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Review

RNA Quality Control in Eukaryotes

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Eukaryotic cells contain numerous RNA quality-control systems that are important for shaping the transcriptome of eukaryotic cells. These systems not only prevent accumulation of nonfunctional RNAs but also regulate normal mRNAs, repress viral and parasitic RNAs, and potentially contribute to the evolution of new RNAs and hence proteins. These quality-control circuits can be viewed as a series of kinetic competitions between steps in normal RNA biogenesis or function and RNA degradation pathways. These RNA quality-control circuits depend on specific adaptor proteins that target aberrant RNAs for degradation as well as the coupling of individual steps in mRNA biogenesis and function.

Introduction

The biogenesis and function of RNAs in eukaryotic cells involves a series of transitions of RNAs between different protein complexes and subcellular compartments. For example, the process of mRNA biogenesis and function involves transcription, capping, splicing, polyadenylation, nuclear-cytoplasmic transport, translation, and degradation in the cytoplasm. During these processes, the mRNA associates in a dynamic manner with various complexes that both catalyze steps in biogenesis or function and promote exchange between the proteins that associate with the mRNA, thereby affecting subsequent events (Dreyfuss et al., 2002; Moore, 2005). Similar complex mechanisms of biogenesis and function occur for small nuclear (sn)RNAs, small nucleolar (sno)RNAs, tRNAs, rRNAs, and micro (mi)RNAs (reviewed in Wolin and Matera, 1999; Nazar, 2004; Kim, 2005). To avoid errors in RNA biogenesis and function, quality-control mechanisms have evolved that preferentially degrade aberrant or nonfunctional RNAs. Here, we discuss the diversity, principles, and consequences of RNA quality control in eukaryotes.

Aberrant RNAs arise by a variety of events. For example, aberrant mRNAs can be caused by mutations in the gene—such as those that create premature translation termination codons—that trigger rapid mRNA degradation (Maquat, 2004). Aberrant RNAs also arise from the inherent error rate of transcription, nuclear pre-RNA processing, or ribonucleoprotein (RNP) assembly of otherwise normal transcripts. For example, transcription of variant 5S ribosomal genes from a large gene family in frog oocytes produces some transcripts that fail to terminate properly; such transcripts then misfold and are targeted for degradation (Shi et al., 1996). Indeed, aberrant RNAs may often be produced from multigene families due to the reduced selective pressure on each individual

Table 1. Quality Control of Cytoplasmic RNAs

RNA	Defect	Basis of Specificity	Consequence of Quality Control
tRNA	Defective tRNA modification	unknown	Rapid tRNA decay (RTD) by an unknown nuclease (Alexandrov et al., 2006)
rRNA	Functional defect in rRNA	unknown	Nonfunctional rRNA decay (NRD), decay of mutant rRNA by an unknown mechanism (LaRiviere et al., 2006)
mRNA	Aberrant translation termination	Recruitment of Upf proteins to termination complex	Nonsense-mediated decay (NMD), rapid deadenylation, decapping, 3′-5′ decay, and some endonuclease cleavages (Isken and Maquat, 2007)
mRNA	No stop codon	Recruitment of Ski7p to elongation stall with no mRNA in A site of the ribosome	Nonstop decay (NSD), Ski7p recruitment of exosome, and rapid 3′-5′ degradation (van Hoof et al., 2002; Frischmeyer et al., 2002)
mRNA	Strong stall in translation elongation	Recruitment of Hbs1p and Dom34p to A site of the ribosome	No-Go decay (NGD), endonucleolytic cleavage, and exonucleolytic decay of fragments (Doma and Parker, 2006)
mRNA	Translation beyond normal stop codon into 3′ UTR	Unknown, removal of 3′ UTR binding proteins?	Ribosome extension-mediated decay (REMD), accelerated deadenylation, and decay (Kong and Liebhaber, 2007; Inada and Aiba, 2005)

