5
mRNA Decay and RNA-degrading Machines in Prokaryotes and Eukaryotes

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5.1
Summary

Research over the past two decades has elucidated the pathways for mRNA decay in *Escherichia coli* and *Saccharomyces cerevisiae*. The study of these model organisms has given us a general overview of mRNA decay in both prokaryotes and eukaryotes. Although the two pathways are largely divergent, some common features are also apparent. Amongst the novel discoveries made in the course of this work was the identification and characterization of the *E. coli* RNA degradosome and the *S. cerevisiae* exosome, which are multienzyme RNA-degrading machines involved in the maturation of stable RNA and the degradation of mRNA. In this chapter, we describe and compare the *E. coli* and *S. cerevisiae* mRNA decay pathways, we discuss the role of RNase E and the RNA degradosome in procaryotic RNA degradation, and then we compare the degradosome to RNase E-based complexes found in other bacteria, and to the eukaryotic exosome.

5.2
Introduction

The ribosome, which decodes genetic information and synthesizes protein, is not the only multicomponent machine that uses RNA as a substrate. Other examples include eukaryotic systems involved in mRNA splicing, 3′-polyadenylation and export from the nucleus as well as eukaryotic and procaryotic complexes involved in RNA maturation and degradation. The *E. coli* RNA degradosome and the *S. cerevisiae* exosome are multienzyme RNA-degrading machines involved in the maturation of stable RNA and the degradation of mRNA. The best-known stable RNAs are the transfer and ribosomal RNAs, which are processed from precursor transcripts to their mature forms. Messenger RNAs are unstable with half-lives in *E. coli* ranging from 30 s to 20 min at 37° C. In eukaryotic cells, mRNA turnover is slower, but the half-lives are usually shorter than the generation time. The instability of mRNA is an important property permitting timely adjustments to changes in growth conditions or to genetically controlled programs of expression. Until recently, transfer and ribosomal RNAs

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were believed to be protected from degradation by their rapid folding and assembly into compact structures. This simplistic view seems unlikely since the RNA-degrading machinery is more robust than imagined previously. Another widely held preconception was that the enzymes involved in the processing of stable RNA are distinct from those in the degradation of mRNA. With the discovery, in *E. coli* and *S. cerevisiae,* that ribonucleases involved in the maturation of ribosomal RNA are also important in the degradation of mRNA, it is now evident that these processes are closely connected. Several articles at the beginning of the references are recommended for reviews on the degradation of mRNA in bacteria [1–4] and eukaryotes [5–7].

5.3 mRNA Decay in *E. coli*

In *E. coli,* the degradation of mRNA is mediated by the combined action of endo- and exoribonucleases (Fig. 5-1A). The endonucleases initiate mRNA decay by creating fragments that are then degraded by the exonucleases. It is now generally believed that the principal endonuclease involved in mRNA decay is RNase E. Arguments supporting this contention have been marshalled in a recent review [8]. In a subsequent step, two enzymes, RNase II and PNPase, degrade the RNA fragments in a 3′→5′ pathway. Enzymes related to RNase II and PNPase are widespread in bacteria and eukaryotes [9, 10]. RNase II is a hydrolytic enzyme producing nucleotide monophosphates (AMP, etc.). PNPase, which is a phosphorylase, uses inorganic phosphate yielding nucleotide diphosphates (ADP, etc.). Although we sometimes speak of PNPase as a phosphate-dependent ribonuclease, this is not strictly correct since nucleases are hydrolytic by definition. A strain of *E. coli* with mutations in the genes encoding RNase II and PNPase, which is conditionally lethal, accumulates mRNA fragments under conditions that are non-permissive for growth [11]. This result is the principal experimental evidence for mRNA fragments as intermediates in decay. The 3′-ends of many bacterial mRNAs, such as those formed by rho-independent termination, are sequestered in stem-loop structures that protect them from degradation. Intercistronic regions in polycistronic transcripts can also harbor protective RNA structures and nascent transcripts have 3′-ends protected by the RNA polymerase. Thus, RNase II and PNPase are believed to be generally incapable of initiating the decay of an intact mRNA. The endonucleolytic cleavage of an mRNA can remove protective RNA structures or sever the nascent transcript from the RNA polymerase, thus producing a single-stranded 3′-end upstream of the cleavage that is a binding site for the exonucleases. Furthermore, the possibility that a cleavage by RNase E might 'trigger' exonuclease-mediated decay of the downstream mRNA fragment is discussed below. The idea that the initial attack by an endonuclease is followed by exonucleolytic decay of mRNA fragments was an important advance in our concept of the degradation of mRNA in *E. coli* [12, 13].

