, it is notable that most organisms also possess a range of nonspecific RNases, in addition to the RNA-processing and degradation factors discussed so far. Among these are mammalian RNase A and fungal RNase T1, with which many readers will be familiar. These are extracellular enzymes that, unlike most intercellular processing enzymes, cleave target RNAs to leave 5′-hydroxyl and 3′-phosphate groups. Humans produce abundant levels of RNase A on skin, in blood, and elsewhere, and it

seems very likely that this acts to reduce contamination with RNAs, whether internally or externally derived, preventing their entry into other RNA-processing pathways.

Kinetic Proofreading in RNA Processing

Two basic questions emerge from studies of many RNP-assembly and RNA-processing pathways: why are they so complicated and, given this complexity, how are "defective" particles distinguished from "normal" intermediates and selectively targeted for degradation? In this section we discuss models for how this might be achieved with particular reference to ribosome synthesis in budding yeast, but the basic premises may hold for other pathways including mRNA processing. Many analyses indicate that surveillance is very active during ribosome synthesis but the pre-ribosomes are too big (~1–20 MDa) to be "seen" by any single surveillance factor. The pathway is also extremely complex—with at least 180 protein factors, 75 snoRNPs, 79 ribosomal proteins, and 7 kb of pre-rRNA that undergoes a multistep processing pathway. The possibilities for errors are clearly enormous, and it seems imperative that surveillance occurs at multiple steps during maturation.

An Equilibrium Model for Surveillance—and Its Problems

A widely accepted general model for surveillance of RNP synthesis is shown in Figure 3A, which we will refer to as an "equilibrium model." In such models the correct binding of factors is discriminated from incorrect binding solely on the basis of the differences in binding energy. Correct binding is energetically favored and in turn favors the next step(s) in the maturation pathway. In contrast, incorrect binding is relatively disfavored, reduces forward processing, and promotes targeting of the RNP for a discard pathway. This basic mechanism has been proposed for several RNA-processing pathways including mRNA maturation (Doma and Parker, 2007).

There are, however, substantial problems with equilibrium models. Consider the case of protein complexes bound incorrectly. Incorrect binding competes with correct binding, and this discrimination is at the heart of all biological processes. All RNA-binding proteins show some nonspecific affinity for RNA and so will bind noncognate sites. These will be of low affinity but on a 7 kb long pre-rRNA are likely to be very numerous. The mass-action effect of numerous incorrect sites may therefore outweigh the greater stability at the single correct site. This probably underlies the observation that most ribosome synthesis factors tested show no clear site specificity in pre-rRNA binding in vitro. At sites that resemble the authentic binding site—near-cognate sites—the binding energy will not differ greatly from the cognate-binding site. There is an energy cost associated with any accurate separation process, due to decreased entropy. So, if the difference in binding energy between cognate and near cognate sites is small, the complexes cannot be efficiently separated.

Other problems arise when we consider mutants defective in ribosome synthesis, in which degradation of pre-ribosomes is greatly elevated (Figure 3B). In an equilibrium model this increase must be driven by mass action and should be accompanied by a great accumulation of precursors unless default degradation levels are very high. However, yeast ribosome synthesis mutants do not generally show strong pre-rRNA accumulation. Mathematical analysis of equilibrium models reveals that achieving sufficiently high rates of degradation when maturation is inhibited requires substantial degradation of "normal" intermediates at each proofreading step (Karbstein, 2009). However, in any multistep pathway high degradation levels would permit very little product formation.

Surveillance via equilibrium binding and competition between degradation and maturation activities does not therefore appear to be sufficient to account for the properties of the pathway.

Kinetic Proofreading in Ribosome Synthesis

An established mechanism for increasing the accuracy of simple biological processes is kinetic proofreading, first proposed by John Hopfield (Hopfield, 1974) (see Figure 3C). Within a proofreading module, the reaction is driven (for example by coupling to hydrolysis of a nucleotide triphosphate) and essentially irreversible. The initial step of equilibrium complex formation does not lead directly to product formation. Instead, a transient high-energy intermediate is generated, which is then resolved into product or degradation pathways. A time delay can therefore be introduced between initial complex formation and activation, to allow discrimination between cognate and noncognate binding based on the enhanced dissociation rate of the latter. In the context of ribosome synthesis, this delay may be particularly important. Major problems will be incorrect binding of factors and the failure of factors to bind in a timely manner, both of which will presumably occur very frequently. We speculate that the time delay before activation of the proofreading system generally takes care of these during normal maturation. Degradation will be a "last resort" when things have gone badly wrong.

Generation of each proofread intermediate absolutely requires an energy input—to avoid the RNA surveillance machinery acting as a Maxwell's Demon, a theoretical (and impossible) entity that can sort molecules without expending energy. To preferentially degrade incorrectly assembled complexes, the system must "know" that the complex is incorrect. But all knowledge comes at a price—in this case the price is the energy required to offset the entropic decrease involved in separating the two populations into correct and incorrect. Because of the energy input, a kinetic proofreading system (Figure 3C) is potentially more accurate than can possibly be achieved by systems utilizing binding equilibria alone. Yeast ribosome synthesis involves 19 RNA-stimulated ATPases (RNA helicases) and 6 GTPases (Henras et al., 2008), and we speculate that these provide the energy for kinetic proofreading.

