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Messenger RNA Degradation in Bacterial Cells

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Abstract

mRNA degradation is an important mechanism for controlling gene expression in bacterial cells. This process involves the orderly action of a battery of cellular endonucleases and exonucleases, some universal and others present only in certain species. They function with the assistance of ancillary enzymes that covalently modify the 5’ or 3’ end of RNA or unwind base-paired regions. Triggered by initiating events at either the 5’ terminus or an internal site, mRNA decay occurs at diverse rates that are transcript-specific and governed by features such as RNA sequence and structure, translating ribosomes, and bound sRNAs or proteins. In response to environmental cues, bacteria are able to orchestrate widespread changes in mRNA lifetimes by modulating the concentration or specific activity of cellular ribonucleases or by unmasking the mRNA-degrading activity of cellular toxins.

Keywords

mRNA stability; ribonuclease; gene regulation; translation; sRNA

I. INTRODUCTION

Critical to survival for all living organisms is the ability to precisely regulate the expression of genetic information in order to produce the proteins needed to navigate the assorted challenges posed by an ever changing environment. This principle holds true for both multicellular organisms and bacteria. In bacterial cells, protein synthesis can be controlled at any of three stages: transcription, translation, and mRNA degradation.

The capacity of cells to degrade mRNA is an evolutionary imperative. The energetic costs of translation and the benefit of recycling ribonucleotides demand a mechanism for rapidly destroying transcripts that are no longer useful. Equally important, rapid mRNA turnover confers a distinct advantage by allowing cells to quickly adapt protein synthesis to sudden environmental challenges, as response times would be much slower if dependent solely on modulating transcription.

Because mRNA degradation is not indiscriminate, it makes an important contribution to differential gene expression. The longevity of individual transcripts can differ significantly,

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their half-lives ranging from seconds to about an hour in bacterial cells, with proportionate effects on protein synthesis. Translational units within the same polycistronic transcript can also differ in stability, allowing co-transcribed genes to be expressed at distinct levels. The ability to alter rates of mRNA degradation is often crucial for the response of cells to environmental cues.

This review will focus on mRNA turnover in bacterial cells, including the ribonucleases and RNA elements that govern mRNA decay, the various pathways by which messages are degraded, and the mechanisms for controlling the lifetimes of transcripts individually and collectively.

II. HISTORICAL PERSPECTIVE

Initial models for mRNA degradation in bacteria were based on a limited number of observations in *Escherichia coli*. For example, no 5’ exoribonuclease activity was ever detected in *E. coli*(40). Furthermore, the action of 3’ exoribonucleases appeared to be blocked by the ubiquitous presence of a stem loop at the 3’ end of mRNAs (118), such that differences in rates of mRNA decay did not seem to be governed by characteristics of the 3’-terminal stem-loop (12). Therefore, logic dictated that mRNA degradation must begin endonucleolytically (3, 12). With the discovery that the endonuclease RNase E controls the decay of most transcripts in *E. coli*(7, 112, 119, 126, 151), a model for endonucleolytic initiation coalesced with this enzyme as the centerpiece. For a majority of mRNAs, degradation was envisioned to begin with internal cleavage by RNase E to yield two decay intermediates. Freed of its protective 3’-terminal stem-loop, the 5’ fragment would be rapidly degraded by 3’ exonucleases, while the 3’ fragment would be degraded through further rounds of RNase E cleavage and 3’ exonuclease degradation.

Although this model accounted for many observations, a number of phenomena remained unexplained. How are stem-loops and other base-paired regions degraded? Why are the 3’ fragments generated by endonucleolytic cleavage typically less stable than their full-length precursors (155)? And if decay begins internally, why was degradation observed to be impeded by base pairing at the 5’ end of transcripts (15, 48)? Equally curious was the discovery that the genomes of a significant number of bacterial species do not encode an RNase E homolog. The realization that there is no universally conserved set of ribonucleolytic enzymes that all bacteria rely upon for mRNA turnover meant that *E. coli* could not be treated as a paradigm for understanding mRNA degradation in all species. Explaining these phenomena required a fuller knowledge of the enzymes responsible for mRNA degradation.