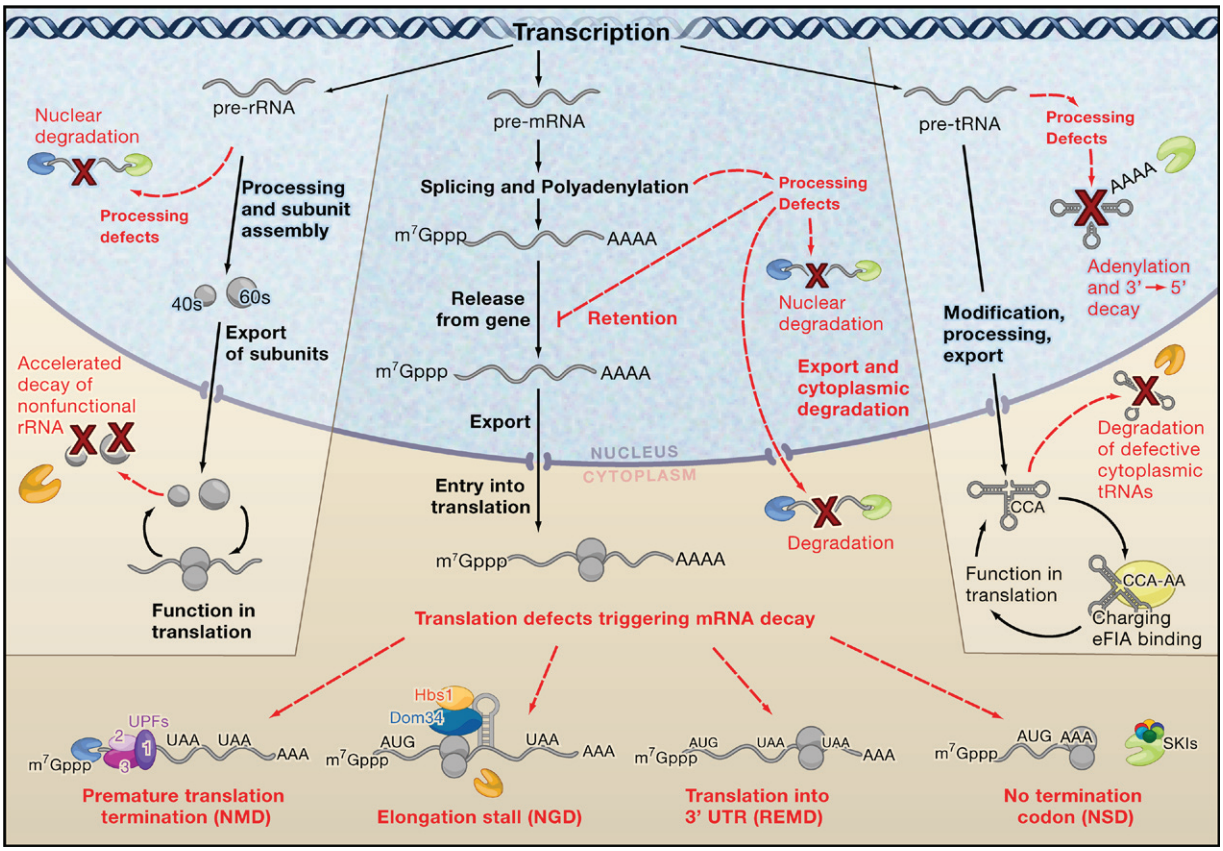

Figure 1. Diversity of RNA Quality-Control Systems in Eukaryotes

The figure depicts some of the known RNA quality-control systems for aberrant rRNA, mRNA, and tRNA in eukaryotic cells. These and additional quality-control mechanisms are summarized in Tables 1 and 2.

gene copy (O'Brien and Wolin, 1994). Aberrant RNAs can also be produced by transcription of intergenic regions, which yields RNAs that lack functional characteristics and are thus rapidly degraded (Thompson and Parker, 2007; Wyers et al., 2005; Davis and Ares, 2006). Finally, quality-control systems also repress the function of, or degrade, parasitic RNAs arising from repetitive elements and transposons.

RNA quality-control mechanisms are known to target aberrant RNAs for degradation by a few conserved nucleases (reviewed in Parker and Song, 2004; Houseley et al., 2006; Isken and Maquat, 2007). Quality control in the cytoplasm is carried out by the exosome comprising a ten-subunit core complex that catalyzes 3′ to 5′ exonucleolytic degradation and Xrn1p, a 5′ to 3′ exonuclease that requires its mRNA target molecules to be decapped. In the nucleus, the exosome plays the major role in RNA quality control, although a paralog of Xrn1p, termed Xrn2/Rat1p in yeast, may also affect nuclear RNA degradation (Fang et al., 2005; Danin-Kreiselman et al., 2003; Bousquet-Antonelli et al., 2000). In the yeast nucleus, the core exosome complex also associates with an additional 3′ to 5′ exonuclease called Rrp6p (Allmang et al., 1999; Burkard and Butler, 2000), whereas in mammals the Rrp6p ortholog, PM/Scl100, is observed in both the cytoplasm and nucleus (Lejeune et al., 2003).