Hopfield (Hopfield, 1974) further proposed that overall accuracy could be enhanced by introducing multiple steps, each of which is essentially irreversible because it is accompanied by the expenditure of energy. Surveillance can occur at each step in this "molecular ratchet," giving very high overall accuracy. We suggest that many steps in ribosome synthesis act as individual kinetic proofreading modules, providing a potential rationale for the Byzantine complexity of the biogenesis pathway.

Conclusions

Almost all organisms analyzed possess multiple, often partially redundant RNA degradation systems that collectively show

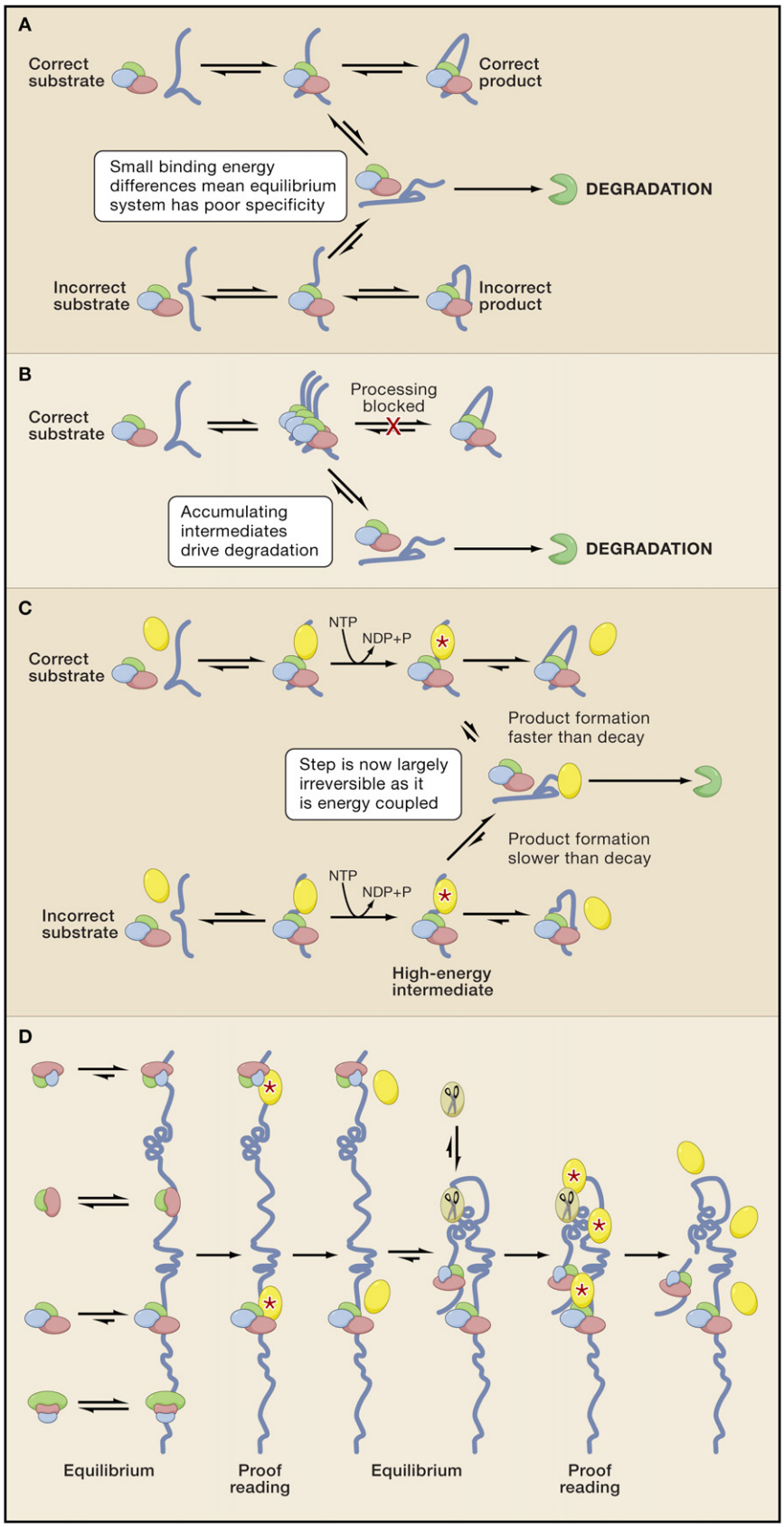

Figure 3. Models for Surveillance during RNA-Protein Complex Formation

(A) Equilibrium model for RNP assembly. Formation of the correct complex is favored over the incorrect complex due to the difference in binding energy of the two resulting substrates. The system can also discriminate toward the correct substrate in formation of the product and toward the incorrect product in a degradative side reaction. This results in a discrimination process whose effectiveness is solely related to the difference in binding energies, which will be low for near-cognate interactions.