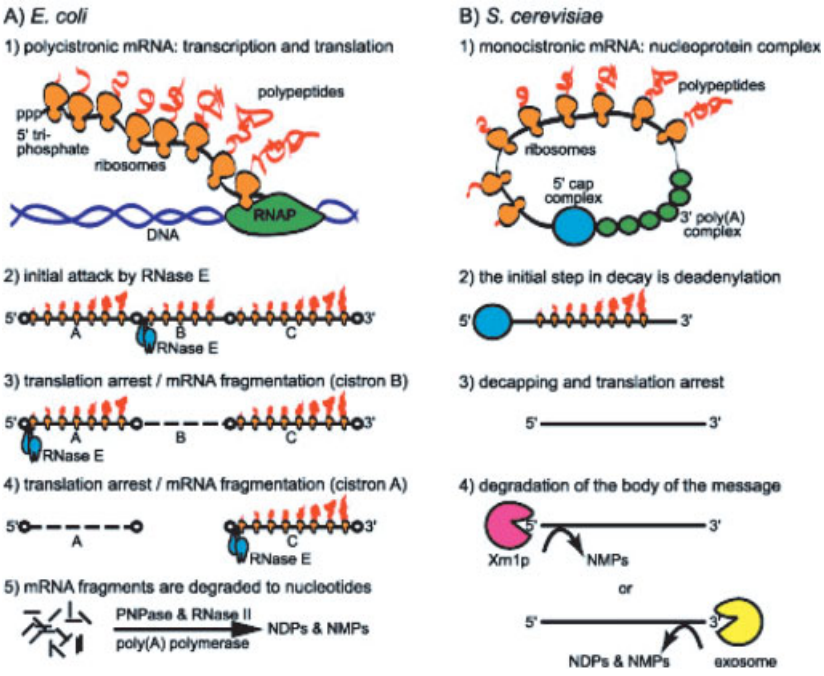

Figure 5-1 Messenger RNA decay in *E. coli* (A) and *S. cerevisiae* (B). The degradation of a hypothetical polycistronic transcript encoding genes A, B, and C is shown in (A). The order of decay depicted here is B, A and then C, but this is an arbitrary choice. The pattern of decay of a real polycistronic transcript in *E. coli* depends on the transcription unit. RNAP (step 1) is the DNA-dependent RNA polymerase. The open circles (steps 2–4) indicate 5′- and 3′-UTRs, and intergenic regions that contain elements controlling translation and mRNA decay. Dashed lines (steps 3–4) indicate cistrons where translation has been arrested and the mRNA has been fragmented by RNase E. The degradation of a hypothetical eukaryotic mRNA is shown in (B). The 5′-cap and 3′-poly(A) complexes are important for translation and mRNA stability. Their removal arrests translation initiation and triggers the degradation of the body of the message. RNase II (A, step 5) and Xrn1p (B, step 4) are hydrolytic enzymes that use water to produce nucleotide monophosphates (NMPs). PNPase (A, step 5) is a phosphorylase that uses inorganic phosphate to produce nucleotide diphosphates (NDPs). The exosome (B, step 4) has both phosphorylytic and hydrolytic activity.

In Fig. 5-1(A) (step 5), poly(A) polymerase assists in the degradation of the mRNA fragments by RNase II and PNPase. RNA 3′-polyadenylation has been implicated in *E. coli* mRNA decay [14–17]. In the PNPase/RNase II double mutant, under non-permissive conditions, the polyadenylation of mRNA decay intermediates is easily detected. *In vivo* and *in vitro* work has shown that 3′-poly(A) addition promotes the exonucleolytic degradation of RNAs whose 3′-ends are sequestered in secondary structure [18–20]. Since the exonucleases are single-strand-specific, the addition of a 3′-poly(A) tail creates a binding site for the exonucleases (see Refs. [21–23] for reviews). Furthermore, *in vitro* experiments using purified PAP I, PNPase and ATP

have demonstrated the degradation of a structured RNA in a reaction involving multiple cycles of polyadenylation [20]. Based on these results, it has been suggested that repeated rounds of polyadenylation and exonucleolytic attack might be necessary for certain hard-to-degrade intermediates. Taken together, this work has shown that RNA 3′-polyadenylation facilitates the decay of certain highly structured mRNA fragments and it suggests that polyadenylation could have a general role in accelerating degradation by the exonucleases.

5.4 mRNA Decay in *S. cerevisiae*

In eukaryotes, the endonucleolytic decay of mRNA appears to be less important although it could be involved in regulating the stability of certain messages [24–26]. Two exonucleolytic pathways have been described in *S. cerevisiae*: 3′→5′ and 5′→3′ pathways [27–31]. Eukaryotic mRNAs are protected by 5′-cap structures and 3′-poly(A) tails. Both elements bind specific proteins that can interact with each other, so that the mRNA is effectively circular (Fig. 5-1B). The cap and poly(A) complexes are important for translation and mRNA stability. Deadenylation by a poly(A)-specific nuclease, followed by decapping, are prerequisites for degradation of the body of the message. Decapping is deadenylation-dependent. In the 5′→3′ degradation pathway, the mRNA is degraded by the exonuclease encoded by *XRN1*, a hydrolytic enzyme producing nucleotide monophosphates. The 3′→5′ degradation pathway involves a multiprotein complex, the exosome (see below), which contains both hydrolytic and phosphorylytic enzymes. Note that since deadenylation promotes decapping and thus the arrest of translation initiation, the 5′→3′ and 3′→5′ pathways are ordered processes in which no new translation can occur during the degradation of the body of the mRNA. In *S. cerevisiae*, the 5′→3′ pathway is apparently the predominant mode of degradation. Whether this is the case in other eukaryotes remains to be clarified. A recent *in vitro* study suggests that the 3′→5′ pathway mediated by the human exosome has an important role in the degradation of short-lived mRNAs encoding certain cytokines and oncogenes [32].

5.5 A Comparison of mRNA Decay in *E. coli* and *S. cerevisiae*

A comparison of the *E. coli* and *S. cerevisiae* pathways in Fig. 5-1 shows that bacterial and eukaryotic mRNA decays are considerably divergent. This probably reflects fundamental differences in the organization of transcription units and the mechanism of translation initiation. *E. coli* does not have a 5′→3′ degradation pathway. The 5′-ends of its mRNA are neither capped nor are there known 5′→3′ ribo-exonucleases. Homologs of the yeast capping enzyme and Xrn1p are found only in other eukaryotes. Thus, capping and the 5′→3′ degradation pathway are apparently specific features of the eukaryotic cell. Eukaryotic messages are generally monocistronic and translation initiation usually involves scanning from the 5′-cap complex to the AUG. Bacterial messages are often polycistronic and translation initiation involves

sequence-specific binding of the ribosome just upstream of the initiating AUG. Owing to this internal mode of entry, the frequency of translation initiation can be independent for each cistron of a polycistronic mRNA. In addition, the inactivation by endonucleolytic cleavage can trigger the decay of a cistron without disrupting neighboring cistrons. Thus, in a bacterial transcription unit with a single promoter, the yield of protein as well as the level of steady-state mRNA can vary considerably from cistron to cistron even though the transcription rate is equivalent. It is difficult to imagine how the independent decay of individual cistrons within a polycistronic transcript could be achieved without the action of an endonuclease. In contrast, there is no obvious advantage in initiating the decay of a monocistronic eukaryotic transcript with an endonuclease although an endonucleolytic cleavage in a 3′-UTR could serve as an alternative pathway for deadenylation.

It has been argued that the *E. coli* mRNA decay pathway outlined in Fig. 5-1(A) is inherently flawed since, in principle, a translated message might be cleaved internally leading to a truncated mRNA without a stop codon and thus a stalled ribosome with an incomplete nascent polypeptide. In the next section, we discuss possible mechanisms whereby many *E. coli* mRNAs may in fact decay via an orderly process in which initiation of decay is coordinated with the arrest of translation, as is the case for eukaryotic mRNAs. Nevertheless, *E. coli* and other bacteria have a mechanism for rescuing ribosomes stalled on an mRNA fragment. The tmRNA, with both tRNA and mRNA function, permits a *trans*-translation step in which the tmRNA initially binds to the stalled ribosome as a tRNA, then it serves as a short mRNA template providing a stop codon [33–35] (see Chap. 11 for more details). Briefly, in this process, the mRNA fragment is released and the truncated polypeptide receives a short C-terminal addition encoded by the tmRNA. The tmRNA-encoded C-terminal tag contains a signal that directs the proteolysis of the incomplete polypeptide. Factors associated with the tmRNA include RNase R, a ribonuclease related to RNase II [36]. Further work is required to establish if RNase R is involved in the decay of the mRNA fragment. It should be noted that a related process, involving the exosome, has been described in yeast [37]. In eukaryotic 'non-stop decay', the exosome degrades the mRNA fragment, although the fate of the stalled ribosome and the associated polypeptide remains to be elucidated. Whereas the tmRNA in *E. coli* can rescue a ribosome stalled at the 3′-end of a non-stop mRNA, it can also release ribosomes that are stalled internally on an intact mRNA. Thus, the emerging view is that the tmRNA has a general role in the rescue of stalled ribosomes [33].