Quality Control of Cytoplasmic RNAs

Several quality-control mechanisms in the cytoplasm degrade eukaryotic mRNAs that have abnormalities in translation (Table 1; Figure 1). An emerging principle is that aberrant mRNAs can be distinguished from normal mRNAs by adaptor proteins that interact with the translation machinery and direct the aberrant mRNAs into a degradation pathway. In a pathway referred to as nonsense-mediated decay (NMD), mRNAs with premature translation termination codons are distinguished from normal mRNAs in a process involving the conserved Upf proteins and their interactions with a translation termination complex (for detailed review, see Isken and Maquat, 2007). Depending on the organism or cell type, NMD can target aberrant mRNAs for decapping and 5′ to 3′ degradation by Xrn1p; endonucleolytic cleavage; or accelerated deadenylation and 3′ to 5′ degradation by the exosome (Isken and Maquat, 2007). In a process referred to as nonstop decay (NSD), ribosomes that have reached the end of mRNAs lacking translation termination codons

Table 2. Nuclear RNA Quality Control

RNA	Defect	Consequences of Quality Control
tRNA (yeast)	Missing modification, processing defects	TRAMP-dependent adenylation and 3′-5′ decay by the exosome (Kadaba et al., 2004; Vanacova et al., 2005; Schneider et al., 2007)
rRNA (yeast and plants)	Stochastic errors or defects in rRNA processing and/or assembly	TRAMP-dependent adenylation and 3′ to 5′ decay by the exosome (Dez et al., 2006; Win et al., 2006; LaCava et al., 2005; J. Ecker and D. Belostotsky, personal communication); retention of immature ribosomes in the nucleus (Dez et al., 2007); adenylation by poly(A) polymerase and Rrp6-dependent decay (Kuai et al., 2004); nuclear 5′ to 3′ decay by Rat1p (Fang et al., 2005)
rRNA (yeast)	Generation of aberrant rRNA by incorporation of 5-fluorouracil	Adenylation and Rrp6p-dependent degradation (Fang et al., 2004)
5S rRNA (yeast)	Mutations or defective processing	Ro protein-dependent decay by unknown mechanism (Shi et al., 1996; Fuchs et al., 2006; O'Brien and Wolin, 1994; Stein et al., 2005); adenylation and 3′ to 5′ degradation (Kadaba et al., 2006)
SnRNAs and snoRNAs (yeast and plants)	Stochastic errors or mutant forms	TRAMP-dependent adenylation and 3′ to 5′ decay by exosome (Kadaba et al., 2006; Egecioglu et al., 2006; Win et al., 2006; LaCava et al., 2005; J. Ecker and D. Belostotsky, personal communication); adenylation and Rrp6p-dependent degradation (Davis and Ares, 2006)
mRNA (yeast)	Hyperadenylation/hypoadenylation; defects in THO/Sub2 complex; defects in 3′ end processing	Rrp6p and/or core exosome-dependent nuclear retention and degradation of RNA (Hilleren et al., 2001; Rougemaille et al., 2007; Libri et al., 2002; Thomsen et al., 2003; Das et al., 2003, 2006; Torchet et al., 2002)
mRNA (mammals)	Failure of polyadenylation or splicing defects	Retention of the mRNA near or at the transcription site (Custodio et al., 1999)
mRNA (mammals)	Absence of introns in a gene that normally contains introns	Accelerated nuclear degradation dependent on 3′ poly(A) tail (Conrad et al., 2006)
mRNA (yeast)	Splicing defect: not recognized by spliceosome	Export and cytoplasmic decapping and 5′ to 3′ decay (Hilleren and Parker, 2003; Legrain and Rosbash, 1989); retention in nucleus by MLP proteins (Sommer and Nehrbass, 2005)
mRNA (yeast)	Splicing defect; trapped lariat intermediate	Nuclear degradation by exosome (Bousquet-Antonelli et al., 2000); debranching, export, and cytoplasmic 5′ to 3′ decay by Xrn1p (Hilleren and Parker, 2003)
mRNA (yeast)	Defect in capping	Export and 5′ to 3′ decay by cytoplasmic Xrn1p (Schwer et al., 1998)
dsRNA (mammals)	Double-stranded RNA	RNA editing and nuclear retention (Zhang and Carmichael, 2001; DeCerbo and Carmichael, 2005)
Intergenic transcripts (yeast and plants)	No known function after transcription	TRAMP-dependent adenylation and 3′ to 5′ decay by the exosome (Thompson and Parker, 2007; Wyers et al., 2005; Davis and Ares, 2006; J. Ecker and D. Belostotsky, personal communication); export and 5′ to 3′ degradation by decapping and Xrn1p (Thompson and Parker, 2007)