(B) Effects of inhibition of processing. As the reactions are in equilibrium, increased accumulation of the precursor will drive increased degradation by mass action. Note, however, that a large increase in flux through the degradation pathway requires a large increase in the precursor pool, whereas this is not observed for most yeast mutants deficient in ribosome synthesis.

(C) Kinetic proofreading. Here, the complexes that form initially are not converted directly to product. Instead, a nonequilibrium reaction generates a transient high-energy intermediate (indicated with an asterisk) that is subsequently converted to the products or channeled into a discard pathway. The proofreading allows discrimination against binding of the incorrect substrate. To obtain high overall fidelity, the proofreading modules can be repeated, as long as each conversion involves an energy input.

(D) Kinetic proofreading in RNP assembly. In this model, any RNP assembly pathway can be schematized as a series of equilibrium-binding steps for RNAs and/or proteins, each separated by proofreading modules. For complex RNPs such as ribosomes, the number of proofreading modules would presumably be quite large. Successive proofreading modules act as a "molecular ratchet" increasing overall fidelity.

great potency in clearing RNAs and RNA-protein complexes that are defective or no longer required. Despite many differences in detail, key features of RNA degradation have been conserved over long evolutionary distances, underlining its consistently high importance. The major use of regulatory RNAs in both bacteria and eukaryotes probably makes it imperative that RNA degradation intermediates not be allowed to accumulate, given that these are likely to have detrimental effects on the regulation of gene expression.

The maturation pathways of many RNPs are strikingly complex, and to a degree that appears to be quite excessive. Pre-tRNAs, pre-snRNAs, and at least some pre-snoRNAs can be exported to the cytoplasm only to be subsequently reimported (Hopper and Shaheen, 2008; Watkins et al., 2007; Yong et al., 2004), and even apparently simple 3′ trimming can turn out, on closer inspection, to contain multiple intermediate steps (see for example Kufel et al., 2003). We speculate that, in part, the evolution of complexity in RNP maturation represents the proliferation of proofreading modules. At the very least, we predict that the presence of kinetic proofreading modules renders such complexity feasible without unacceptable error rates during assembly and processing.

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REFERENCES

Ahola, T., den Boon, J.A., and Ahlquist, P. (2000). Helicase and capping enzyme active site mutations in brome mosaic virus protein 1a cause defects in template recruitment, negative-strand RNA synthesis, and viral RNA capping. J. Virol. *74*, 8803–8811.

Allmang, C., Petfalski, E., Podtelejnikov, A., Mann, M., Tollervey, D., and Mitchell, P. (1999). The yeast exosome and human PM-Scl are related complexes of 3′->5′ exonucleases. Genes Dev. *13*, 2148–2158.

Amaral, P.P., Dinger, M.E., Mercer, T.R., and Mattick, J.S. (2008). The eukaryotic genome as an RNA machine. Science *319*, 1787–1789.

Ansel, K.M., Pastor, W.A., Rath, N., Lapan, A.D., Glasmacher, E., Wolf, C., Smith, L.C., Papadopoulou, N., Lamperti, E.D., Tahiliani, M., et al. (2008). Mouse Eri1 interacts with the ribosome and catalyzes 5.8S rRNA processing. Nat. Struct. Mol. Biol. *15*, 523–530.

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D.A., and Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. Science *315*, 1709–1712.

Beau, I., Esclatine, A., and Codogno, P. (2008). Lost to translation: when autophagy targets mature ribosomes. Trends Cell Biol. *18*, 311–314.

Beckham, C.J., Light, H.R., Nissan, T.A., Ahlquist, P., Parker, R., and Noueiry, A. (2007). Interactions between brome mosaic virus RNAs and cytoplasmic processing bodies. J. Virol. *81*, 9759–9768.

Beggs, J.D. (2005). Lsm proteins and RNA processing. Biochem. Soc. Trans. *33*, 433–438.

Bernstein, J., Patterson, D.N., Wilson, G.M., and Toth, E.A. (2008). Characterization of the essential activities of Saccharomyces cerevisiae Mtr4p, a 3′->5′ helicase partner of the nuclear exosome. J. Biol. Chem. *283*, 4930–4942.

Birney, E., Stamatoyannopoulos, J.A., Dutta, A., Guigo, R., Gingeras, T.R., Margulies, E.H., Weng, Z., Snyder, M., Dermitzakis, E.T., Thurman, R.E., et al. (2007). Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature *447*, 799–816.

Brandhorst, B.P., and McConkey, E.H. (1974). Stability of nuclear RNA in mammalian cells. J. Mol. Biol. *85*, 451–463.

Brouns, S.J., Jore, M.M., Lundgren, M., Westra, E.R., Slijkhuis, R.J., Snijders, A.P., Dickman, M.J., Makarova, K.S., Koonin, E.V., and van der Oost, J. (2008). Small CRISPR RNAs guide antiviral defense in prokaryotes. Science *321*, 960–964.