5.6
RNase E Specificity: A Role in Translation Arrest?

The RNase E of *E. coli* is a single-strand specific endo-ribonuclease with a preference for AU-rich sequences [38–41]. *In vitro* work has shown that although RNase E is an endonuclease, its activity is influenced by the 5′-end of the RNA [42]. The hybridization of an oligonucleotide to the 5′-end of a small RNA substrate inhibits endonucleolytic cleavage by RNase E at a downstream site suggesting that single-stranded 5′-ends facilitate substrate binding. These results could explain previous *in vivo* work

which showed that RNA stem loops, sequestering the 5′-end into double-stranded structure, impede RNase E-mediated degradation [43]. Furthermore, the *in vitro* work showed that the initial rate of cleavage is faster with a 5′-monophosphate end than with a 5′-triphosphate, and that covalently closed circular RNAs are resistant to cleavage by RNase E. Thus, RNase E 'senses' RNA topology presumably by recognition of the 5′-end although other results suggests that the 3′-end could be involved as well [44]. The remarkable stability of covalently closed circular mRNA *in vivo* is further evidence for the importance of the RNA ends in controlling degradation [45]. Nevertheless, mRNA that is fully protected at the 5′-end by a stem-loop structure can still be degraded in a slow RNase E-dependent pathway that apparently involves 'internal entry', i.e., via an end-independent mechanism [46].

The interaction of RNase E with the ends of its substrate could help to give an overall direction to mRNA decay. The model in Fig. 5-2 shows how RNase E could help to reinforce a 5′→3′ directionality if the initial cleavage is in the 5′-end. RNase E, which is known to be oligomeric, is generally presumed to be a dimer. After the initial cleavage, RNase E is envisioned to remain tethered to the 5′-end of the decay product (Fig. 5-2). Since the enzyme is dimeric, it could remain bound to the 5′-end and simultaneously interact with a downstream site by 'looping out' the intervening RNA [1]. Repeated cycles of tethering and looping could produce a rapid, processive reaction in which RNase E fragments the message. If the initial cleavage inactivates translation, this could help to resolve the translation-decay conflict by facilitating RNase E cleavage as the ribosomes clear from the message. A 5′→3′ directionality of mRNA decay was first proposed by Apirion as a means of avoiding conflicts between translation and decay [12]. This directionality is indeed evident in long cistrons such as *lacZ*, in which the 5′ region starts decaying before the 3′-end is made [47]. The localization of RNase E to the inner periphery of the *E. coli* cell [48] could also contribute to this directionality, since the most recently synthesized RNA, being associated with the nucleoid in the interior of the cell, might not be accessible to RNase E: it is the 5′-end of the growing transcript that would first reach RNase E.

The model in Fig. 5-2 is probably an oversimplification. Recent work with short synthetic RNAs suggests that RNase E has a preference for distal cleavage sites giving an overall 3′→5′ directionality *in vitro* [49]. The basis for this preference is not known but it could involve recognition of the 3′-end by RNase E. Furthermore, our understanding of the decay of two well-studied mRNAs is at odds with the model in Fig. 5-2. In the *rpsO* message, the initial cleavage by RNase E is 10 nucleotides downstream of the translation termination codon and in the *rpsT* message, in the 3′ half of the coding sequence [50, 51]. Thus, there is no evidence that RNase E cleaves the 5′-UTRs of these messages. Whether our understanding of the *rpsO* and *rpsT* messages can be generalized to other messages is not clear since both are short transcripts whose translation is self-regulated by the binding of their cognate proteins to translational operators in their 5′-UTRs. Regardless of these considerations, other mechanisms for avoiding the translation-degradation conflict are possible. For instance, a relatively minor modification of the scheme depicted in Fig. 5-2 would be that RNase E binds to, but does not cleave, the translation-initiation region in an initial step that arrests translation. Indeed, it has been proposed that RNase E (or other RNA-binding

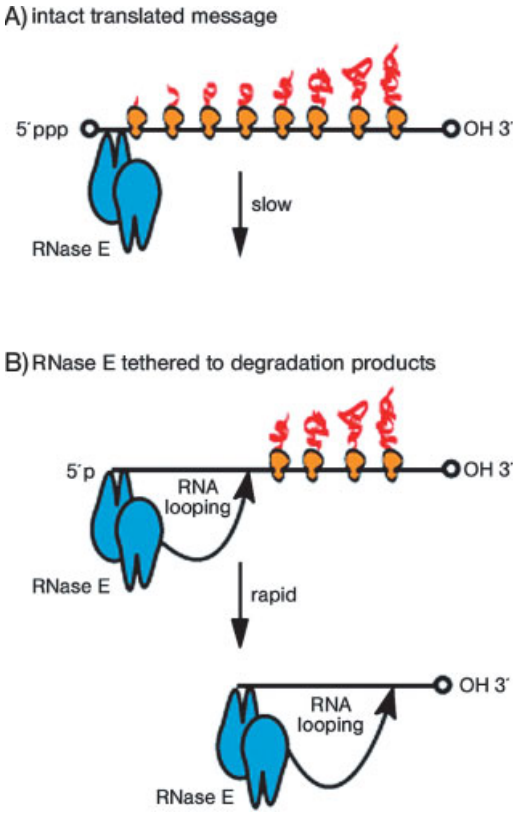

Figure 5-2 A hypothetical scheme in which a slow initial cleavage by dimeric RNase E leads to a rapid processive fragmentation of the mRNA (see text). In this model, the initial cleavage in the 5′-UTR inactivates translation initiation. The subsequent cleavages by RNase E tethered to the monophosphate 5′-end of the decay products occur as the ribosomes clear from the transcript. In this process, RNase E remains bound to the 5′-monophosphate end in a reaction that involves the 'looping out' of the RNA substrate as RNase E searches for cleavage sites. For simplicity, the mRNA is represented as a monocistronic transcript with protective structures in the 5′- and 3′-UTRs (open circles), but this model could also apply to a cistron within a polycistronic message.

proteins) could arrest translation and sequester the message in an inactive form before initiating nucleolytic degradation [52]. Translation initiation sites are unstructured and by definition contain binding sites for the ribosomal protein S1. RNase E is single-strand-specific and contains an S1 RNA-binding domain. Thus, 5′-UTRs and intergenic regions containing translation-initiation regions could be targets for RNase E binding. Other non-nucleolytic models for translation arrest have been proposed. For instance, it has been suggested that decay might be initiated by a 'collapse'

of the translation-initiation region into RNA secondary structure that inhibits ribosome binding [53, 54]. In research on *E. coli* mRNA decay, sorting out the connection between mRNA degradation and translation arrest is an important challenge.