recruit the exosome through the action of Ski7p—a paralog of the eEF1A (eukaryotic translation elongation factor 1A)—and eRF3 (eukaryotic release factor 3) proteins, which interact with the ribosomal A site during elongation or termination, respectively (Frischmeyer et al., 2002; van Hoof et al., 2002). This suggests that Ski7p recognizes the empty A site produced when a ribosome reaches the 3′ end of an mRNA. Similarly, when mRNAs have strong pauses in elongation, the mRNA is targeted for endonucleolytic cleavage in a process referred to as No-Go decay (NGD) (Doma and Parker, 2006). NGD is promoted by the Hbs1 and Dom34 proteins, which are paralogs of the translation termination factors eRF3 and eRF1 (eukaryotic release factor 1) and presumably interact with the stalled ribosome. Finally, when ribosomes inappropriately translate and then terminate within the 3′ UTR at least some mRNAs are destabilized in a process referred to as ribosome extension-mediated decay (REMD) (Inada and Aiba, 2005; Kong and Liebhaber, 2007).

Pathways also exist to degrade cytoplasmic rRNA and tRNAs that are defective in function. Specifically, yeast rRNAs incorporated into ribosomes that are defective in either peptide bond formation or the initiation of translation are degraded faster than wild-type rRNAs (LaRiviere et al., 2006). Similarly, yeast tRNAs that are missing multiple modifications are more rapidly degraded (Alexandrov et al., 2006). The manner by which these defective

rRNAs and tRNAs are targeted for degradation remains to be determined. One possibility is that ribosomes or tRNAs that are not engaged in translation are more susceptible to general nucleases, perhaps because of the absence of appropriate interacting proteins.

Quality Control of Nuclear RNAs

Numerous quality-control systems target nuclear RNAs that are defective in RNA-processing events (summarized in Table 2, Figure 1). These nuclear quality-control systems lead to three related mechanisms for preventing the function of the aberrant RNA. First, some defective RNAs are exported to the cytoplasm for degradation. For example, mutant yeast pre-mRNAs that are trapped as lariats prior to the second step of pre-mRNA splicing are debranched and exported to the cytoplasm for 5′ to 3′ digestion by Xrn1p (Hilleren and Parker, 2003). Similarly, some apparently nonfunctional intergenic transcripts appear to be exported and degraded in the cytoplasm (Thompson and Parker, 2007).

Aberrant or unprocessed nuclear RNAs can also be retained within the nucleus (Table 2). Nuclear retention may be important both to give time for RNA processing to be completed and to allow for a kinetically unfavorable nuclear degradation pathway to degrade the RNA (see below). Examples of nuclear retention of aberrant mRNAs include the retention of mRNAs with defects in splicing or polyadenylation in both yeast and mammalian cells (Custodio et al., 1999; Hilleren et al., 2001; Jensen et al., 2001). Interestingly, these aberrant RNAs are retained in the vicinity of the gene (Custodio et al., 1999; Thomsen et al., 2003), which has the potential to have feedback effects on transcription (see below). Similarly, long double-stranded (ds)RNAs that are extensively edited to contain inosines can be retained within the nucleus by binding a nuclear lamin-associated complex consisting of p54nrb, PSF, and matrin 3 (Zhang and Carmichael, 2001; DeCerbo and Carmichael, 2005).

Some aberrant RNAs appear to be degraded within the nucleus (Table 2). This conclusion is based on the observation that lesions in the nuclear 5′ to 3′ exonuclease Xrn2p, Rrp6p, and/or core exosome components show increased levels of several RNAs, processing intermediates, or intergenic transcripts. For example, yeast or plants defective in nuclear exosome function accumulate precursors of tRNAs, snoRNAs, snRNAs, and rRNAs, suggesting that some of these precursors are normally targeted for degradation by the exosome and are only revealed when the degradation process is blocked (Allmang et al., 2000; Kadaba et al., 2004, 2006; van Hoof et al., 2000; Egecioglu et al., 2006; J. Ecker and D. Belostotsky, personal communication). Evidence that at least the defective ribosomal subunits are degraded in the nucleus is that when degradation is inhibited, the defective ribosomal subunits are observed to accumulate in nuclear foci (Dez et al., 2006). Similarly, when mammalian intron-containing mRNAs are lacking their introns, the resulting aberrant mRNAs are more rapidly degraded and accumulate in the nucleus when degradation is inhibited (Conrad et al., 2006).