Buhler, M., Spies, N., Bartel, D.P., and Moazed, D. (2008). TRAMP-mediated RNA surveillance prevents spurious entry of RNAs into the Schizosaccharomyces pombe siRNA pathway. Nat. Struct. Mol. Biol. *15*, 1015–1023.

Carroll, K.L., Ghirlando, R., Ames, J.M., and Corden, J.L. (2007). Interaction of yeast RNA-binding proteins Nrd1 and Nab3 with RNA polymerase II terminator elements. RNA *13*, 361–373.

Celesnik, H., Deana, A., and Belasco, J.G. (2007). Initiation of RNA decay in *Escherichia coli* by 5′ pyrophosphate removal. Mol. Cell *27*, 79–90.

Chandran, V., Poljak, L., Vanzo, N.F., Leroy, A., Miguel, R.N., Fernandez-Recio, J., Parkinson, J., Burns, C., Carpousis, A.J., and Luisi, B.F. (2007). Recognition and cooperation between the ATP-dependent RNA helicase RhlB and ribonuclease RNase E. J. Mol. Biol. *367*, 113–132.

Chapman, K.B., and Boeke, J.D. (1991). Isolation and characterization of the gene encoding yeast debranching enzyme. Cell *65*, 483–492.

Chernyakov, I., Whipple, J.M., Kotelawala, L., Grayhack, E.J., and Phizicky, E.M. (2008). Degradation of several hypomodified mature tRNA species in Saccharomyces cerevisiae is mediated by Met22 and the 5′-3′ exonucleases Rat1 and Xrn1. Genes Dev. *22*, 1369–1380.

Copela, L.A., Fernandez, C.F., Sherrer, R.L., and Wolin, S.L. (2008). Competition between the Rex1 exonuclease and the La protein affects both Trf4p-mediated RNA quality control and pre-tRNA maturation. RNA *14*, 1214–1227.

Cordin, O., Banroques, J., Tanner, N.K., and Linder, P. (2006). The DEAD-box protein family of RNA helicases. Gene *367*, 17–37.

Core, L.J., Waterfall, J.J., and Lis, J.T. (2008). Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. Science *322*, 1845–1848.

Davis, C.A., and Ares, M.J. (2006). Accumulation of unstable promoter-associated transcripts upon loss of the nuclear exosome subunit Rrp6p in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA *103*, 3262–3267.

de la Sierra-Gallay, I.L., Zig, L., Jamalli, A., and Putzer, H. (2008). Structural insights into the dual activity of RNase J. Nat. Struct. Mol. Biol. *15*, 206–212.

Deana, A., Celesnik, H., and Belasco, J.G. (2008). The bacterial enzyme RppH triggers messenger RNA degradation by 5′ pyrophosphate removal. Nature *451*, 355–358.

Deshmukh, M.V., Jones, B.N., Quang-Dang, D.U., Flinders, J., Floor, S.N., Kim, C., Jemielity, J., Kalek, M., Darzynkiewicz, E., and Gross, J.D. (2008). mRNA decapping is promoted by an RNA-binding channel in Dcp2. Mol. Cell *29*, 324–336.

Deutscher, M.P. (2006). Degradation of RNA in bacteria: comparison of mRNA and stable RNA. Nucleic Acids Res. *34*, 659–666.

Diederichs, S., and Haber, D.A. (2007). Dual role for argonautes in microRNA processing and posttranscriptional regulation of microRNA expression. Cell *131*, 1097–1108.

Doma, M.K., and Parker, R. (2007). RNA quality control in eukaryotes. Cell *131*, 660–668.

Dziembowski, A., Lorentzen, E., Conti, E., and Seraphin, B. (2007). A single subunit, Dis3, is essentially responsible for yeast exosome core activity. Nat. Struct. Mol. Biol. *14*, 15–22.

Egloff, S., O'Reilly, D., Chapman, R.D., Taylor, A., Tanzhaus, K., Pitts, L., Eick, D., and Murphy, S. (2007). Serine-7 of the RNA polymerase II CTD is specifically required for snRNA gene expression. Science *318*, 1777–1779.

Eulalio, A., Huntzinger, E., and Izaurralde, E. (2008). Getting to the root of miRNA-mediated gene silencing. Cell *132*, 9–14.

Eulalio, A., Huntzinger, E., Nishihara, T., Rehwinkel, J., Fauser, M., and Izaurralde, E. (2009). Deadenylation is a widespread effect of miRNA regulation. RNA *15*, 21–32.

Franks, T.M., and Lykke-Andersen, J. (2008). The control of mRNA decapping and P-body formation. Mol. Cell *32*, 605–615.

Fukuda, T., Yamagata, K., Fujiyama, S., Matsumoto, T., Koshida, I., Yoshimura, K., Mihara, M., Naitou, M., Endoh, H., Nakamura, T., et al. (2007). DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. Nat. Cell Biol. *9*, 604–611.