The ideas presented in this section are based principally on the study of short RNA substrates *in vitro* or short monocistronic messages *in vivo*. How these ideas apply to more complicated polycistronic mRNAs remains to be elaborated; however, it seems probable that the intergenic regions of polycistronic transcripts will contain elements that act as initiators of the decay as well as elements that serve as barriers preventing the spread of decay from a cistron to its neighbors.

5.7 The *E. coli* RNA degradosome

Two temperature-sensitive mutations, now known as *rne3071* and *rne1*, were identified because of their effect on the maturation of 5S ribosomal RNA [55] and the degradation of mRNA [56]. Subsequent studies showed that both mutations are in the structural gene for RNase E [57–59]. It is now generally accepted that RNase E has a role in both the maturation of ribosomal RNA and the degradation of mRNA. Recent work has shown that RNase E is also essential for tRNA maturation and evidence from these studies suggests that tRNA deficiency is the ultimate cause of lethality in RNase E mutant strains [60, 61]. It is striking that RNase E appears to have a role in the processing and degradation of nearly every transcript in *E. coli*. RNase E is a large, multidomain enzyme that is part of a complex called the RNA degradosome. Figure 5-3 shows a schematic representation of the primary structure of RNase E. Its

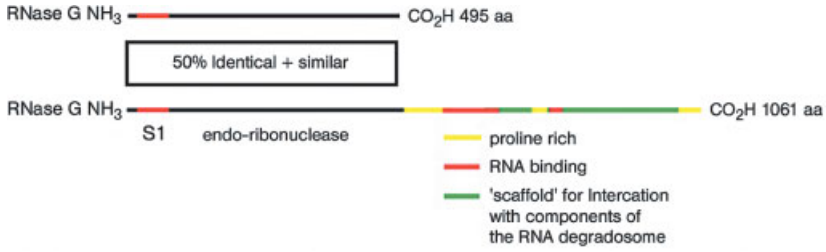

Figure 5-3 The RNase E/G family of enzymes. The RNase E and RNase G of *E. coli* are paralogues in which the N-terminal S1 RNA-binding domain and the endo-ribonuclease catalytic site are conserved [59]. RNase E differs from RNase G by its long C-terminal non-catalytic region containing proline 'hinge' regions, sites that bind RNA, and a 'scaffold' involved in protein–protein interactions with other components of the RNA degradosome (see text). Homologs of RNase G (catalytic domain only) and RNase E (non-catalytic extensions) are found throughout the eubacterial kingdom and in the plant chloroplast [71, 72, 110].

nucleolytic activity resides in the N-terminal half of the protein, which also contains an S1 RNA-binding domain [62–66]. The C-terminal half (CTH) of the protein contains several proline-rich regions, two arginine-rich RNA-binding regions and sites for protein–protein interactions with the other components of the RNA degradosome. *E. coli* encodes a paralogue, now called RNase G, that is about half the size of RNase E [67–69]. Although their catalytic domains are related, RNase G lacks the region corresponding to the CTH of RNase E (Fig. 5-3). It is noteworthy that RNase G is also 5′-end-dependent. This thus appears to be a general property of the RNase E/G family and the determinants involved in the 5′-end preference are apparently part of the conserved N-terminal catalytic domain. Proteins related to RNases E and G are found throughout the eubacterial kingdom and in certain plants [70–72]. The plant homologs are presumably in the chloroplast, which is an organelle of eubacterial origin. The 'RNase E/G' family can be divided into two groups: the large RNase E-like enzymes that can form degradosome complexes and the small RNase G-like proteins that presumably act alone. Although related, these enzymes are not functionally equivalent since in *E. coli*, RNase E is essential for viability whereas RNase G is dispensable. Nevertheless, it has been shown recently that if RNase G is overexpressed, it can complement a knockout of the gene encoding RNase E [73].

A multienzyme complex, now called the RNA degradosome, was discovered during the purification and characterization of *E. coli* RNase E [39, 74–76]. The major components of the RNA degradosome include RNase E, PNPase, and the DEAD-box RNA helicase, RhlB [77–79]. The RNA degradosome also contains enolase, a glycolytic enzyme, as an integral component. Associated proteins, present in substoichiometric amounts, include polyphosphate kinase (PPK), DnaK, and GroEL. Inter-actions with other enzymes such as *E. coli* poly(A) polymerase and the ribosomal protein S1 have also been reported [80, 81]. The role of enolase, PPK and other associated proteins in the degradation of mRNA remains to be clarified. The non-catalytic CTH of RNase E has been shown to contain the protein 'scaffold' upon which the other components of the RNA degradosome assemble [70, 82]. A functional 'minimal' degradosome containing RNase E, RhlB, and PNPase can be reconstituted from purified components [83, 84].

The association of RNase E and PNPase in a complex could provide a direct physical link for their cooperation in degradation. Indeed, there is *in vivo* evidence for coordination between endonucleolytic cleavage at the 5′-end by RNase E and attack at the 3′-end by PNPase [19, 21, 85]. RNA I, a small 108 nucleotide molecule, is a repressor of ColE1 plasmid replication with a short lifetime similar to that of an mRNA. Although the primary transcript with a 5′-triphosphate end and 3′-RNA stem-loop structure is resistant to PNPase attack, removal of five nucleotides from the 5′-end by RNase E triggers the decay of the RNA I-$_5$ intermediate in a pathway that involves 3′-polyadenylation and degradation by PNPase. It has been suggested that poly(A) polymerase and PNPase might be recruited to RNA I-$_5$ by their interaction with RNase E in the degradosome. Indeed, with the *rne131* mutant (see below), which disrupts the RNA degradosome, there is significant stabilization of the RNA I-$_5$ intermediate (M. Dreyfus, unpublished results). The decay of other small regulatory RNAs, such as

Sok, controlling R1 plasmid partition, and CopA, controlling R1 plasmid replication, are also controlled by an initial cleavage at the 5′-end by RNase E followed by a 3′ attack by PNPase [86, 87]. Although it is tempting to believe that the decay of these small regulatory RNAs could serve as a model for mRNA degradation, as discussed above, there is little evidence supporting the idea that RNase E initiates decay by cleavage in the 5′-end of an mRNA. However, a coordination between RNase E cleavage and subsequent steps involving polyadenylation and PNPase attack could be part of a pathway mediating the degradation of structured mRNA decay intermediates or mRNA 3′-end-fragments blocked by stem-loop structures.

