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All Things Must Pass: Contrasts and Commonalities in Eukaryotic and Bacterial mRNA Decay

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Abstract

Despite its universal importance for controlling gene expression, messenger RNA degradation was initially thought to occur by disparate mechanisms in eukaryotes and bacteria. This conclusion was based on differences in the structures used by such organisms to protect mRNA termini and in the ribonucleases and modifying enzymes originally implicated in message decay. Subsequent discoveries have identified a number of striking parallels between the cellular factors and molecular events that govern mRNA degradation in those two kingdoms of life. Nevertheless, some key distinctions remain, the most fundamental of which may be related to the dissimilar mechanisms by which eukaryotes and bacteria control translation initiation.

Protein synthesis is one of the most important biochemical processes in all living cells. It is also among the costliest, requiring substantial investments of energy and resources. Therefore, to maximize their competitive advantage in an ever-changing environment, all organisms must precisely regulate this process so as to produce exactly the proteins that are needed in just the right amounts. Achieving that end requires an ability to degrade mRNA so that patterns of protein synthesis can be altered rapidly. In so doing, cells recycle ribonucleotides for incorporation into new RNA molecules.

mRNA degradation directly affects protein synthesis through its impact on the concentration of mRNA available for translation. Its influence on the expression of individual genes reflects the diverse lifetimes of mRNAs, whose half-lives can differ by as much as two orders of magnitude in the same cell. For example, in rapidly dividing bacterial cells, mRNA half-lives typically range from a fraction of a minute to as long as an hour, whereas in the cells of higher eukaryotes, which divide less frequently, those half-lives range from several minutes to more than a day. The lifetimes of mRNAs often are not invariant but are instead modulated in response to the changing needs of cells for the proteins those messages encode.

Because of the many real and presumed differences between bacterial and eukaryotic mRNAs and the enzymes available to degrade them, it was initially thought that the mechanisms by which messages are degraded in these two kingdoms of life were quite different as well. One by one, those distinctions have fallen by the wayside as a result of new discoveries that have revealed unexpected mechanistic parallels. These parallels, and the distinctions that remain, are the subject of this review. After summarizing earlier views that mRNA decay is generally governed by endonucleolytic events in bacteria and exonucleolytic events in eukaryotes, more recent evidence for the importance of 3’- and 5’-terminal degradative phenomena in bacterial cells and of internal cleavage in eukaryotic cells will be described. The influence of quality-control mechanisms and noncoding RNAs

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on bacterial and eukaryotic mRNA degradation will also be compared. Finally, possible explanations for some fundamental disparities between mRNA decay in bacteria and eukaryotes will be addressed. mRNA turnover in archaea will not be reviewed here because much less is known about it.

BREAKDOWN: FIRST IMPRESSIONS

The models initially conceived to explain mRNA decay were strongly influenced by differences in the structure of bacterial and eukaryotic mRNAs and by early studies of mRNA degradation in two model organisms: *Escherichia coli* and *Saccharomyces cerevisiae*.

Shapes of Things: mRNA Structural Dissimilarities

The structure and organization of bacterial and eukaryotic mRNAs differ in a number of significant ways (Figure 1). For example, while eukaryotic messages are capped at the 5' end with a methylated guanosine connected via a 5'-5' triphosphate linkage (m^7GpppN…), their bacterial counterparts begin with a simple 5'-terminal triphosphate (pppN…). Furthermore, eukaryotic mRNAs typically end with a long, 3'-terminal poly(A) tail that is added post-transcriptionally, whereas few if any additional nucleotides are found at the 3' terminus of bacterial messages, which instead typically end with a stem-loop structure. The protein complexes that assemble on the 5'-terminal cap and 3'-terminal poly(A) tail of eukaryotic mRNAs (eukaryotic translation initiation factor 4F [eIF4F] and poly(A)-binding protein [PABP], respectively) can interact with one another[1], thereby causing messages to assume a noncovalent closed-loop conformation that is not thought to be characteristic of bacterial transcripts.

The mechanisms by which ribosomes are recruited to bacterial and eukaryotic mRNAs are also quite different. In bacteria, ribosome binding is generally mediated by a complementary sequence element (the Shine-Dalgarno element) located just upstream of the initiation codon [2] (Figure 1A). The internal location of the signals that govern translation initiation makes it possible for a single polycistronic bacterial transcript to contain multiple translational units, each with its own ribosome-binding site and protein product. By contrast, eukaryotic ribosomes ordinarily are recruited to mRNA via the affinity of the small ribosomal subunit for the multiprotein complex (comprising eIF4F and eIF3) that assembles on the cap, leading to translation initiation at a nearby AUG codon [3, 4] (Figure 1B). As a result, most eukaryotic mRNAs encode only one protein. Although ribosomes are sometimes recruited directly to the initiation codon of eukaryotic messages by a cap-independent process involving an internal ribosome entry site (IRES), this alternative mechanism of translation initiation is uncommon [5, 6].

Go Your Own Way: Supposed Pathway Differences

Early studies suggested that the principal pathways for mRNA decay in bacteria and eukaryotes were quite different due to disparities in RNA structure, degradative enzymes, and the location of elements controlling mRNA stability.

The conventional model for mRNA degradation in bacterial cells (Figure 2A) was based entirely on studies in *E. coli* and strongly influenced by the kinds of ribonucleases that are present in that organism. *E. coli* contains a number of endonucleases and 3' exonucleases (Table 1) but appears to lack any 5' exonuclease capable of degrading RNA from the 5' terminus. Because exonucleolytic digestion of *E. coli* mRNA from the 3' end is impeded by the stem-loop structure typically present there, it was concluded that degradation of bacterial messages must begin with endonucleolytic cleavage at one or more internal sites to produce a pair of short-lived decay intermediates [7, 8]. Lacking a protective 3' stem-loop, the 5'

fragment thereby generated would be susceptible to 3’ exonuclease attack, while the 3’ fragment was assumed to undergo additional cycles of endonuclease cleavage and 3’ exonuclease digestion. Subsequent studies revealed that the endonuclease most important for mRNA turnover in *E. coli* is RNase E [9–14], a low-specificity ribonuclease that cleaves RNA in single-stranded regions that are AU-rich [15]. Further investigation indicated that the rate at which RNase E degrades mRNA in *E. coli* is frequently determined by characteristics of the 5’ untranslated region (UTR), such as base pairing at the 5’ terminus and efficient ribosome binding, both of which have a protective effect [16–19]. Four *E. coli* 3’ exonucleases – polynucleotide phosphorylase (PNPase), RNase II, RNase R, and oligoribonuclease – were also implicated in mRNA degradation as scavengers of RNA fragments lacking protection at the 3’ end [20–22]. Interestingly, RNase E and PNPase associate with one another as subunits of the RNA degradosome, a multiprotein complex important for RNA processing and degradation that also contains an RNA helicase (RhlB) and a glycolytic enzyme (enolase) [23].

The standard model for mRNA degradation in eukaryotes (Figure 2B) was based primarily on studies in *S. cerevisiae* and mammalian cells, where a variety of degradative enzymes have been identified, including both 3’ and 5’ exonucleases, endonucleases, deadenylases, and decapping enzymes (Table 1). Although mRNA decay in those organisms is sometimes observed to begin with endonucleolytic cleavage or decapping, the most common mechanism of eukaryotic mRNA turnover appears to involve deadenylation as a first step [24, 25], an event whose rate is typically governed by discrete elements within the 3’ UTR or coding region [24, 26]. Loss of the 3’ poly(A) tail and the PABP bound there triggers mRNA degradation by either of two mechanisms. On the one hand, by disrupting the mRNA closed loop, deadenylation facilitates 5’ cap removal by the decapping enzyme Dcp2, yielding a 5’-monophosphorylated decay intermediate that is then rapidly degraded by the monophosphate-dependent 5’ exoribonuclease Xrn1 [27, 28]. In addition, poly(A) tail removal renders messages more susceptible to 3’-terminal attack by the exosome, a multisubunit 3’ exonuclease that is present in both the cytoplasm and the nucleus and that can readily degrade RNA whose 3’ end is not protected by PABP [29, 30].