Gabel, H.W., and Ruvkun, G. (2008). The exonuclease ERI-1 has a conserved dual role in 5.8S rRNA processing and RNAi. Nat. Struct. Mol. Biol. *15*, 531–533.

Garneau, N.L., Sokoloski, K.J., Opyrchal, M., Neff, C.P., Wilusz, C.J., and Wilusz, J. (2008). The 3′ untranslated region of sindbis virus represses deadenylation of viral transcripts in mosquito and mammalian cells. J. Virol. *82*, 880–892.

Gatfield, D., and Izaurralde, E. (2004). Nonsense-mediated messenger RNA decay is initiated by endonucleolytic cleavage in Drosophila. Nature *429*, 575–578.

Ghaemmaghami, S., Huh, W.K., Bower, K., Howson, R.W., Belle, A., Dephoure, N., O'Shea, E.K., and Weissman, J.S. (2003). Global analysis of protein expression in yeast. Nature *425*, 737–741.

Glisovic, T., Bachorik, J.L., Yong, J., and Dreyfuss, G. (2008). RNA-binding proteins and post-transcriptional gene regulation. FEBS Lett. *582*, 1977–1986.

Gudipati, R.K., Villa, T., Boulay, J., and Libri, D. (2008). Phosphorylation of the RNA polymerase II C-terminal domain dictates transcription termination choice. Nat. Struct. Mol. Biol. *15*, 786–794.

Hale, C., Kleppe, K., Terns, R.M., and Terns, M.P. (2008). Prokaryotic silencing (psi)RNAs in Pyrococcus furiosus. RNA *14*, 2572–2579.

Han, J., Kim, D., and Morris, K.V. (2007). Promoter-associated RNA is required for RNA-directed transcriptional gene silencing in human cells. Proc. Natl. Acad. Sci. USA *104*, 12422–12427.

He, Y., Vogelstein, B., Velculescu, V.E., Papadopoulos, N., and Kinzler, K.W. (2008). The antisense transcriptomes of human cells. Science *322*, 1855–1857.

Henras, A., Soudet, J., Gérus, M., Lebaron, S., Caizergues-Ferrer, M., Mougin, A., and Henry, Y. (2008). The post-transcriptional steps of eukaryotic ribosome biogenesis. Cell. Mol. Life Sci. *65*, 2334–2359.

Hong, Y., Cole, T.E., Brasier, C.M., and Buck, K.W. (1998). Novel structures of two virus-like RNA elements from a diseased isolate of the Dutch elm disease fungus, Ophiostoma novo-ulmi. Virology *242*, 80–89.

Hopfield, J.J. (1974). Kinetic proofreading: a new mechanism for reducing errors in biosynthetic processes requiring high specificity. Proc. Natl. Acad. Sci. USA *71*, 4135–4139.

Hopper, A.K., and Shaheen, H.H. (2008). A decade of surprises for tRNA nuclear-cytoplasmic dynamics. Trends Cell Biol. *18*, 98–104.

Houseley, J., LaCava, J., and Tollervey, D. (2006). RNA-quality control by the exosome. Nat. Rev. Mol. Cell Biol. *7*, 529–539.

Houseley, J., Kotovic, K., El Hage, A., and Tollervey, D. (2007). Trf4 targets ncRNAs from telomeric and rDNA spacer regions and functions in rDNA copy number control. EMBO J. *26*, 4996–5006.

Houseley, J., Rubbi, L., Grunstein, M., Tollervey, D., and Vogelauer, M. (2008). A ncRNA modulates histone modification and mRNA induction in the yeast GAL gene cluster. Mol. Cell *32*, 685–695.

Huang, H.Y., Chang, H.Y., Chou, C.H., Tseng, C.P., Ho, S.Y., Yang, C.D., Ju, Y.W., and Huang, H.D. (2008). sRNAMap: genomic maps for small non-coding RNAs, their regulators and their targets in microbial genomes. Nucleic Acids Res. *37*, D150–D154.

Huntzinger, E., Kashima, I., Fauser, M., Sauliere, J., and Izaurralde, E. (2008). SMG6 is the catalytic endonuclease that cleaves mRNAs containing nonsense codons in metazoan. RNA *14*, 2609–2617.

Isken, O., and Maquat, L.E. (2008). The multiple lives of NMD factors: balancing roles in gene and genome regulation. Nat. Rev. Genet. *9*, 699–712.

Jochl, C., Rederstorff, M., Hertel, J., Stadler, P.F., Hofacker, I.L., Schrettl, M., Haas, H., and Huttenhofer, A. (2008). Small ncRNA transcriptome analysis from Aspergillus fumigatus suggests a novel mechanism for regulation of protein synthesis. Nucleic Acids Res. *36*, 2677–2689.

Kaczanowska, M., and Ryden-Aulin, M. (2007). Ribosome biogenesis and the translation process in Escherichia coli. Microbiol. Mol. Biol. Rev. *71*, 477–494.