The nuclease activity of RNase E is essential, but strains expressing protein with C-terminal truncations are viable. An allele, now known as *rne131*, directing the synthesis of a protein lacking the non-catalytic part of RNase E, was isolated in a screen for extragenic suppressors of a temperature-sensitive *mukB* allele [88]. The suppression resulted from overexpression of the mutant MukB protein. Several other *rne* mutants were obtained, all of them resulting in a truncated protein. The *rne131* mutation was extensively characterized in a subsequent study which showed that the maturation of 5S ribosomal RNA was normal, whereas there was a small but detectable slowdown in the decay of bulk mRNA [89]. It was also demonstrated that certain messages such as the endogenous *thrS* mRNA or messages synthesized by bacteriophage T7 RNA polymerase were preferentially stabilized compared with bulk mRNA. These messages, in which the coupling of transcription and translation is disrupted, might be degraded by RNase E in an alternative pathway distinct from that involving normally translated mRNA (see the next paragraph). Recent work with mutants disrupting various regions in the non-catalytic part of RNase E has demonstrated that it contains both positive and negative elements affecting mRNA-degrading activity [90]. This work also showed that (i) the autoregulation of RNase E synthesis (see the next section) compensates, at least partly, for the defective activity of the mutant enzyme *in vivo*; (ii) the *rne131* mutant has a significant growth defect in the absence of autoregulation; and (iii) even with autoregulation, the mutant strains are less fit than an isogenic wild-type strain in growth competition experiments. Thus, it seems reasonable to suggest that the non-catalytic part of RNase E is involved in fine-tuning RNA-degrading activity although the specific role of each element remains to be elucidated.

It is noteworthy that bacteriophage T7 expresses a protein kinase that phosphorylates a number of *E. coli* proteins including two components of the RNA degradosome, RNase E and RhlB [91]. The target in RNase E, which is heavily phosphorylated, is the non-catalytic region containing the RNA-binding domains and protein scaffold. Bacteriophage T7 encodes its own RNA polymerase. In uninfected cells, mRNA synthesized by this polymerase is exceptionally sensitive to inactivation by RNase E [92]. Since the transcription-elongation rate of the T7 RNA polymerase is 5- to 10-fold faster than its *E. coli* counterpart, it largely exceeds the rate of translation elongation. This leads to long stretches of ribosome-free mRNA proximal to the RNA polymerase. The inactivation of these messages by RNase E appears to involve 'internal entry', i.e., via a 5′-end-independent mechanism [46, 91].

Expression of the T7 protein kinase in uninfected cells stabilizes mRNA transcribed by the T7 RNA polymerase [91]. Thus, during a T7 infection, phosphorylation could help to stabilize the bacteriophage messages although this has not been demonstrated directly. It should be interesting to elucidate the mechanism by which the phosphorylation of RNase E controls 5′-end-independent mRNA decay and to ask if there are cellular protein kinases, perhaps regulated as part of a signal transduction pathway, which modulate RNase E activity depending on conditions of growth or stress.

5.8 The Autoregulation of RNase E and PNPase Synthesis: A Link between Bulk Translation and mRNA Stability

The expressions of RNase E and PNPase are both autoregulated in posttranscriptional pathways that involve the control of mRNA stability via elements in the 5′-untranslated region (UTR) of their messages (Fig. 5-4). The mRNA encoding RNase E contains a 361 nt 5′-UTR region that controls the stability of the *rne* message in response to RNase E levels [93, 94]. Experiments in which fusions were constructed between the 5′-UTR and a *lacZ* reporter gene demonstrated that the *rne* leader regulates functional stability by a mechanism that 'senses' RNase E activity in the cell. More recent work has identified a stem-loop structure in the *rne* leader that is essential for autoregulation and it has been proposed that binding to this site tethers RNase E to the mRNA and promotes its degradation [95, 96]. In the presence of high levels of RNase E, its mRNA would be destabilized thus decreasing expression, whereas low levels would provoke mRNA stabilization and increased expression. The control of RNase E expression can be viewed as a homeostasis that assures adequate RNase E activity. Indeed, recent *in vivo* results have confirmed that this autoregulatory system responds to changes in the demand for RNase E activity [97, 98]. This work shows that the RNase E message is particularly sensitive to changes in RNase E concentration, i.e., it is only partially inactivated at concentrations where other cellular targets are already saturated. How this is achieved remains to be elucidated, but it suggests that the activity of RNase E on its own message is weaker than its activity on other messages or precursors of stable RNA.

PNPase is expressed as part of a polycistronic transcript that begins with the *rpsO* gene encoding the small ribosomal protein, S15 [99]. Maturation of the *pnp* message involves RNase III processing of a double-stranded RNA structure encoded in the intergenic space between *rpsO* and *pnp*. The processing by RNase III is essential for autoregulation [100] and recent work has revealed a novel mechanism for the control of PNPase expression [101]. RNase III processing yields a mature *pnp* mRNA with its 5′-UTR hybridized to an oligoribonucleotide that stabilizes the mRNA (Fig. 5-4B). The degradation of this oligoribonucleotide by PNPase destroys the duplex thus exposing the 5′-monophosphate end and destabilizing the *pnp* message, which is presumably degraded by RNase E although this has not been established. Thus, the oligoribonucleotide acts as a sensor of the level of exonucleolytic activity in the cell.