An important aspect of RNA quality control in the nucleus are specialized polyadenylation complexes referred to as TRAMP complexes, which contain a non-canonical poly(A) polymerase (Trf4 or Trf5 in yeast), an RNA-binding protein (Air1 or Air2), and an RNA helicase (Mtr4) (LaCava et al., 2005). Multiple lines of evidence argue that TRAMP complexes adenylate aberrant RNAs and thereby stimulate 3′ to 5′ degradation by Rrp6p and/or the exosome by providing a single-stranded extension for nuclease loading. For example, many of the aberrant RNAs or precursors that accumulate in yeast strains or plants defective in nuclear exosome function contain 3′ poly(A) tails, which are often dependent on the action of Trf4 and/or Trf5 (Kadaba et al., 2004, 2006; Dez et al., 2006; Egecioglu et al., 2006; LaCava et al., 2005; Wyers et al., 2005; J. Ecker and D. Belostotsky, personal communication). Moreover, experiments in vitro have shown that polyadenylation of defective tRNAs can promote their degradation by the exosome (Vanacova et al., 2005). The role of polyadenylation in stimulating RNA decay by 3′ to 5′ exonucleases—which is similar to the role of polyadenylation in prokaryotes—is a conserved mechanism to target RNAs for 3′ to 5′ destruction (Dreyfus and Regnier, 2002). The features that dictate preferential polyadenylation, and thereby degradation, of some RNAs by the TRAMP complex are unresolved. Possibilities include the specific recruitment of the TRAMP complex by RNA-binding proteins preferentially bound to aberrant RNAs. Alternatively, TRAMP complexes may polyadenylate any exposed RNA 3′ end at some rate such that whether an RNA is a substrate for polyadenylation and degradation may simply be a kinetic competition with normal RNA processing and export (see below).

Several perplexing observations indicate that our understanding of the functions of the TRAMP and/or exosome complexes in nuclear RNA metabolism is incomplete. First, strains lacking Trf4 and Rrp6 show increased levels, but also increased RNA decay rates, of the intergenic Srg1 transcript, which is primarily degraded in the cytoplasm (Thompson and Parker, 2007). Second, in addition to their role in nuclear degradation, Rrp6p and the nuclear core exosome are required for retention of mRNAs with aberrant 3′ end processing in the vicinity of the gene (Hilleren et al., 2001; Rougemaille et al., 2007; Thomsen et al., 2003; Libri et al., 2002). Finally, the observable population of yeast mRNAs with defects in poly(A) tail lengths, or defects in the THO/Sub2 complex, which promotes mRNP biogenesis and export, are surprisingly stable (Mandart and Parker, 1995; Hilleren and Parker, 2001; Rougemaille et al., 2007). One possible explanation for these observations is that aberrant transcripts retained at the gene have a feedback effect to decrease transcription, perhaps by forming RNA-DNA hybrids and decreasing transcriptional elongation (Huertas and Aguilera, 2003), although recent nuclear run-on

experiments argue against this model (Rougemaille et al., 2007). Alternatively, nuclear degradation may occur in competition with assembly of the RNA-processing machinery on nascent transcripts. In this view, either transcripts are degraded so rapidly that they cannot be observed in the steady-state population (Thompson and Parker, 2007; Rougemaille et al., 2007) or the transcripts associate with the processing machinery and are stable until processed, which would prevent ongoing degradation of transcripts undergoing processing.

Quality Control of Parasitic RNAs

RNA quality-control systems, some of which involve the TRAMP and exosome complexes, also play a role in limiting the expression of RNAs from repetitive elements, transposons, and viruses. For example, the host antiviral protein ZAP specifically recruits the exosome to some viral RNAs and thereby promotes their degradation (Guo et al., 2007). Moreover, because dsRNA is often a signature of parasitic or viral transcripts, metazoan cells have evolved numerous mechanisms to degrade or repress the function of dsRNA molecules including activation of the RNaseL endonuclease (Malathi et al., 2007), activation of the PKR protein kinase (Garcia et al., 2006), and a response to extracellular dsRNA that activates transcription of antiviral genes (Alexopoulou et al., 2001). Nuclear dsRNA can also undergo extensive adenosine to inosine editing and then be selectively retained within the nucleus (DeCerbo and Carmichael, 2005). Most eukaryotic cells also respond to dsRNA by RNA interference (RNAi), wherein small-interfering (si)RNAs are generated from the dsRNA and then assemble into the *R*NA-*i*nduced silencing *c*omplex (RISC). The RISC:siRNA complex then silences the expression of RNAs with sequences complementary to the siRNA by RNA degradation, or in some cases in plants and some fungi, siRNAs-directed methylation helps to silence repetitive regions of the genome (reviewed in Meister and Tuschl, 2004; Grewal and Jia, 2007).