MAYBE I’M AMAZED: UNEXPECTED PARALLELS

In spite of its superficial appeal, the conventional model for bacterial mRNA degradation had a number of worrisome shortcomings. For one thing, it could not explain how stem-loop structures, either at the 3’ end or upstream of an RNase E cleavage site, would ever be degraded or why the 3’ product of initial endonucleolytic cleavage is typically so much more labile that its intact precursor [8, 31]. In addition, the model could not account for the stabilizing influence of stem-loop structures at the 5’ terminus of bacterial mRNAs [16, 17, 32–35] or the ability of a stalled ribosome to selectively prolong the lifetime of the downstream mRNA segment in *Bacillus subtilis* [36]. Finally, it offered no explanation for the striking absence of an RNase E sequence homolog in many bacterial species. These inadequacies of the standard model, as well as interest in how the stabilities of bacterial and eukaryotic messages are regulated, prompted additional investigations that revealed some unexpected similarities between mRNA degradation in those two kingdoms of life.

The End: 3’-Terminal Degradative Events

Despite the widespread belief that 3’ polyadenylation was a characteristic unique to eukaryotic messages, *E. coli* had long been known to contain a poly(A) polymerase [37, 38] and polyadenylated RNA [39, 40]. However, it was not until many years later that the important role of polyadenylation in bacterial RNA decay was recognized [41, 42]. It is now clear that the transient addition of poly(A) tails to bacterial RNAs is crucial for the 3’ exonucleolytic degradation of stem-loop structures in decay intermediates [43–45]. Although exonucleases such as PNPase and RNase R are hindered when they encounter a significant

stem-loop, they are nevertheless capable of inefficiently degrading such structures, but only if a single-stranded RNA segment is present downstream for them to start with [22, 43]. (By contrast, RNase II seems unable to degrade structured RNA under any circumstances [46, 47].) Ordinarily the poly(A) tails added to bacterial RNAs are barely detectable because they are promptly digested by 3' exonucleases [44, 48]. However, their repeated addition provides those enzymes multiple opportunities to degrade through structured regions, and eventually they succeed, sometimes with the aid of an RNA helicase (such as RhlB, which assists PNPase) [22, 43, 46] (Figure 3). Bacteria that lack a poly(A) polymerase can use PNPase operating in reverse (i. e., synthetically rather than degradatively) to add heteropolymeric 3'-terminal tails that serve a similar purpose [49]. Interestingly, this pathway for 3'-exonucleolytic degradation appears to be conserved in organelles such as chloroplasts [50], which are thought to be evolutionary descendents of bacteria.

The destabilizing influence of poly(A) on mRNA decay intermediates in bacteria may seem at odds with its stabilizing effect on messages in the cytoplasm of eukaryotic cells, where it must be removed by a specialized deadenylase (Ccr4-Not, Pan2-Pan3, or PARN (which is present only in vertebrates and insects) [51] (Table 1)) as a prelude to mRNA decay. However, recent data indicate that in eukaryotic nuclei a pair of noncanonical poly(A) polymerases, the TRAMP subunits Trf4 and Trf5, may have a function similar to that of bacterial poly(A) polymerase, in that they facilitate 3' exonucleolytic degradation of defective RNAs by exosomes [52, 53]. Interestingly, poly(A) addition by Trf4 or Trf5 appears to be necessary for the TRAMP complex to accelerate the decay of some but not all of its RNA targets [53–55].

The nuclear exosome and bacterial PNPase not only share a propensity to degrade polyadenylated RNA but also bear a striking structural resemblance to one another despite superficial differences in their subunit composition. The three identical subunits of PNPase each comprise two RNase PH-like domains and two RNA-binding domains (one each from the KH and S1 families) that together form a homotrimeric ring surrounding a central channel [56]. An apparent product of divergent evolution, the core of the eukaryotic exosome contains an analogous set of protein domains organized into nine different polypeptides – six distinct RNase PH-like subunits and three distinct subunits containing KH and S1 domains – that assemble to form a similar toroidal structure [29, 57]. However, unlike the bacterial enzyme, which contains one catalytically active RNase PH domain per subunit (three per trimer), none of the corresponding subunits of the core exosome in yeast or humans retains activity. Instead, those exosomes appear to rely for their exonucleolytic activity on either of two associated proteins, Rrp6 or Rrp44 (also known as Dis3) [57, 58]. Moreover, unlike PNPase, which degrades RNA phosphorolytically to produce nucleoside diphosphates as reaction products, both Rrp6 and Rrp44/Dis3 are hydrolytic ribonucleases that yield nucleoside monophosphate products. Despite these catalytic differences, exosomes and PNPase both depend on assistance from an RNA helicase (Ski2 or Mtr4 in *S. cerevisiae* and RhlB in *E. coli*) for their efficient function [46, 59, 60].

Both Sides Now: 5'-Terminal Degradative Events

A key distinction between mRNA degradation in *E. coli* and eukaryotic cells is the apparent absence of a 5'-to-3' exoribonuclease in the former, where mRNA degradation was thought to begin with endonucleolytic cleavage. Therefore, the discovery that 5'-terminal stem-loop structures can protect triphosphorylated primary transcripts from RNase E-mediated degradation in *E. coli* came as quite a surprise, as no mechanism was known that could account for that effect [16, 17, 32, 33].

A clue as to a possible explanation for that phenomenon came later when it was determined that RNase E has a strong preference for RNA substrates bearing a single phosphate at the 5' end and will cut such RNAs at internal sites more than an order of magnitude faster than it

will cut their triphosphorylated counterparts [61]. The influence of 5’ phosphorylation on endonucleolytic cleavage by RNase E is a consequence of a discrete enzyme pocket where monophosphorylated 5’ ends can bind and promote downstream cleavage [62]. This property was immediately accepted as the reason why the monophosphorylated 3’ products of internal cleavage are typically so much more labile than their intact triphosphorylated precursors. However, it could not account for the stabilizing influence of 5’-terminal stem-loop structures on primary transcripts, whose triphosphorylated 5’ ends are incapable of binding to RNase E.

This conundrum eventually led to the discovery of an important alternative decay pathway in which internal cleavage by RNase E is triggered by a prior event at the 5’ end: the conversion of the 5’-terminal triphosphate to a monophosphate by RppH, an *E. coli* RNA pyrophosphohydrolase that preferentially acts on 5’ termini that are single-stranded [63, 64] (Figure 4A). Interestingly, pyrophosphate removal from bacterial transcripts and the decapping of eukaryotic mRNAs not only bear a striking structural resemblance to one another (triphosphate cleavage to generate a monosphosphorylated product) but also are catalyzed by evolutionarily related enzymes (RppH and Dcp2, respectively (Table 1)) that are both members of the Nudix hydrolase family [28, 64]. Moreover, the functional consequences of the two events are similar, as each involves removing a protective group to render RNA more susceptible to digestion by a 5’-monophosphate-dependent ribonuclease (the endonuclease RNase E or the 5’ exonuclease Xrn1) [27, 61, 63, 65].