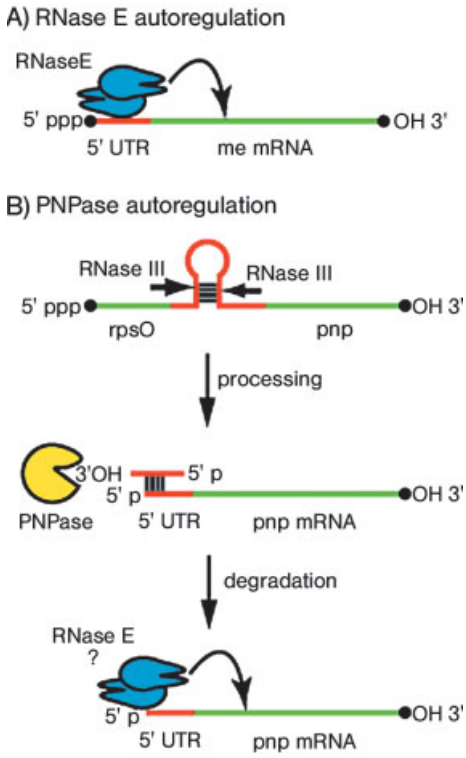

Figure 5-4 The autoregulation of RNase E (A) and PNPase (B) synthesis. The green regions represent coding sequences whereas the red represent untranslated regions (UTRs). In the mechanisms described in both panels, autoregulation involves a process that 'senses' nuclease activity in the cell and controls expression by modulating mRNA stability. In (A), the *rne* mRNA has a long, 361 nt, 5′-UTR that is specifically targeted by RNase E in a process involving 'tethering' to an element in the 5′-UTR (see text). In (B), PNPase expression is regulated in a two-step process. The primary polycistronic transcript encoding *rpsO* and *pnp* is processed to a mature *pnp* message in a pathway that involves degradation of the *rpsO* message (not shown) and the processing of an intergenic RNA stem loop by RNase III. The product of RNase III cleavage is drawn to emphasize that the processed message has a 5′-end that is protected in a double-stranded RNA structure. The oligoribonucleotide hybridized to the 5′-UTR of the *pnp* mRNA has a short protruding 3′-end that is sensitive to attack by PNPase. Degradation of the protective oligoribonucleotide by PNPase promotes the decay of the *pnp* mRNA, which could be mediated by RNase E although this has not been demonstrated.

It should be mentioned that the other major exo-ribonuclease in *E. coli* mRNA decay, RNase II, is also autoregulated and that there is cross-regulation between RNase II and PNPase [102]. The effect of RNase II on PNPase expression could involve degradation of the oligoribonucleotide that stabilizes the *pnp* message although this remains to be tested. The mechanism by which RNase II is autoregulated has not yet been elucidated.

The fact that the stability of the *rne* and *pnp* mRNAs varies with the concentration of their cognate proteins suggests that PNPase and RNase E are never present in excess in the cell. Rather, these proteins must be able to adjust continuously their concentration through autoregulation. Consistent with this view, a burst in the synthesis of an RNase E substrate causes a transient stabilization of the *rne* mRNA until the RNase E pool has expanded to meet the new demand [97]. Similarly, even though poly(A) tails usually destabilize mRNA fragments, overexpression of poly(A) polymerase leads, paradoxically, to the stabilization of the *pnp* and *rne* mRNAs, presumably because the need to degrade the extra poly(A) tails increases the demand for PNPase and RNase E [103]. Interestingly, the homeostatic regulation of RNase E and PNPase expression may be responsible for a seemingly unrelated phenomenon, i.e., the well-known stabilization of bulk mRNA that follows a block in translation. This phenomenon is generally attributed to a protection of mRNAs by stalled ribosomes. However, even untranslated mRNAs are protected from degradation under these circumstances, showing that the stabilization must somehow reflect the reduced activity of the degradation machinery itself. In particular, RNA I and its RNase E cleavage product, RNA I-5, are stabilized, suggesting that both RNase E and PNPase are inhibited under these circumstances [85]. The homeostasy of RNase E and PNPase can provide a straightforward explanation for these effects. Following a translation block, the synthesis of ribosomal RNA is known to be boosted. Moreover, the newly synthesized ribosomal RNA is unstable since it cannot assemble into ribosomes due to the lack of new ribosomal proteins [104]. This results in an increased demand for RNase E and PNPase under conditions where the pools of these enzymes cannot expand. Their titration by the ribosomal RNA thus explains the stabilization of bulk mRNA [85, 97]. Interestingly, a block in translation also causes stabilization of many and perhaps most mRNAs in yeast and higher eukaryotic cells [105]. It will be interesting to learn if the expression of components of the eukaryotic mRNA degradation machinery is also autoregulated.

5.9
RNA-degrading Machines in other Organisms

Several other degradosome-like complexes have been identified and characterized over the past decade (Table 5-1). All act in a 3′→5′ degradation pathway. An RNase E-based complex has been characterized in *Rhodobacter capsulatus*, which is a photosynthetic Gram-negative bacteria that is only distantly related to *E. coli* [106]. Although a PNPase-like activity co-purified with this complex, none of the major polypeptides identified by protein sequencing corresponded to a PNPase homolog.

Table 5-1 RNA-degrading multienzyme complexes

	Degradosome ***E. coli***	**Degradosome** ***R. captulatus***	**?** **chloroplast**	***S. cerevisiae*** **mitochondria**	**Exosome** ***S. cerevisiae*** **nucleus/cytoplasm**
Integral proteins	PNPase 3′→5′phosphorylase (PH1, PH2, S1, KH)		100RNP 3′→5′ phosphorylase (PH1, PH2, S1, KH)	Dss1p 3′→5′ ribonuclease (RNase II homolog)	Rrp41p, 42p, 43p, 45p, 46p, Mtr3p 3′→5′ phosphorylase
	RNase E endonuclease, scaffold	RNase E endonuclease, scaffold	?	p75 ?	Rrp4p, 6p, 40p, 44p, Clsp4p 3′→5′ ribonuclease S1, KH, RNA binding
	RhlB RNA helicase	Rhl1, Rhl2 RNA helicase		Suv3p RNA helicase	
	enolase glycolytic enzyme	Rho transcription termination			
Associated proteins	DnaK, GroEL chaperons	PNPase?	?	?	Mtr4p, Ski2p RNA helicases
	PPK, PAP, S1 specificity?				Ski3p, Ski8p specificity?

Thus, it has been suggested that PNPase might only be loosely associated with this complex. Curiously, two DEAD-box RNA helicases and the transcription termination factor Rho, which is also an RNA helicase, were shown to be associated with RNase E. The role of multiple helicases in the complex is unclear although these proteins might act as adaptors that target the degradosome to specific substrates. The link with Rho is intriguing. In *E. coli*, Rho is an essential factor that is responsible for rho-dependent transcription termination [107]. One manifestation of Rho activity is 'polarity', a phenomenon in which a mutation terminating translation within a cistron provokes transcription termination. The association of Rho with RNase E suggests a link between rho-dependent transcription termination and mRNA degradation, which could involve targeting the degradosome to rho-terminated mRNA. Message decay in the plant chloroplast, an organelle of eubacterial origin, has also been suggested to involve a degradosome. However, despite an earlier report of a degradosome-like association, 100RNP, which is a PNPase homolog, appears to be a hexamer of identical subunits forming a large 600 kDa enzyme [108]. Whether other enzymes associate with the chloroplast PNPase is an open question. In *Streptomyces coelicolor*, a Gram-positive bacteria, an RNase E-like activity was described several years ago [109] and an authentic homolog, RNase ES, has recently been identified [110]. Intriguingly, RNase ES has been shown to associate physically with the PNPase from *S. coelicolor*, suggesting the existence of a degradosome-like complex. Further work will be required to characterize the putative *Streptomyces* degradosome including the identification of other proteins that associate with RNase ES. Considering the very large evolutionary distance between *E. coli* and *S. coelicolor*, these results suggest that the physical association of RNase E and PNPase to form degradosome-like complexes might be widespread in bacteria.