Metazoans also express a class of 24–29 nucleotide RNAs called rasiRNA (repeat associated small-interfering RNA) or piRNAs (piwi-associated RNA) that function to silence transposons, repetitive sequences, and some heterochromatic regions (reviewed in Hartig et al., 2007; Saito et al., 2006). Generally, numerous piRNAs are produced from genomic clusters, often have homology to repetitive elements, and assemble with members of the PIWI protein family, which are related to the Argonaute proteins that are the core component of the RISC complex for siRNAs and miRNAs. The mechanisms by which piRNAs and PIWI proteins silence their targets are not yet clear but may involve aspects of RNA degradation as well as chromatin modifications and repression of transcription. Thus, this system of piRNAs represents a mechanism to repress the function of transposons and repetitive RNAs and, like other quality-control systems, is also likely to regulate some normal mRNAs.

Recent observations also suggest connections between silencing by small RNAs and degradation of RNA by the TRAMP and exosome complexes. For example, in fission yeast, efficient silencing of centromeric regions by small RNAs involves TRAMP-dependent targeting of some transcripts to Rrp6p and/or the exosome (Buhler et al., 2007). Defects in the exosome also reduce silencing at the mating type locus, suggesting that silencing of heterochromatic regions may sometimes involve RNA degradation of nascent transcripts (Buhler et al., 2007). Moreover, in *Chlamydomonas reinhardtii*, a noncanonical poly(A) polymerase is required for efficient decay of siRNA-targeted transcripts and appears to stimulate their exosome-dependent decay (Ibrahim et al., 2006). Finally, *Arabidopsis* plants defective in exosome function accumulate transcripts from regions highly coincident with repetitive elements and DNA methylation including multiple small RNA-producing loci (J. Ecker and D. Belostotsky, personal communication), suggesting that RNA degradation may commonly contribute to silencing of heterochromatic regions.

Kinetic Competition and Quality Control

A key to effective quality-control systems is accurate mechanisms to distinguish between aberrant and normal RNAs. A unifying principle is that quality-control circuits for RNA often can be viewed as kinetic competitions between the rate of a normal reaction in the life of an RNA and a quality-control event targeting the RNA for degradation (Figure 2). For example, the process of NGD appears to be the result of a competition between translation elongation and interaction of the Hbs1/Dom34 complex with the stalled ribosome (Doma and Parker, 2006). Note that if an aberrant RNA undergoes multiple cycles of quality control to determine whether it is normal or aberrant, then the overall effectiveness of quality control can be quite high even if the absolute difference in the rate between degradation and function at each cycle is small.

Examination of quality-control systems reveals recurring mechanistic features, often used in combinations, by which the efficiency of quality control and its cost can be optimized. First, some aberrant RNAs or RNPs have features that decrease the rate of the normal forward reaction, thereby giving more time for quality control (Figure 2B(i)). For example, assembly of the spliceosome, but failure to complete splicing, impedes nuclear mRNA export, at least in yeast (Legrain and Rosbash, 1989). Therefore, the completion of pre-mRNA processing prevents nuclear RNA degradation by removing inhibitors of export from the transcript. Second, some normal RNAs have features that promote the downstream normal event (Figure 2B(ii)). For example, nuclear pre-mRNA splicing also exerts a positive effect on transport to the cytoplasm by delivering export factors to the mRNA (Zhou et al., 2000; Cheng et al., 2006; Reed and Cheng, 2005). Finally, some aberrant RNAs have features that promote their recognition

by specific adaptor proteins that funnel those RNAs into the quality-control fate (Figure 2B(iii)). For example, the Ro protein binds to variant and misfolded 5S RNAs that contain an aberrant 3′ extension and may target them for degradation (O'Brien and Wolin, 1994; Shi et al., 1996; Stein et al., 2005; Fuchs et al., 2006).

An important feature of kinetic competitions leading to quality control is that any defect leading to a delay in the normal forward reaction will trigger quality control. In this manner, quality-control systems do not need to recognize specific defective features of an RNA or RNP but instead can function on a broad range of defects affecting the rate of a given step in biogenesis or function. One example of this phenomenon is in yeast where a variety of defects in proteins affecting 3′ end processing, hypoadenylation, or hyperadenylation of mRNAs all lead to retention of the aberrant mRNAs at the gene in a manner that can be suppressed by loss of Rrp6p (Hilleren et al., 2001; Rougemaille et al., 2007; Thomsen et al., 2003; Libri et al., 2002).