Remarkably, despite its central role in mRNA decay in *E. coli*, RNase E is absent from a number of bacterial species, including many firmicutes such as *Bacillus subtilis* and *Staphylococcus aureus* and even some proteobacteria such as *Helicobacter pylori* [66]. Moreover, in contrast to what is observed in *E. coli*, a ribosome stalled on a *B. subtilis* transcript can protect the entire downstream RNA segment (but not the upstream segment) from degradation, suggesting a 5’-to-3’ directionality for message degradation in that organism [36]. These mysteries were solved when it was discovered that, almost invariably, bacteria lacking RNase E instead contain the 5’-to-3’ exonuclease RNase J and/or the endonuclease RNase Y, two enzymes that are absent from *E. coli* (Table 1) [66–69]. In *B. subtilis*, RNase J and RNase Y appear to play important roles in mRNA turnover [66, 70]. Moreover, like Xrn1 and RNase E, these two bacterial ribonucleases preferentially degrade RNA substrates that bear only one phosphate at the 5’ end [66, 68]. This property suggests that digestion of primary transcripts by RNase J or RNase Y may well be preceded by a deprotection step that generates a monophosphorylated intermediate. Interestingly, RNase J itself is capable of catalyzing that prior step, as it can act not only as an exonuclease but also, less efficiently, as a 5’-end-independent endonuclease [71], cleaving RNA at internal sites to generate 3’ fragments that it can then degrade exonucleolytically (Figure 4B). Alternatively, it has been hypothesized that exonucleolytic attack by RNase J may in many instances be triggered by pyrophosphate removal from primary transcripts to generate a monophosphorylated 5’ end [63]. If so, the latter pathway would closely resemble the degradation mechanism that often ensues after deadenylation in eukaryotic cells: decapping followed by 5’ exonuclease digestion.

It has recently been reported that decapping and subsequent degradation of eukaryotic mRNAs can also be stimulated by the addition of an oligo(U) tract at the 3’ end [72, 73]. No analogous oligo(U)-dependent decay pathway has been described in bacteria; nor do bacteria contain a homolog of the eukaryotic poly(U) polymerases Cid1 and Zcchc11 [73–75].

Heartbreaker: Internal Cleavage of mRNA

In bacteria, endonucleases have long been thought to play a major role in mRNA degradation, particularly RNase E and its homolog RNase G (both of which cleave RNA in

single-stranded regions [15, 76]), RNase III (specific for double-stranded RNA [77]), and more recently RNase Y and RNase J (both specific for single-stranded RNA [66, 67]). By contrast, the important contribution of endonucleases to mRNA decay in eukaryotic cells was long overlooked as deprotection of the 5' and 3' ends grabbed the lion's share of attention.

One of the earliest documented examples of endonucleolytic initiation of eukaryotic mRNA decay was the regulated degradation of transferrin receptor mRNA, whose vulnerability to site-specific cleavage within the 3' untranslated region is controlled by iron [78]. Although the enzyme responsible for that cleavage remains unknown, the recent implication of a number of metazoan endonucleases in mRNA turnover has revived interest in internal cleavage as a pathway for initiating mRNA decay in eukaryotes. Foremost among these endonucleases are the Argonaute (Ago) proteins important for RNA interference, many of which cleave messages at sites targeted by perfectly complementary small interfering RNAs (siRNAs) and microRNAs (miRNAs) [79, 80]. Internal cleavage has also been implicated in nonsense-mediated decay and no-go decay, quality-control pathways for rapidly degrading translationally defective transcripts [81, 82] (see below). For example, in metazoans degradation via the former pathway is often initiated by SMG6, an endonuclease with a catalytically active PIN domain [83, 84]. Lacking protection at one end or the other, the resulting RNA fragments are susceptible to swift exonucleolytic digestion. Interestingly, in addition to its RNase II-like 3' exonuclease domain [85], the exosome-associated protein Rrp44/Dis3 has a PIN domain that is capable of cleaving RNA endonucleolytically [86, 87]. Thus, not only do the exosome core and PNPase resemble one another structurally, but also each can form a multimeric complex with an endonuclease (Rrp44/Dis3 or RNase E, respectively). Other eukaryotic endonucleases that have been implicated in mRNA degradation include Zc3h12a, a PIN-domain ribonuclease induced by stimulation of Toll-like receptors [88], Swt1, a PIN-domain ribonuclease important for nuclear mRNP surveillance [89], RNase L, an enzyme involved in the host antiviral response [90], IRE-1, a mediator of the unfolded protein response [91, 92], and PMR1, a ribonuclease thought to contribute to hormone-dependent changes in mRNA stability [93]. Nevertheless, the contribution of endonucleases to mRNA turnover appears to be more limited in eukaryotes than in bacteria.

You're No Good: Quality Control via mRNA Degradation

Both bacteria and eukaryotes have evolved quality-control pathways for rapidly degrading messages that are unfit for protein synthesis due to defects in translation. In this manner, cells are able to minimize the synthesis of abnormal proteins, many of which may be toxic, while freeing ribosomes for more productive uses.

An example of such a translational defect is a premature termination codon (PTC, also known as a nonsense codon), which can arise by a number of mechanisms, including genetic mutation, transcription or translation initiation at a cryptic site, and aberrant or incomplete splicing. In both bacterial and eukaryotic cells, messages that contain a PTC are generally degraded much faster than their wild-type counterparts [94, 95]. However, the mechanisms that govern nonsense-mediated mRNA decay (NMD) in these two kingdoms of life appear to be quite different from one another.

The specificity of NMD is dependent on the ability of cells to differentiate between PTCs and normal termination codons. To make this distinction, eukaryotic organisms rely on their capacity to recognize that the 3' UTR downstream of a PTC is abnormal. The distinguishing characteristics of such a misconfigured 3' UTR are not fully understood, but they appear to include an unusually long distance between the stop codon and the poly(A) tail and/or the presence there of one or more exon junctions, which are uncommon in natural 3' UTRs [96–99]. By contrast, although little is known about PTC recognition in bacteria, the

highly variable number of translational units and the rarity of introns in bacterial mRNAs would seem to make distance measurements and splice sites downstream of stop codons unreliable gauges of normalcy.

The mechanism of NMD in eukaryotes and bacteria is also different. In *E. coli*, it is thought to begin with 5'-end-independent RNase E cleavage at internal sites exposed by the premature release of ribosomes [19, 33] (Figure 5A), whereas a variety of triggering mechanisms have been reported in eukaryotes, including decapping, deadenylation, and internal cleavage near the site of premature translation termination [81, 100–102] (Figure 5B).

A number of proteins required for NMD in eukaryotes have been identified, including three UPF proteins and, in metazoans, several SMG proteins [95]. Functions have been assigned to some of these proteins, such as the UPFs, which form a surveillance complex important for PTC recognition [99, 103–106], and the endonuclease SMG6, which can cleave defective mRNAs near the site of premature translation termination [83, 84]. By contrast, no ribonuclease or ancillary protein with a specialized role in NMD has been identified in bacteria, which lack homologs of the UPF and SMG proteins. Instead, the recognition and rapid turnover of PTC-containing transcripts in bacteria appears to be accomplished by the ordinary cellular apparatus for RNA degradation.

Another category of defective mRNAs are those that cannot release ribosomes due to the lack of an in-frame translation termination codon. Messages of this kind can arise by aberrant cleavage and polyadenylation within the coding region (eukaryotes) or degradation of the 3'-terminal portion of a transcript (eukaryotes or bacteria). In both types of organisms, the resulting "non-stop" mRNAs are rapidly degraded by 3' exonucleases [107, 108]; however, the mechanism by which the RNA 3' end becomes exposed to exonuclease attack is quite different in each case. Bacteria utilize a process called "trans-translation" to free that end of the message from the ribosome trapped there [109]. This mechanism of ribosome release depends on tmRNA, a specialized RNA that has properties of both a tRNA and an mRNA. An aminoacylated tmRNA molecule binds to the empty A-site of the stalled ribosome and serves first as an acceptor for peptidyl transfer and then as a template for renewed translation, which ends when the ribosome encounters a termination codon in the tmRNA and dissociates. Exonuclease digestion of the message then ensues. Lacking a homolog of tmRNA, eukaryotes rely instead on the exosome-associated protein Ski7 to stimulate the exonucleolytic degradation of non-stop mRNAs [107]. The mechanism by which Ski7 accomplishes this feat is not yet clear.