In the yeast *S. cerevisiae*, two complexes have been described, the mtEXO complex and the exosome. The mtEXO complex (Table 5-1), located in the mitochondria, is required for the degradation of introns [111, 112]. Dss1p, in the mtEXO complex, is an exoribonuclease related to RNase II. The RNA helicase Suv3p, an integral component, is required for mtEXO activity both *in vitro* and *in vivo*. The yeast exosome (Table 5-1), with both hydrolytic and phosphorylytic activity, exists in a cytoplasmic form that degrades mRNA and a nuclear form that processes ribosomal RNA and small nuclear RNAs [6]. The nuclear form is also involved in the degradation of pre-mRNA and the rescue of read through transcripts that fail to be cleaved and polyadenylated at the normal processing site [113, 114]. It is from their function in ribosomal RNA processing that many of the components of the exosome derive their "Rrp" nomenclature. A number of co-factors are named "Ski" for the observed super killer phenotype due to overexpression of a toxin from an endogenous RNA. Mtr4p and Ski2p are DEvH-box RNA helicases. Exosome-like complexes have been found in a broad spectrum of eukaryotes ranging from humans to trypanosomes, thus suggesting that they are a highly conserved feature of eukaryotic stable RNA maturation and mRNA decay [115, 116].

A key similarity between eubacterial PNPase and the yeast exosome is that they both have phosphorylytic activity. In addition to PNPase, *E. coli* has a second phosphorylytic RNA-degrading enzyme, RNase PH, which is implicated in the maturation

of tRNA [117, 118]. This phosphorylase is the founder of a superfamily of RNase PH-like enzymes, which include PNPase as well as components of the catalytic core of the eukaryotic exosome [10, 119]. The PNPase of *E. coli* is a trimer of identical subunits. Sequence analysis has suggested that each subunit contains two RNase PH-like domains acquired as the result of an ancient gene-duplication-fusion event [119]. In addition to the two RNase PH domains, PNPase also contains S1 and KH RNA-binding domains in the C-terminal region of the protein (Fig. 5-5A). The recent crystal structure of the PNPase from *Streptomyces antibioticus* has revealed for the first time the architecture of the catalytic site of a member of the RNase PH superfamily [120]. This work shows that the RNase PH domains in the PNPase monomer fold independently and pack closely together to form an intramolecular dimer. These dimers then assemble into a ring structure. Thus, the catalytic core of the PNPase trimer can be viewed as a hexameric ring assembled from the RNase PH domains (Fig. 5-5B). In the X-ray diffraction pattern, the S1 and KH domains were not

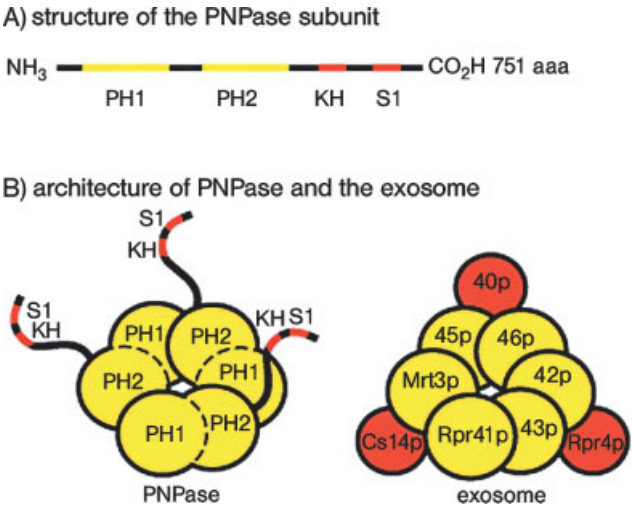

Figure 5-5 The eubacterial PNPase and the yeast exosome. (A) The *E. coli* PNPase subunit contains two RNase PH domains (yellow: PH1 and PH2) and C-terminal KH and S1 RNA-binding domains (red). It has been suggested, based on protein sequence comparisons, that the *pnp* gene arose from a duplication fusion of an ancient gene encoding an RNase PH-like enzyme (see text). (B) X-ray analysis [120, 121] has revealed that the catalytic core of PNPase can be viewed as a hexameric ring of RNase PH domains. In the model for PNPase presented here, the PH domains are in yellow; dashed lines indicate the domain boundaries; bold lines, the subunit boundaries. The 'tails' represent the C-terminal extensions containing the KH and S1 RNA-binding domains (red). The hexameric domain organization of the eubacterial PNPase can serve as a model for the phosphorylytic core of the yeast exosome, which is composed of six RNase PH-like subunits (yellow). The order of the exosome subunits in the hexameric ring (Rrp41p, Rrp43p, Rrp42p, Rrp46p, Rrp45p, and Mrt3p) is taken from a recent prediction [122]. Proteins associated with the exosome core, such as Rpr4p, Rpr40p, and Cs14p (red), contain S1 and KH RNA-binding domains that could serve the same function as the KH and S1 domains that are an integral part of the PNPase subunit.

detected suggesting that they could be part of a flexible structure. Nevertheless, molecular modelling indicates that the S1 and KH domains can form a 'crown', capping the catalytic core, which might serve to 'feed' RNA into the active site. It has been suggested that the ring structure of the catalytic core of PNPase could serve as a model for the organization of the RNase PH-like enzymes in the exosome [121]. A low-resolution structure of the yeast exosome, based on electron microscopy, and the mapping of protein–protein interactions by two-hybrid analysis supports this contention [122,123]. To transform PNPase into the exosome, the RNase PH domains forming the catalytic core of PNPase are replaced by the Rpr41p, 42p, etc., subunits (Fig. 5-5B). It is interesting to note that the non-phosphorylytic subunits of the exosome, Rpr4p, Rpr40p and Cs14p, contain RNA-binding motifs, including the S1 and KH domains found in eubacterial PNPase. Thus, whereas in PNPase, the PH1, PH2, S1 and KH domains are fused into a single polypeptide, in the exosome these domains exist in separate polypeptides. These considerations suggest that PNPase and the exosome might have evolved from an ancient phosphorylytic enzyme with a hexameric ring structure. Considering protein sequence alignments, an archeal 'exosome' has been predicted [124]. Two RNase PH-like proteins as well as a protein related to Rpr4p have been identified as part of an 'operon' in several different archeabacteria. It will be interesting to learn if these proteins actually assemble into an exosome-like complex.