Consequences of Quality Control

One consequence of RNA quality control is that some "normal" RNAs will be subjected to degradation by quality control. For example, approximately 1% of the yeast mRNAs with a wild-type intron are estimated to be degraded at the second step of pre-mRNA splicing (Hilleren and Parker, 2003). Although these discarded mRNAs could represent errors in splicing such as the use of aberrant 5′ splice sites or branch points, they could simply reveal the cost of this specific quality-control circuit. Moreover, cells use quality-control circuits to control the levels of normal transcripts. For example, microarray analysis has revealed that levels of several "normal" mRNAs are reduced by the NMD pathway (reviewed in Isken and Maquat, 2007). Similarly, the levels of the yeast histone mRNAs are reduced in abundance by the TRAMP and nuclear exosome systems (Reis and Campbell, 2007). Cells also use the regulation of pre-mRNA splicing to produce mRNAs that are targeted to NMD, thereby downregulating the levels of transcripts from specific genes (Matlin et al., 2005). Thus, quality-control systems play a role in determining the levels of accumulation of normal RNAs.

The existence of RNA quality-control systems argues that numerous steps in RNA biogenesis have relatively low fidelity and a substantial population of transcripts is rapidly degraded. One example of this phenomenon is the process of 3′ end formation and polyadenylation of mRNAs in yeast. In yeast cDNA databases, approximately 1% of the ESTs correspond to mRNAs aberrantly polyadenylated within the coding region (Graber et al., 1999). Because these "nonstop" mRNAs are degraded at least ten-fold faster than the normal mRNA pool, this implies that up to 10% of the polyadenylation reactions occur inappropriately within coding regions of genes (van Hoof et al., 2002). In addition, strains defective in NMD accumulate mRNAs from a number of genes where

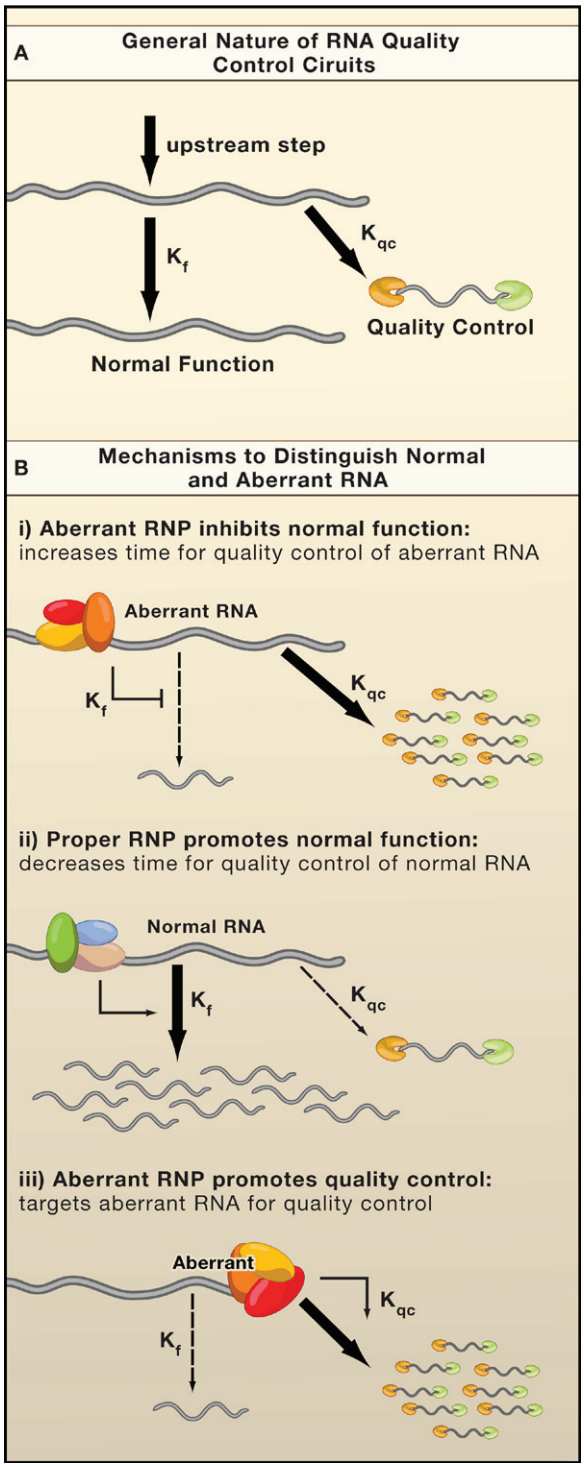

Figure 2. Kinetic Competition and RNA Quality Control

(A) A general model for quality-control circuits as a kinetic competition between the rate of normal function in the life of an RNA and a quality-control event that targets the RNA for degradation.