A third type of surveillance mechanism, "no-go" decay, degrades mRNAs on which ribosomes have become stalled during translation. In both *S. cerevisiae* and *E. coli*, such ribosomal pausing can often induce endonucleolytic cleavage at a nearby site [82, 110-112]. However, in neither case has the ribonuclease responsible for cleavage been identified, prompting speculation that ribosomes themselves might possess an intrinsic, but as yet unproven, endonuclease activity [110–113].

Every Little Thing: Destabilization by Noncoding RNAs

Short noncoding RNAs that control gene expression by base pairing with complementary sites in mRNA were first discovered in bacteria [114]. Subsequently they were found to play a crucial regulatory role in eukaryotic organisms as well [115, 116]. In both kingdoms of life, noncoding RNAs influence the translation and longevity of mRNAs to which they bind. However, despite similar regulatory outcomes, the mechanisms by which those outcomes are achieved are rather different.

Metazoan organisms produce two major kinds of noncoding RNAs that act post-transcriptionally to control gene expression: miRNAs and siRNAs. These ~22-nucleotide RNAs differ somewhat in their biogenesis [117] but not in their regulatory potential, which depends on the degree of complementarity of their mRNA targets. When miRNAs or siRNAs base pair with a message to which they are partially complementary, they typically impair its translation while also hastening poly(A) removal and thereby destabilizing the message [115, 116, 118, 119] (Figure 6A). These two effects seem to be largely independent of one another and to contribute additively to downregulation [118, 119]. Base pairing with perfectly complementary elements also results in faster decay and diminished translation; however, in this case decay is triggered by endonucleolytic cleavage at the site where the noncoding RNA binds rather than by deadenylation [120–122] (Figure 6B). Each of these effects results not from an intrinsic activity of the bound miRNA or siRNA but rather from the properties of two associated proteins that accompany it to its mRNA target: Ago, an endonuclease specific for fully base paired RNA duplexes [79, 80], and TNRC6 (also known as GW182), which mediates translational repression and deadenylation by mechanisms that are poorly understood [123–125].

Unlike miRNAs and siRNAs, which are processed from long precursor transcripts, small noncoding RNAs (sRNAs) in bacteria typically are primary transcripts of diverse lengths (tens to hundreds of nucleotides). Chaperoned to their complementary mRNA targets by the Sm-like RNA-binding protein Hfq, bacterial sRNAs usually repress translation, often by competing directly with ribosomes for binding to sites of translation initiation (Figure 6C) but sometimes by binding at a distance and inhibiting translation by mechanisms that are not well understood [126–129]. However, they occasionally have the opposite effect, antagonizing translational repression by disrupting intramolecular base pairing that would otherwise occlude the ribosome binding site [130–132]. When they inhibit translation, bacterial sRNAs also destabilize their mRNA targets, apparently as a secondary consequence of diminished ribosomal protection [133, 134] (Figure 6C). Nevertheless, sRNAs sometimes destabilize mRNA as a primary effect that is not linked to translation, facilitating cleavage by either RNase III (when the two RNAs form a long, well paired duplex) (Figure 6D) or RNase E (when they do not) [135–137]. In contrast to eukaryotic miRNAs and siRNAs, which determine specificity but rely entirely on specialized protein cofactors (Ago and TNRC6/GW182) to effect gene regulation, bacterial sRNAs bound to their targets can function as both specificity determinants and effectors, downregulating gene expression by acting either alone or in conjunction with components of the cell's generalized machinery for RNA degradation. Moreover, whereas the target specificity of eukaryotic miRNAs is defined primarily by the sequence of nucleotides near the 5' end owing to the manner in which those RNAs are bound by Ago [138–140], the location of the RNA segment that determines the specificity of bacterial sRNAs can vary.

Recent studies have identified a distinct class of short (~30–70 nucleotide) noncoding RNAs that may be able to downregulate gene expression by a mechanism that more closely resembles siRNA-directed RNA cleavage in eukaryotes. These regulatory RNAs are processed from long transcripts of CRISPR loci. In the archaeon *Pyrococcus furiosus*, CRISPR RNAs guide the hexameric Cmr complexes with which they associate to bind complementary RNAs and cleave them at a specific site within the base-paired region [141] (Figure 6E). By this and other means, CRISPR RNAs are thought to help their host resist invasion by viruses and plasmids. Eubacterial species that produce both CRISPR RNAs and Cmr proteins (e. g., *Bacillus halodurans* and *Thermus thermophilus* but not *E. coli* [142]) are expected to share this defense mechanism. Notwithstanding the functional similarities of Cmr and Ago proteins, their sequences and cleavage site specificities are distinct [141].

WE JUST DISAGREE: KEY DIFFERENCES

Despite the growing number of parallels now evident between the mechanisms of mRNA degradation in bacteria and eukaryotes, a number of notable distinctions remain, some of which may be causally interrelated. Perhaps the most fundamental is the relative importance of internal versus terminal degradative events. Low-specificity endonucleases play a major role in bacterial mRNA decay. By contrast, in eukaryotes, where mRNA degradation is dominated by 3'- and 5'-terminal events (deadenylation, decapping, and exonuclease digestion), the contribution of endonucleases appears to be much more limited, and those that do participate seem to act with greater specificity than their bacterial counterparts. This difference appears to have had a number of important consequences. Foremost among these is that steric protection by ribosomes is generally crucial for the longevity of bacterial messages but relatively unimportant for eukaryotic mRNA stability. Thus, whereas inefficient translation initiation need not doom eukaryotic messages to rapid degradation [118, 143], a poor ribosome binding site almost always hastens bacterial mRNA decay, presumably by increasing the spacing between translating ribosomes and thereby exposing potential endonuclease cleavage sites in or near the protein-coding region [144]. This difference might also explain why eukaryotes had to evolve a specialized machinery to recognize and degrade mRNAs that contain premature termination codons, while bacteria seem to have managed to achieve that end by employing the same proteins used to degrade ordinary messages. Furthermore, it may help to clarify why the 3' UTRs of eukaryotic messages, which contain binding sites for proteins and noncoding RNAs that regulate translation, cellular localization, and decay, can be hundreds or even thousands of nucleotides long without adversely affecting mRNA stability, whereas intercistronic and 3' untranslated regions in bacterial transcripts are generally much shorter.

Conversely, eukaryotes depend heavily on deadenylation to govern rates of cytoplasmic mRNA decay. That reliance has necessitated the protective influence of poly(A)-binding protein, without which rapid and uncontrollable degradation would ensue, and the existence of specialized deadenylases capable of degrading PABP-associated poly(A) tails in an orderly manner. By contrast to its stabilizing effect in the cytoplasm of eukaryotes, poly(A) appears to serve only a destabilizing function in bacteria despite its affinity for the bacterial RNA-binding protein Hfq, whose ability to impede the exonucleolytic destruction of poly(A) tails [145] is not sufficient for those tails to persist at significant steady-state lengths in vivo.