5.10 DEAD-box ATPases

The identification of the DEAD-box ATPase, RhlB, in the *E. coli* degradosome was one of the first indications that RNA helicases could have an active role in the degradation of mRNA. The DEAD-box proteins are a family of putative ATP-dependent RNA helicases that have a conserved core sequence containing eight motifs including the amino acids D-E-A-D [125, 126]. Members of this family have been implicated in a variety of processes involving RNA including ribosome assembly, translation initiation and RNA splicing. The advantage of having an RNA helicase in an RNA-degrading complex was demonstrated *in vitro* with the RNA degradosome [77, 83]. RNAs with internally structured regions often impede the progress of enzymes such as PNPase, forcing the enzyme to pause. RhlB in the degradosome facilitates PNPase-mediated degradation of structured substrates in an ATP-hydrolysis-dependent reaction that is believed to involve the unwinding of RNA double strands. The ATPase activity of RhlB is strongly activated by its interaction with the CTH of RNase E and polypeptides derived from this region can form a complex with RhlB that is capable of unwinding short RNA helices *in vitro* [82]. Thus, the interaction between RNase E and RhlB controls ATPase activity and it could serve to give RhlB specificity via its physical association with an RNA-degrading complex. Since the degradosome, exosome and mtEXO complex all contain putative RNA helicases (Table 5-1), a role for these enzymes appears to be a common feature of complexes involved in 3′→5′ RNA degradation.

In the mtEXO complex, Suv3p, which is a DEAD-box ATPase, is required for RNA-degrading activity both *in vitro* and *in vivo*, and a requirement for ATP hydrolysis has clearly been demonstrated *in vitro* [112, 127]. Thus, Suv3p appears to be providing more than a simple RNA helicase function. It is conceivable that Suv3p could serve as a 'motor' that translocates the RNA substrate. The PNPase of *E. coli* has RNA-degrading activity by itself. However, the enzyme works close to the chemical equilibrium for the reaction. In the presence of low phosphate and high nucleotide concentrations, it can catalyse the reverse reaction, i.e., synthesis of polynucleotides from nucleotide diphosphates. Indeed, in mutant strains of *E. coli* deficient in poly(A) polymerase activity, an RNA synthetic activity attributed to PNPase has been described [128]. It was suggested that under certain 'micro conditions' within the cell, PNPase could work synthetically for brief periods in which short 3′ extensions are added to mRNA decay intermediates. This raises the issue of whether RhlB might have a role in regulating the degradative versus synthetic activity of PNPase. Although RhlB cannot alter the chemical equilibrium of the phosphorolysis reaction, it is conceivable that the energy of ATP hydrolysis could be coupled with the activity of PNPase in a kinetic control that promotes degradation.

5.11 Perspective

In *E. coli*, mRNAs are often polycistronic, and transcription and translation are coupled. Decay is initiated by endonucleases that fragment the mRNA. The principal endonuclease in *E. coli* mRNA decay is RNase E, which is 5′-end-dependent. Nevertheless, recent work suggests that RNase E can also initiate the decay of untranslated or poorly translated mRNA via an 'internal entry' pathway that is 5′-end-independent. RNase II and PNPase, the principal exonucleases in *E. coli* mRNA decay, degrade mRNA fragments to nucleotides in a 3′→5′ pathway. Poly(A) polymerase, which can add 3′ single-strand extensions to mRNA fragments, facilitates attack by the exonucleases. Internal regions of RNA structure that impede exonuclease activity can be unwound by RNA helicases. Thus, the main points for the control of degradation of *E. coli* mRNA are RNA structures in 5′- and 3′-UTRs, and in the intergenic regions of polycistronic messages, which modulate the activity of RNase E and the exonucleases. Whether *E. coli* is a model organism for the entire eubacterial kingdom is debatable. Notably, *B. subtilis* and related Gram-positive bacteria do not have identifiable RNase E homologs [71, 72]. RNase II, which is hydrolytic, and PNPase, which is phosphorylytic, seem to have redundant functions. Nevertheless, related proteins are widespread in the eubacteria and the eukaryotes, suggesting that there must be some advantage in having both types of 3′→5′ exonucleases.

The pathway of mRNA decay in *S. cerevisiae* differs considerably from that of *E. coli*. In yeast and other eukaryotes, the messages, which are monocistronic, are part of ribonucleoprotein complexes containing a wide diversity of RNA-binding proteins [129]. The 5′-cap and 3′-poly(A) structures are important for translation and mRNA stability. Messenger RNA decay is an orderly process in which 3′ deadenylation promotes 5′ decapping; this in turn leads to the arrest of translation initiation

and the degradation of the body of the mRNA. Thus, translation and mRNA stability are intimately linked. Nonsense-mediated mRNA decay, in which messages with premature stop codons are targeted for degradation, and non-stop mRNA decay, in which messages lacking stop codons are targeted for degradation, are examples of the importance of 'translatability' in mRNA stability [130–132]. The body of the mRNA is degraded to nucleotides by two distinct exonucleolytic pathways: 3′→5′ degradation involving the exosome and 5′→3′ degradation involving Xrn1p. The mRNA 5′-cap and the 5′→3′ exoribonuclease Xrn1p are specific features of the eukaryotic mRNA decay pathway. Many of the components involved in mRNA decay in yeast appear to be conserved in higher eukaryotes. It is thus generally believed that the yeast system will serve as a general model for mRNA decay. In higher eukaryotes, the lifetime of specific mRNAs can vary from minutes to days. The challenge now confronting researchers interested in eukaryotic mRNA decay is, in the framework of the established pathways, to elucidate how lifetimes are controlled. The eukaryotic mRNA-binding proteins probably to have a critical role in controlling the stability of specific messages.

In *E. coli*, RNase E and PNPase associate into a complex known as the RNA degradosome, which also contains the DEAD-box ATPase, RhlB. Related complexes, which are RNase E-based, have been described in other eubacteria suggesting that the degradosome-like machinery might be widespread. PNPase is a member of the RNase PH superfamily of phosphorylytic RNA-degrading enzymes, which includes six subunits of the yeast exosome. The crystal structure of a eubacterial PNPase and a recent low-resolution structure of the yeast exosome suggest that the RNase PH-like domains in these complexes have a conserved structure and that they assemble into a conserved hexameric ring architecture. Thus, despite considerable differences between mRNA decay in *E. coli* and *S. cerevisiae*, the core of the phosphorylytic RNA-degrading machinery appears to be conserved in eubacteria and eukaryotes.

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