(B) Three ways in which aberrant RNAs can be preferentially channeled into different quality-control fates. (i) Aberrant RNAs are defective in normal function. (ii) Normal mRNAs promote normal function and thereby reduce quality control. (iii) Aberrant ribonucleoproteins (RNPs) recruit quality-control machinery.

the transcript has been aberrantly polyadenylated at sites distal to the normal poly(A) site (Jungwirth et al., 2001). These observations indicate that polyadenylation occurs at a variety of both premature and distal sites, but these mRNAs are then rapidly degraded by either nonstop or NMD mechanisms. Thus, RNA processing creates a diverse pool of transcripts; only those that survive quality control accumulate to substantial levels. Note that the ability to degrade the products of errors in RNA processing allows the cell to maintain a more flexible RNA-processing machinery, which allows for increased regulation of RNA-processing patterns and for the evolution of new RNAs.

This "Darwinian" view of mRNA biogenesis suggests that quality-control mechanisms might also function as an evolutionary capacitor, which is a system that silences the phenotypic consequences of mutations, thereby allowing a population to build up greater genetic diversity, which can then be revealed at a later time (Rutherford and Lindquist, 1998). In this case, mRNA quality control allows for the accumulation of mutations, or alternate RNA-processing patterns, by silencing their phenotypic consequences. However, under conditions where mRNA quality control is compromised, or additional mutations occur that allow specific mRNAs to evade a limiting quality-control system, the phenotypic consequences would be revealed. Consistent with a possible role of aberrant RNAs in evolving new functions, some intergenic transcripts in yeast are exported to the cytoplasm and even associate with polysomes (Thompson and Parker, 2007). Thus, mRNA quality-control systems may not only have important roles to play in the day-to-day operation of the cell but may also make long-term contributions to evolutionary change.

Future Issues in Quality Control

Over the last five years, a wide range of RNA quality-control mechanisms in eukaryotic cells have been revealed. It is now important to refine our understanding of the molecular mechanisms that allow these systems to distinguish between "normal" and "aberrant" RNAs. This will allow insight into the specificity of gene expression and will have medical implications. For example, one class of disease-causing mutations results in the formation of nonsense codons that lead to degradation of the mutant mRNA (Mendell and Dietz, 2001). Indeed, clinical trials are currently underway to test drugs that promote read-through of nonsense codons for the treatment of muscular dystrophy and cystic fibrosis (Welch et al., 2007). Moreover, defects in RNA quality-control systems may lead to a variety of human diseases. For example, mutations in the human Upf3b protein, which is part of the NMD pathway, have been shown to lead to mental retardation (Tarpey et al., 2007).

One anticipates that the genomic scale and diversity of eukaryotic RNA quality-control mechanisms will continue to expand. Transcripts from large stretches of eukaryotic genomes now can be detected at low levels (reviewed in Bickel and Morris, 2006), many of which may be substrates for RNA quality-control mechanisms. Indeed, recent experiments in *Arabidopsis* using tiling microarrays—which use oligonucleotides to detect transcripts from any region of the genome—to examine the amount and diversity of RNAs present when exosome function is inhibited have revealed significant populations of transcripts that appear to be produced but rapidly degraded and hence are only observed at very low levels under normal conditions (J. Ecker and D. Belostotsky, personal communication).

One expects that new classes of quality-control mechanisms will emerge where defects in RNPs lead not to degradation of the transcript but to repair of the defect. For example, RNA polymerase II can repair defects in transcriptional elongation by degrading the 3′ end of the nascent transcript, resulting in restarting elongation a few nucleotides back on the template (Fish and Kane, 2002). Similarly, in yeast it has been suggested that the exosome can process aberrant 3′ extended mRNAs in yeast to restore their function (Torchet et al., 2002). Moreover, systems may exist to repair chemical damage to RNA such as demethylation of methyl lesions (Falnes et al., 2007). Indeed, as our understanding of the diversity of RNA quality-control pathways matures, the relevant question may become: What stage of RNA biogenesis and function is not subject to quality control?

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