The evolutionary imperatives for these differences may be related to the distinct mechanisms by which eukaryotes and bacteria control translation initiation. In eukaryotic organisms, ribosome binding is ordinarily governed by a protein complex (eIF4F) that associates with both the 5'-terminal cap and the PABP on the 3'-terminal poly(A) tail. Deadenylation disrupts those interactions, thereby inhibiting translation [3, 4]. Employing poly(A) tail loss also as the principal mechanism for triggering mRNA degradation allows this decrease in translational activity to be tightly coupled to the destruction of eukaryotic messages. By contrast, the reliance of bacteria on internal ribosome binding sites rather than terminal structures to control translation initiation has enabled them to coordinate the expression of genes by organizing them into co-transcribed polycistronic operons. Although removing pyrophosphate from the 5' end or adding poly(A) to the 3' end may trigger exonucleolytic mRNA degradation, a heavy reliance on endonucleolytic cleavage (sometimes triggered by pyrophosphate removal) makes it possible for bacteria to selectively degrade discrete segments of polycistronic transcripts, irrespective of the position of those segments. Consequently, a translational unit anywhere within such a transcript may be either longer or shorter lived than the others [146]. Together with individualized signals for translational control, such segmental differences in mRNA stability are an important

mechanism by which bacteria can differentially regulate the expression of genes within operons. This kind of degradative flexibility would be of little use in eukaryotes, where the mechanism of translation initiation prevents most mRNAs from encoding more than one polypeptide.

CHANGES: EVOLVING PERCEPTIONS

It is clear that what had once seemed a broad divide between the degradation mechanisms of mRNA in bacteria and eukaryotes has been steadily shrinking in recent years due to a growing awareness of the importance of 5'- and 3'-terminal events (such as pyrophosphate removal, 5' exonuclease attack, and polyadenylation) in bacteria and internal cleavage (such as during RNA interference or NMD) in eukaryotes. This gap is likely to narrow further as new findings reveal previously unrecognized parallels. For example, the recent discovery of CRISPR RNA-guided RNA cleavage in archaea suggests that eubacteria that produce CRISPR RNAs and Cmr proteins will be found to have a similar ability to trigger mRNA degradation by a mechanism very reminiscent of siRNA-guided mRNA cleavage in eukaryotes. Nonetheless, although infrequent exceptions can be found to almost any rule, some important distinctions are likely to persist due to fundamental differences in how mRNAs are synthesized, compartmentalized, and translated in bacterial and eukaryotic cells. Thus, the pervasive importance of low-specificity endonucleases for bacterial mRNA decay seems unlikely to be replicated in eukaryotes. The valuable insights that can be derived from such comparisons ensure that studies of the mechanisms of mRNA degradation in these two kingdoms of life will continue to inform one another.

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GLOSSARY

Argonaute/Ago	A member of a family of proteins that contain PAZ and PIWI domains and help to mediate RNA interference by binding siRNAs and miRNAs and delivering them to complementary mRNAs
A-site	The ribosomal site where aminoacylated tRNA binds
CRISPR	A cluster of regularly interspaced short palindromic repeats in bacterial or archaeal DNA
CRISPR RNA	A bacterial or archaeal regulatory RNA, ~30-60 nucleotides long, that is processed from the transcript of clustered regularly interspaced short palindromic repeats (CRISPRs) in chromosomal DNA
Deadenylase	A 3' exonuclease specific for degrading poly(A) tails
eIF3	A multisubunit eukaryotic translation initiation factor that binds to cap-associated eIF4F and guides small ribosomal subunits to the translation initiation codon
eIF4F	A multisubunit eukaryotic translation initiation factor that binds the mRNA cap, eIF3, and poly(A)-binding protein
Endonuclease	An enzyme that cleaves RNA or DNA at an internal position

eRF1 and eRF3	Eukaryotic release factors that mediate translation termination at UAA, UAG, and UGA codons
Exon junction	A site in a spliced eukaryotic mRNA where an intron was excised and the two flanking exons were joined
Exon junction complex	A protein complex deposited on exon junctions during splicing and transported with mRNA to the cytoplasm
Exonuclease	An enzyme that degrades RNA or DNA by removing mononucleotides sequentially from the 5’ or 3’ end
Exosome	A protein complex that, in eukaryotes, comprises a nine-subunit core and other associated polypeptides and that utilizes its 3’ exonuclease activity and to a lesser extent its endonuclease activity to function in RNA processing and decay in the nucleus and cytoplasm
Hfq	A bacterial RNA-binding protein, homologous to eukaryotic Sm and Lsm proteins, that acts as a chaperone for many sRNAs and can also bind to mRNA and poly(A) tails
KH domain	A member of a family of RNA-binding domains homologous to the RNA-binding domains of heterogeneous nuclear ribonucleoprotein K (hnRNP K)
miRNA	A microRNA, ~22 nucleotides long, that binds to Ago and mediates translational repression and accelerated degradation of complementary mRNAs
mRNP	A complex of mRNA and the proteins with which it associates
NMD	Nonsense-mediated degradation of mRNAs that contain a premature termination codon
Nudix hydrolase	A member of a family of hydrolytic enzymes that share a characteristic sequence motif and catalyze the hydrolysis of substrates containing a nucleoside diphosphate as a constituent unit
PIN domain	A member of a family of homologous protein domains that have endoribonuclease activity and are present in both eukaryotes and bacteria
PNPase	Polynucleotide phosphorylase, a phosphorolytic bacterial 3’ exoribonuclease that can also act synthetically to add heteropolymeric tails to RNA
Poly(A)-binding protein	A protein that binds 3’ poly(A) tails and eIF4F, thereby facilitating translation initiation and protecting mRNA from attack by exosomes and decapping enzymes
Polycistronic mRNA	An mRNA that contains multiple translational units, each encoding a distinct protein
Premature termination codon (PTC)	An in-frame UAA, UAG, or UGA triplet that causes ribosomes to terminate translation upstream of the normal termination codon

RNA degradosome	A bacterial protein complex comprising multiple enzymes important for RNA processing and degradation (e. g., RNase E, a 3’ exonuclease, and an RNA helicase)
RNA interference	A eukaryotic regulatory process in which siRNAs or miRNAs repress gene expression by inhibiting the translation and accelerating the degradation of complementary mRNAs
RNA pyrophosphohydrolase	An enzyme that can remove the γ and β phosphates from the 5’ end of a triphosphorylated transcript, converting it to a 5’-monophosphorylated RNA
RNase PH	A phosphorolytic 3’ exoribonuclease important for the maturation of the 3’ ends of bacterial tRNAs
Rrp6	A hydrolytic 3’ exoribonuclease associated with nuclear exosomes
Rrp44/Dis3	A bifunctional exosome-associated ribonuclease that contains both a hydrolytic 3’ exonuclease domain and an endonucleolytic PIN domain
S1 domain	A member of a family of RNA-binding domains homologous to the RNA-binding domains of ribosomal protein S1
Shine-Dalgarno element	A bacterial mRNA element that guides translation initiation at a downstream start codon by base pairing with the 3’ end of 16S rRNA.
siRNA	A small interfering RNA, ~22 nucleotides long, that binds to Ago and mediates translational repression and accelerated degradation of complementary mRNAs
SMG1	A serine/threonine kinase that helps to trigger NMD in metazoans by phosphorylating UPF1
Sm protein	A eukaryotic RNA-binding protein that assembles into a multimeric ring and binds RNA (e. g., spliceosomal snRNA) in single-stranded regions that typically are U-rich.
sRNA	A noncoding bacterial RNA that influences the translation and/or degradation of complementary mRNAs to which it binds
tmRNA	A bifunctional aminoacylated RNA that has properties of both a tRNA and an mRNA and mediates the release of ribosomes from bacterial mRNAs that lack an in-frame translation termination codon
TNRC6/GW182	A member of a family of proteins that bind to Argonaute and help to mediate the effects of miRNAs and siRNAs on translation and deadenylation
TRAMP	A eukaryotic polyadenylation complex, comprising Trf, Air, and Mtr4 proteins, that facilitates the 3’-exonucleolytic degradation of defective RNAs by nuclear exosomes