Kadaba, S., Wang, X., and Anderson, J.T. (2006). Nuclear RNA surveillance in Saccharomyces cerevisiae: Trf4p-dependent polyadenylation of nascent hypomethylated tRNA and an aberrant form of 5S rRNA. RNA *12*, 508–521.

Karbstein, K. (2009). Mechanisms of quality control in eukaryotic ribosome assembly. In Wiley Encyclopedia of Chemical Biology, T.P. Begley, ed. (Oxford: John Wiley & Sons).

Kashima, T., Rao, N., David, C.J., and Manley, J.L. (2007). hnRNP A1 functions with specificity in repression of SMN2 exon 7 splicing. Hum. Mol. Genet. *16*, 3149–3159.

Kim, J.H., and Richter, J.D. (2006). Opposing polymerase-deadenylase activities regulate cytoplasmic polyadenylation. Mol. Cell *24*, 173–183.

Kim, M., Vasiljeva, L., Rando, O.J., Zhelkovsky, A., Moore, C., and Buratowski, S. (2006). Distinct pathways for snoRNA and mRNA termination. Mol. Cell *24*, 723–734.

Kraft, C., Deplazes, A., Sohrmann, M., and Peter, M. (2008). Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. Nat. Cell Biol. *10*, 602–610.

Kufel, J., Allmang, C., Verdone, L., Beggs, J., and Tollervey, D. (2003). A complex pathway for 3′ processing of the yeast U3 snoRNA. Nucleic Acids Res. *31*, 6788–6797.

Kwak, J.E., Drier, E., Barbee, S.A., Ramaswami, M., Yin, J.C., and Wickens, M. (2008). GLD2 poly(A) polymerase is required for long-term memory. Proc. Natl. Acad. Sci. USA *105*, 14644–14649.

LaCava, J., Houseley, J., Saveanu, C., Petfalski, E., Thompson, E., Jacquier, A., and Tollervey, D. (2005). RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. Cell *21*, 713–724.

LaRiviere, F.J., Cole, S.E., Ferullo, D.J., and Moore, M.J. (2006). A late-acting quality control process for mature eukaryotic rRNAs. Mol. Cell *24*, 619–626.

Lebreton, A., Tomecki, R., Dziembowski, A., and Séraphin, B. (2008). Endonucleolytic RNA cleavage by a eukaryotic exosome. Nature *456*, 993–996.

Lee, H.J., Lee, J., Shin, M.K., and Ryu, W.S. (2008). Polyadenylation is dispensable for encapsidation and reverse transcription of hepatitis B viral pregenomic RNA. Mol. Cells *25*, 545–552.

Liu, Q., Greimann, J.C., and Lima, C.D. (2006). Reconstitution, activities, and structure of the eukaryotic RNA exosome. Cell *127*, 1223–1237.

Lorentzen, E., and Conti, E. (2006). The exosome and the proteasome: nano-compartments for degradation. Cell *125*, 651–654.

Lorentzen, E., Walter, P., Fribourg, S., Evguenieva-Hackenberg, E., Klug, G., and Conti, E. (2005). The archaeal exosome core is a hexameric ring structure with three catalytic subunits. Nat. Struct. Mol. Biol. *12*, 575–581.

Luke, B., Panza, A., Redon, S., Iglesias, N., Li, Z., and Lingner, J. (2008). The Rat1p 5′ to 3′ exonuclease degrades telomeric repeat-containing RNA and promotes telomere elongation in *Saccharomyces cerevisiae*. Mol. Cell *32*, 465–477.

Maeda, N., Kasukawa, T., Oyama, R., Gough, J., Frith, M., Engstrom, P.G., Lenhard, B., Aturaliya, R.N., Batalov, S., Beisel, K.W., et al. (2006). Transcript annotation in FANTOM3: mouse gene catalog based on physical cDNAs. PLoS Genet. *2*, e62. 10.1371/journal.pgen.0020062.

Mathy, N., Benard, L., Pellegrini, O., Daou, R., Wen, T., and Condon, C. (2007). 5′-to-3′ exoribonuclease activity in bacteria: role of RNase J1 in rRNA maturation and 5′ stability of mRNA. Cell *129*, 681–692.

Milligan, L., Decourty, L., Saveanu, C., Rappsilber, J., Ceulemans, H., Jacquier, A., and Tollervey, D. (2008). A yeast exosome cofactor, Mpp6, functions in RNA surveillance and in the degradation of noncoding RNA transcripts. Mol. Cell. Biol. *28*, 5446–5457.

Mohanty, B.K., and Kushner, S.R. (2000). Polynucleotide phosphorylase functions both as a 3′-> 5′ exonuclease and a poly(A) polymerase in Escherichia coli. Proc. Natl. Acad. Sci. USA *97*, 11966–11971.

Morlando, M., Ballarino, M., Gromak, N., Pagano, F., Bozzoni, I., and Proudfoot, N.J. (2008). Primary microRNA transcripts are processed co-transcriptionally. Nat. Struct. Mol. Biol. *15*, 902–909.