UPF protein	One of three proteins (UPF1, UPF2, and UPF3) that are important for the recognition of premature termination codons during nonsense-mediated mRNA decay in eukaryotic cells
UTR	An mRNA segment that is not translated by ribosomes and does not encode a protein

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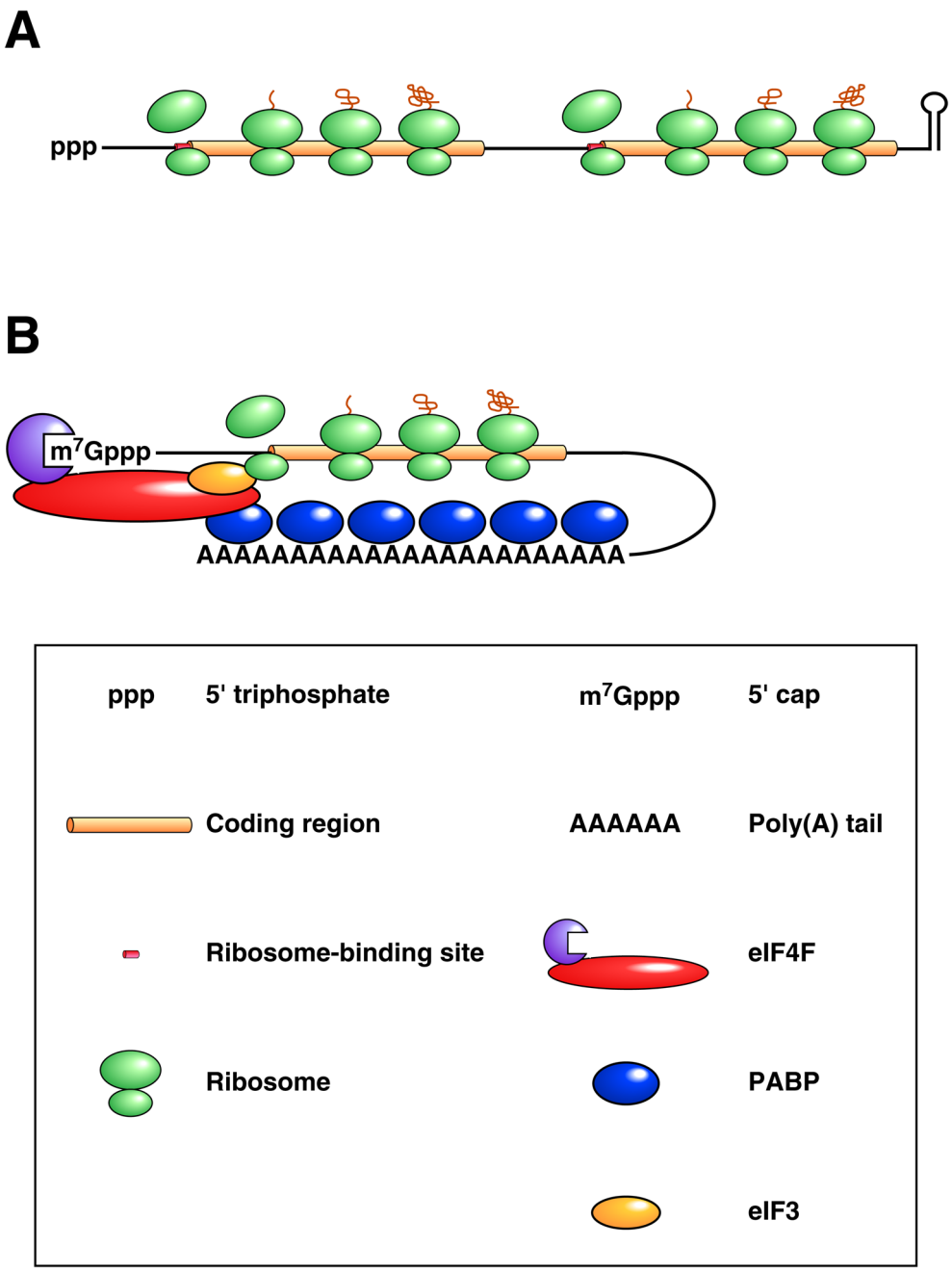

Figure 1. Differences in the structure and translation of bacterial and eukaryotic mRNAs

(A) Bacterial mRNA. A translated dicistronic transcript that begins with a triphosphate and ends with a stem-loop is depicted. Ribosome binding to the beginning of each translational unit is aided by base pairing between the 3' end of 16S ribosomal RNA (a component of the small ribosomal subunit) and a Shine-Dalgarno element adjacent to the initiation codon (collectively termed the ribosome binding site). (B) Eukaryotic mRNA. A message undergoing cap-dependent translation is shown. Ribosome binding to the translation initiation codon is guided by the affinity of the small ribosomal subunit for eukaryotic initiation factor 3 (eIF3), a protein multimer recruited to mRNA by the cap-binding complex

eIF4F, whose affinity for mRNA is enhanced by its ability to bind PABP associated with the 3’ poly(A) tail.

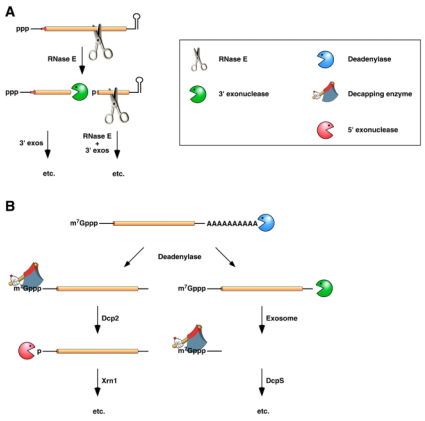

Figure 2. Conventional pathways for mRNA degradation in *E. coli* and in eukaryotic cells

(A) mRNA decay in *E. coli*. In this pathway, serial internal cleavage by RNase E generates degradation intermediates whose lack of base pairing at the 3' end renders them susceptible to attack by the 3' exonucleases polynucleotide phosphorylase, RNase II, RNase R, and (for very short RNA fragments) oligoribonuclease. By contrast, the intact transcript resists exonucleolytic degradation because it is protected by a 3'-terminal stem-loop, which hinders such attack. (B) mRNA decay in eukaryotic cells. In this pathway, poly(A) tail removal by a deadenylase (Ccr4-Not, Pan2-Pan3, or PARN) yields a deadenylated intermediate susceptible both to decapping by Dcp2 and to 3'-exonucleolytic degradation by exosomes. The decapped RNA generated by Dcp2 is then degraded by the 5'-exonuclease Xrn1, whereas the 5'-terminal RNA fragment that results from extensive exosome digestion undergoes cap removal by an alternative decapping enzyme (DcpS) specific for oligonucleotides [147]. These pathways were deduced from early studies of mRNA degradation in *E. coli*, *S. cerevisiae*, and mammalian cells. Ribosomes, PABP, and translation factors have been omitted from this figure for the sake of simplicity.

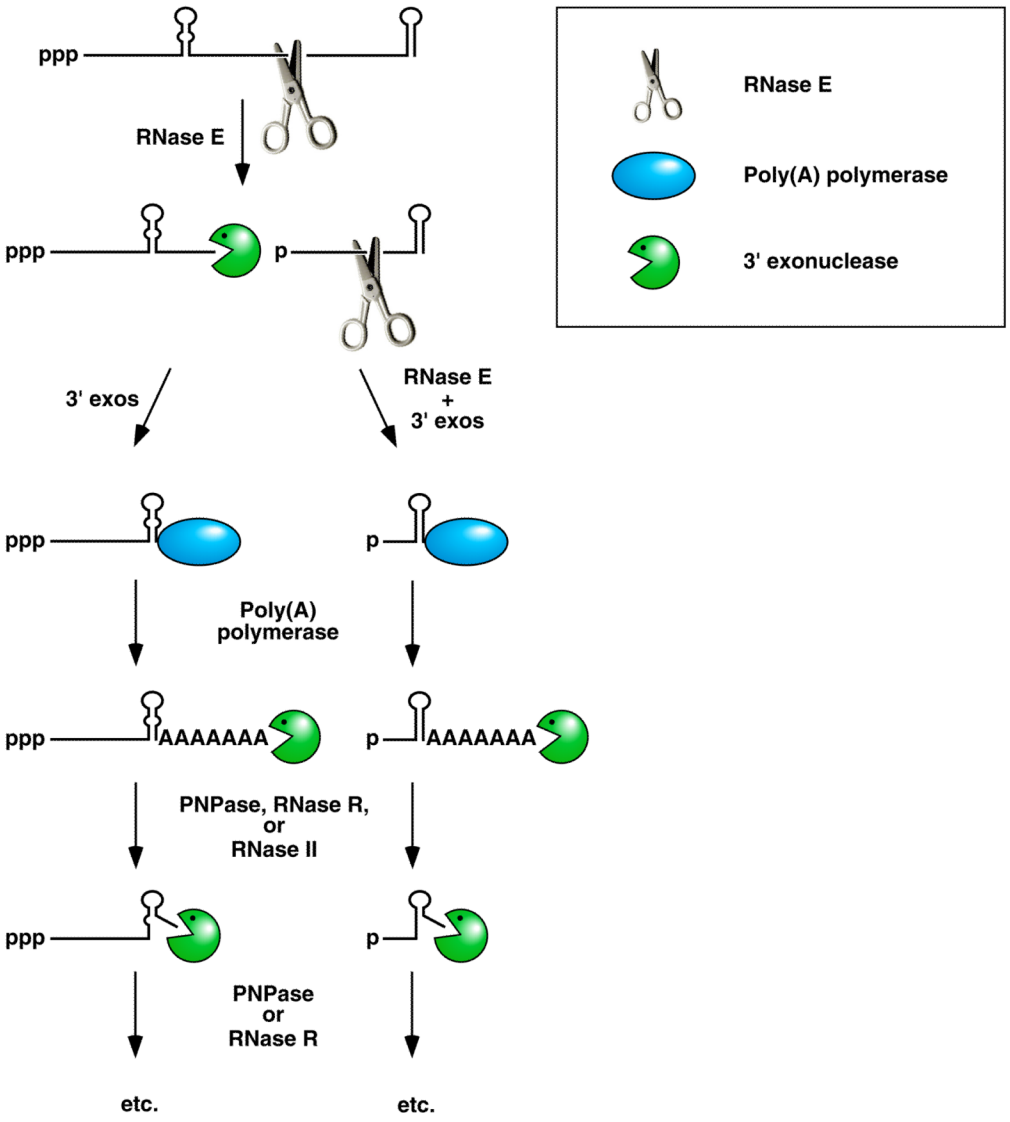

Figure 3. Facilitation of the 3'-exonucleolytic degradation of bacterial mRNA decay intermediates by polyadenylation

Endonucleolytic cleavage of mRNA by RNase E generates multiple fragments, one of which ends with the original 3'-terminal stem-loop. The others undergo 3'-exonucleolytic attack by PNPase, RNase R, and/or RNase II until an upstream stem-loop is encountered, which interrupts further degradation due to the preference of those ribonucleases for 3' ends that are unpaired. The resulting decay intermediates are then polyadenylated by poly(A) polymerase, thereby enabling the exonucleases to re-engage. The repeated addition of single-stranded poly(A) tails to the 3' ends of these intermediates provides multiple opportunities for PNPase and RNase R to overcome structural impediments to exonucleolytic degradation, and eventually they succeed. The ability of PNPase to digest base-paired RNA is enhanced by its association with the RNA helicase RhlB, whereas RNase R requires no such assistance. By contrast, RNase II can degrade poly(A) and other kinds of unstructured RNA but not structured RNA. Ribosomes and coding regions have been omitted from this figure for the sake of simplicity.

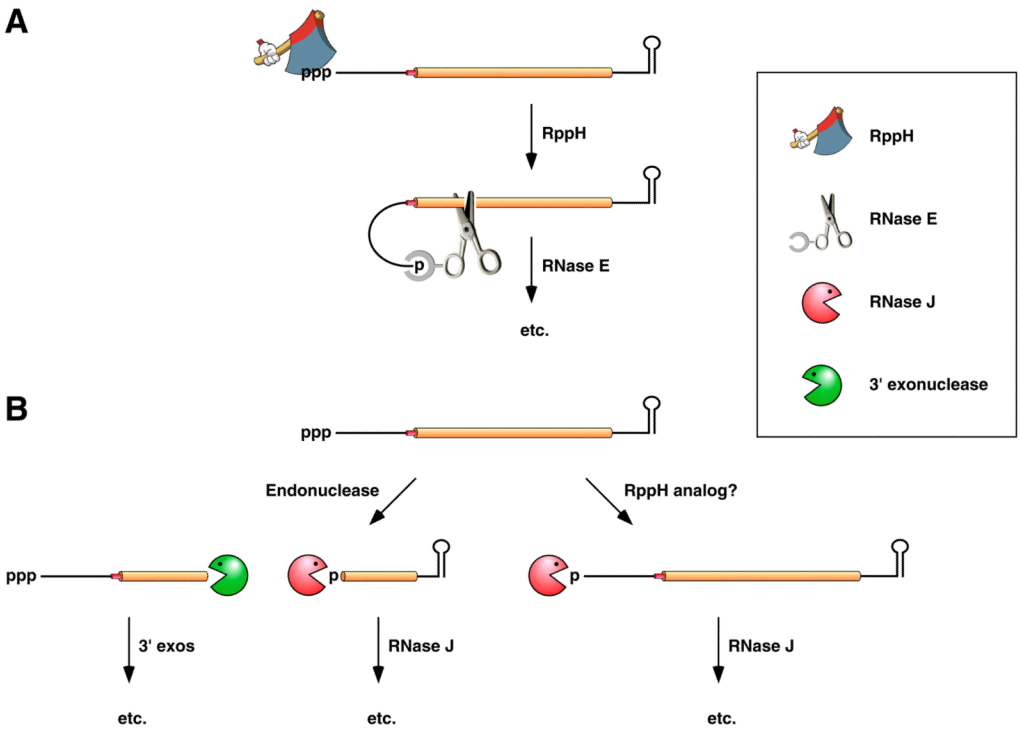

Figure 4. Pathways for 5’-end-dependent mRNA degradation in bacteria

(A) 5’-end-dependent mRNA decay in bacteria that contain the endonuclease RNase E or a homolog thereof. Pyrophosphate removal by RppH generates a 5’-terminal monophosphate that binds to a discrete pocket on the surface of RNase E, thereby facilitating mRNA cleavage at a downstream location by the active site of that enzyme. In *E. coli*, RNase E cleavage of primary transcripts can also occur by an alternative, 5’-end-independent mechanism that does not require prior pyrophosphate removal (Figure 2A) [19, 33, 148]. (B) 5’-end-dependent mRNA decay in bacteria that contain the 5’ exonuclease RNase J. Internal cleavage by an endonuclease generates a monophosphorylated intermediate susceptible to 5’-to-3’ digestion by RNase J, whose exonucleolytic activity is impeded by a 5’ triphosphate. Alternatively, it is possible that 5’-exonucleolytic digestion by RNase J may be triggered by pyrophosphate removal from primary transcripts by an as yet unidentified RppH analog. Ribosomes have been omitted from this figure for the sake of simplicity.