Mroczek, S., and Kufel, J. (2008). Apoptotic signals induce specific degradation of ribosomal RNA in yeast. Nucleic Acids Res. *36*, 2874–2888.

Mullen, T.E., and Marzluff, W.F. (2008). Degradation of histone mRNA requires oligouridylation followed by decapping and simultaneous degradation of the mRNA both 5′ to 3′ and 3′ to 5′. Genes Dev. *22*, 50–65.

Neil, H., Malabat, C., d'Aubenton-Carafa, Y., Xu, Z., Steinmetz, L.M., and Jacquier, A. (2009). Widespread bidirectional promoters are the major source of cryptic transcripts in yeast. Nature. Published online January 25, 2009. 10.1038/nature07747.

Noueiry, A.O., Diez, J., Falk, S.P., Chen, J., and Ahlquist, P. (2003). Yeast Lsm1p-7p/Pat1p deadenylation-dependent mRNA-decapping factors are required for brome mosaic virus genomic RNA translation. Mol. Cell. Biol. *23*, 4094–4106.

Peng, Y., Liu, X., and Schoenberg, D.R. (2008). The 90-kDa heat shock protein stabilizes the polysomal ribonuclease 1 mRNA endonuclease to degradation by the 26S proteasome. Mol. Biol. Cell *19*, 546–552.

Portnoy, V., Evguenieva-Hackenberg, E., Klein, F., Walter, P., Lorentzen, E., Klug, G., and Schuster, G. (2005). RNA polyadenylation in Archaea: not observed in Haloferax while the exosome polynucleotidylates RNA in Sulfolobus. EMBO Rep. *6*, 1188–1193.

Preker, P., Nielsen, J., Kammler, S., Lykke-Andersen, S., Christensen, M.S., Mapendano, C.K., Schierup, M.H., and Jensen, T.H. (2008). RNA exosome depletion reveals transcription upstream of active human promoters. Science *322*, 1851–1854.

Rajkowitsch, L., Chen, D., Stampfl, S., Semrad, K., Waldsich, C., Mayer, O., Jantsch, M.F., Konrat, R., Bläsi, U., and Schroeder, R. (2008). RNA chaperones, RNA annealers and RNA helicases. RNA Biol. *4*, 118–130.

Ramachandran, V., and Chen, X. (2008). Degradation of microRNAs by a family of exoribonucleases in Arabidopsis. Science *321*, 1490–1492.

Rissland, O.S., and Norbury, C.J. (2008). The Cid1 poly(U) polymerase. Biochim. Biophys. Acta *1779*, 286–294.

Rougemaille, M., Dieppois, G., Kisseleva-Romanova, E., Gudipati, R.K., Lemoine, S., Blugeon, C., Boulay, J., Jensen, T.H., Stutz, F., Devaux, F., and Libri, D. (2008a). THO/Sub2p functions to coordinates 3′-end processing with gene-nuclear pore association. Cell *135*, 308–321.

Rougemaille, M., Villa, T., Gudipati, R.K., and Libri, D. (2008b). mRNA journey to the cytoplasm: attire required. Biol. Cell *100*, 327–342.

Rouhana, L., Wang, L., Buter, N., Kwak, J.E., Schiltz, C.A., Gonzalez, T., Kelley, A.E., Landry, C.F., and Wickens, M. (2005). Vertebrate GLD2 poly(A) polymerases in the germline and the brain. RNA *11*, 1117–1130.

Schaeffer, D., Tsanova, B., Barbas, A., Reis, F.P., Dastidar, E.G., Sanchez-Rotunno, M., Arraiano, C.M., and van Hoof, A. (2008). The exosome contains domains with specific endoribonuclease, exoribonuclease and cytoplasmic mRNA decay activities. Nat. Struct. Mol. Biol. *16*, 56–62.

Schneider, C., Anderson, J.T., and Tollervey, D. (2007). The exosome subunit Rrp44 plays a direct role in RNA substrate recognition. Mol. Cell *27*, 324–331.

Schneider, C., Leung, E., Brown, J., and Tollervey, D. (2009). The exosome subunit Rrp44 has endonuclease activity and shows functional redundancy with the cytoplasmic 5′-3′ exonuclease Xrn1. Nucleic Acids Res. Published online January 7, 2009. 10.1093/nar/gkn1020.

Schwede, A., Ellis, L., Luther, J., Carrington, M., Stoecklin, G., and Clayton, C. (2008). A role for Caf1 in mRNA deadenylation and decay in trypanosomes and human cells. Nucleic Acids Res. *36*, 3374–3388.

Seila, A.C., Calabrese, J.M., Levine, S.S., Yeo, G.W., Rahl, P.B., Flynn, R.A., Young, R.A., and Sharp, P.A. (2008). Divergent transcription from active promoters. Science *322*, 1849–1851.

She, M., Decker, C.J., Svergun, D.I., Round, A., Chen, N., Muhlrad, D., Parker, R., and Song, H. (2008). Structural basis of Dcp2 recognition and activation by Dcp1. Mol. Cell *29*, 337–349.