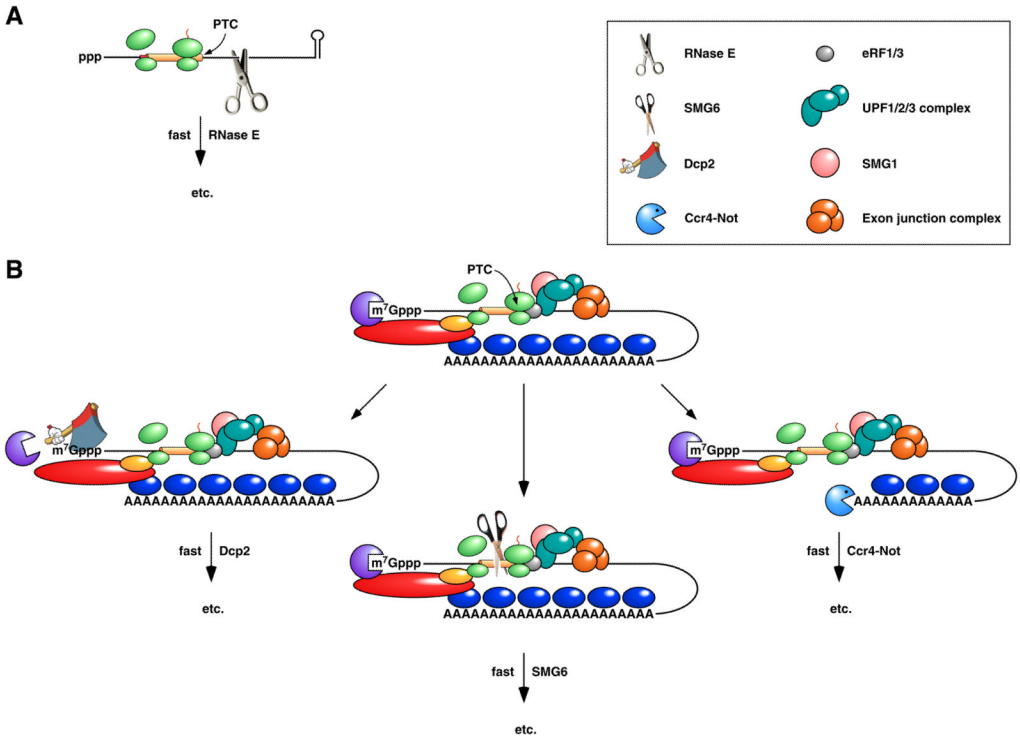

Figure 5. Pathways for the rapid degradation of bacterial and eukaryotic mRNAs that contain a premature termination codon

(A) Rapid degradation of a premature termination codon (PTC)-containing mRNA in *E. coli*. Premature translation termination and the resulting loss of ribosome protection downstream of the PTC expose the mRNA to internal cleavage by RNase E. (B) Nonsense-mediated decay (NMD) in metazoans. Premature translation termination results in an unusually long 3' UTR, often in conjunction with an exon junction downstream of the PTC. The assembly of the PTC surveillance proteins UPF1, UPF2, and UPF3 at the site of translation termination is guided by the presence there of termination factors eRF1 and eRF3 and can be enhanced by the interaction of UPF3 with an exon junction complex, a heteromultimer deposited on exon junctions during splicing, transported with mRNA to the cytoplasm, and displaced by translating ribosomes only if bound in the coding region. Phosphorylation of UPF1 by the serine/threonine kinase SMG1 triggers nonsense-mediated decay via any of three pathways: deadenylation-independent decapping by Dcp2 (left), endonucleolytic cleavage by SMG6 (centre), or poly(A) tail removal by Ccr4-Not (right). Deadenylation-independent decapping or poly(A) removal leads to degradation via the pathways depicted in Figure 2B. Endonucleolytic cleavage leads to 5'-exonucleolytic degradation of the 3' fragment by Xrn1 and degradation of the 5' fragment via the pathways depicted in Figure 2B.

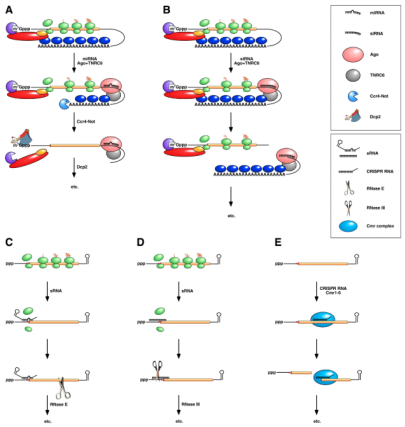

Figure 6. Post-transcriptional downregulation by noncoding RNAs in eukaryotes and bacteria

(Top) Repression by miRNAs in vertebrates and insects. (A) A miRNA associated with Ago and TNRC6 (also known as GW182) binds to the 3' UTR of a message to which it is partially complementary, impeding (though not abolishing) translation and accelerating poly(A) tail removal by the deadenylase Ccr4-Not. The deadenylated mRNA is then decapped by Dcp2 and degraded exonucleolytically by Xrn1 and possibly also by exosomes. (B) An siRNA associated with Ago and TNRC6/GW182 binds to a message to which it is fully complementary, either within the 3' UTR or somewhere upstream, and directs endonucleolytic cleavage there by Ago. Endonucleolytic cleavage leads to 5'-exonucleolytic degradation of the 3' fragment by Xrn1 and degradation of the 5' fragment via the pathways depicted in Figure 2B. Note that miRNAs and siRNAs have the same regulatory potential, their mode of action being determined by the degree of complementarity of their mRNA targets and the activity of the Ago and TNRC6/GW182 proteins with which they associate. (Bottom) Repression by sRNAs and CRISPR RNAs in bacteria. (C) sRNA binding to a partially complementary mRNA impairs translation initiation, often by occluding the ribosome binding site. No longer protected by ribosomes, the message becomes vulnerable to attack by RNase E. (D) Antisense sRNA binding to a fully complementary mRNA can create a long, perfectly paired duplex susceptible to cleavage by RNase III. (E) A Cmr-associated CRISPR RNA directs endonucleolytic cleavage of a complementary message by one of the six Cmr proteins. Ribosomes have been omitted from this panel because the effect of CRISPR RNAs on translation has not been investigated.

Table 1

Enzymes of broad importance for cytoplasmic mRNA decay

	Enzyme	Specificity/Function
Endonucleases		
Bacteria:	RNase E*, RNase G*	Single-stranded RNA
	RNase III	Double-stranded RNA
	RNase J	Single-stranded RNA
	RNase Y	Single-stranded RNA
	Cmr complex	mRNA-CRISPR RNA duplexes
Eukaryotes:	Ago	mRNA-siRNA or mRNA-miRNA duplexes that are fully paired
	SMG6	PTC-containing mRNAs
3' Exonucleases		
Bacteria:	Polynucleotide phosphorylase	Single-stranded 3' end
	RNase R	Single-stranded 3' end
	RNase II	Single-stranded 3' end
	Oligoribonuclease	RNA oligonucleotides
Eukaryotes:	Exosome	3' end not protected by PABP
5' Exonucleases		
Bacteria:	RNase J	Monophosphorylated 5' end
Eukaryotes:	Xrn1	Monophosphorylated 5' end
5'-end modification		
Bacteria:	RppH	Pyrophosphate removal
Eukaryotes:	Dcp2	Decapping of RNA polynucleotides
	DcpS	Decapping of RNA oligonucleotides
3'-end modification		
Bacteria:	Poly(A) polymerase (PcnB)	Polyadenylation
	Polynucleotide phosphorylase	Heteropolymeric tail addition
Eukaryotes:	Ccr4-Not	Deadenylation
	Pan2-Pan3	Deadenylation
	PARN	Deadenylation
	Cid1#, Zcchc11#	Oligouridylation

*,#Homologous enzymes.