Shyu, A.B., Wilkinson, M.F., and van Hoof, A. (2008). Messenger RNA regulation: to translate or to degrade. EMBO J. *27*, 471–481.

Sittka, A., Lucchini, S., Papenfort, K., Sharma, C.M., Rolle, K., Binnewies, T.T., Hinton, J.C., and Vogel, J. (2008). Deep sequencing analysis of small noncoding RNA and mRNA targets of the global post-transcriptional regulator, Hfq. PLoS Genet. *4*, e1000163. 10.1371/journal.pgen.1000163.

Skruzny, M., Schneider, C., Racz, A., Weng, J., Tollervey, D., and Hurt, E. (2008). An endoribonuclease functionally linked to perinuclear mRNP quality control associates with the nuclear pore complexes. PLoS Biol. *7*, e8. 10.1371/journal.pbio.1000008.

Slomovic, S., Laufer, D., Geiger, D., and Schuster, G. (2006). Polyadenylation of ribosomal RNA in human cells. Nucleic Acids Res. *34*, 2966–2975.

Stalder, L., and Muhlemann, O. (2008). The meaning of nonsense. Trends Cell Biol. *18*, 315–321.

Staley, J.P., and Guthrie, C. (1998). Mechanical devices of the spliceosome: motors, clocks, springs, and things. Cell *92*, 315–326.

Stevenson, A.L., and Norbury, C.J. (2006). The Cid1 family of non-canonical poly(A) polymerases. Yeast *23*, 991–1000.

Struhl, K. (2007). Transcriptional noise and the fidelity of initiation by RNA polymerase II. Nat. Struct. Mol. Biol. *14*, 103–105.

Symmons, M.F., Williams, M.G., Luisi, B.F., Jones, G.H., and Carpousis, A.J. (2002). Running rings around RNA: a superfamily of phosphate-dependent RNases. Trends Biochem. Sci. *27*, 11–18.

Taghbalout, A., and Rothfield, L. (2008). RNaseE and RNA helicase B play central roles in the cytoskeletal organization of the RNA degradosome. J. Biol. Chem. *283*, 13850–13855.

Thompson, D.M., Lu, C., Green, P.J., and Parker, R. (2008). tRNA cleavage is a conserved response to oxidative stress in eukaryotes. RNA *14*, 2095–2103.

Urban, J.H., and Vogel, J. (2008). Two seemingly homologous noncoding RNAs act hierarchically to activate glmS mRNA translation. PLoS Biol. *6*, e64. 10.1371/journal.pbio.0060064.

Vanácová, S., Wolf, J., Martin, G., Blank, D., Dettwiler, S., Friedlein, A., Langen, H., Keith, G., and Keller, W. (2005). A new yeast poly(A) polymerase complex involved in RNA quality control. PLoS Biol. *3*, e189. 10.1371/journal.pbio.0030189.

Vasiljeva, L., Kim, M., Mutschler, H., Buratowski, S., and Meinhart, A. (2008). The Nrd1-Nab3-Sen1 termination complex interacts with the Ser5-phosphorylated RNA polymerase II C-terminal domain. Nat. Struct. Mol. Biol. *15*, 795–804.

Vasudevan, S., and Steitz, J.A. (2007). AU-rich-element-mediated upregulation of translation by FXR1 and argonaute 2. Cell *128*, 1105–1118.

Venables, J.P., Koh, C.S., Froehlich, U., Lapointe, E., Couture, S., Inkel, L., Bramard, A., Paquet, E.R., Watier, V., Durand, M., et al. (2008). Multiple and specific mRNA processing targets for the major human hnRNP proteins. Mol. Cell. Biol. *28*, 6033–6043.

Watkins, N.J., Lemm, I., and Luhrmann, R. (2007). Involvement of nuclear import and export factors in U8 box C/D snoRNP biogenesis. Mol. Cell. Biol. *27*, 7018–7027.

West, S., Gromak, N., Norbury, C.J., and Proudfoot, N.J. (2006). Adenylation and exosome-mediated degradation of co-transcriptionally cleaved pre-messenger RNA in human cells. Mol. Cell *17*, 437–443.

West, S., Proudfoot, N.J., and Dye, M.J. (2008). Molecular dissection of mammalian RNA polymerase II transcriptional termination. Mol. Cell *29*, 600–610.

Wierzbicki, A.T., Haag, J.R., and Pikaard, C.S. (2008). Noncoding transcription by RNA polymerase Pol IVb/Pol V mediates transcriptional silencing of overlapping and adjacent genes. Cell *135*, 635–648.

Wu, L., and Belasco, J.G. (2008). Let me count the ways: mechanisms of gene regulation by miRNAs and siRNAs. Mol. Cell *29*, 1–7.

Yong, J., Wan, L., and Dreyfuss, G. (2004). Why do cells need an assembly machine for RNA-protein complexes? Trends Cell Biol. *14*, 226–232.