III. BACTERIAL RIBONUCLEASES

Bacteria utilize a large arsenal of ribonucleolytic enzymes to carry out mRNA degradation, many of which are present only in certain bacterial clades.

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Endoribonucleases

RNase E and its homolog RNase G—Among bacterial ribonucleases, RNase E is one of the most important for governing rates of mRNA decay. Initially discovered for its role in ribosomal RNA maturation in *E. coli*(4), this endonuclease was later implicated in mRNA degradation when it was observed that bulk mRNA stability and the half-lives of many individual transcripts increase significantly when a temperature-sensitive RNase E mutant is shifted to non-permissive temperatures (7, 112, 119, 126, 151).

Each subunit of an *E. coli* RNase E homotetramer consists of a well conserved amino-terminal domain that houses the catalytic site and a poorly conserved carboxy-terminal domain that includes a membrane-binding helix, two arginine-rich RNA-binding domains, and a region that serves as a scaffold for the assembly of a ribonucleolytic complex called the RNA degradosome (Figure 1)(78, 108, 153). RNase E cuts RNA internally within single-stranded regions that are AU-rich, but with little sequence specificity (110). Despite being an endonuclease that can cleave RNA far from the 5’ terminus, RNase E displays a marked preference for RNAs whose 5’ end is monophosphorylated and unpaired (99). Comparison of monophosphorylated RNAs with their triphosphorylated counterparts has shown their difference in reactivity *in vitro* to typically be greater than an order of magnitude (76). This phenomenon is explained by the presence of a discrete 5’-end binding pocket in the catalytic domain, which serves as a phosphorylation sensor able to accommodate a 5’ monophosphate but not a 5’ triphosphate(20).

The essential nature of RNase E makes it difficult to determine the full extent of its role in mRNA turnover, but it appears that the vast majority of *E. coli* mRNAs decay by an RNase E-dependent mechanism. Interestingly, in addition to RNase E, *E. coli* also contains a nonessential paralog, RNase G. RNase G closely resembles the amino-terminal catalytic domain of RNase E, sharing almost 50% similarity in amino acid sequence as well as a comparable 5’-monophosphate dependence and cleavage site preference (76, 109, 152). Nevertheless, overexpression of RNase G cannot fully compensate for the absence of RNase E (34, 84). The effect on the *E. coli* transcriptome of deleting the RNase G gene is rather modest, likely due to the relatively low cellular concentration of this enzyme (only 1% as abundant as RNase E (84)).

RNase Y—In species that lack an RNase E homolog, RNase Y can fulfill the role of an endonuclease that mediates mRNA degradation. This enzyme consists of a transmembrane domain, a disordered coil-coiled domain, an RNA-binding KH domain, and a catalytic HD domain (Figure 1)(86). Although RNase Y is structurally distinct from RNase E, the two ribonucleases share certain characteristics. For example, both are membrane-associated and cleave RNA internally and with little sequence specificity within single-stranded regions that are AU-rich (141). On the other hand, unlike RNase E, the membrane-binding domain is essential for RNase Y function (86).

Multiple studies have implicated RNase Y as a major regulator of RNA metabolism. In *Bacillus subtilis*, which lacks RNase E, a large percentage of the transcriptome is affected by RNase Y depletion (44, 82, 88). Furthermore, in *Streptococcus pyogenes* and

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Staphylococcus aureus, RNase Yhas been shown to be important for controlling the expression of virulence genes (27, 101).

RNase III—Unlike RNase E/G and RNase Y, RNase III cuts RNA within double-stranded regions (138). By this means, RNase III plays a general role in the maturation of ribosomal RNA and a more selective role in the processing and degradation of mRNAs, sRNAs, and CRISPR RNAs(38, 106).

RNase III is a dimer of identical subunits, each comprising an endonucleolytic domain and a double-stranded RNA-binding domain (Figure 1)(14). The two centrally located catalytic sites function independently of one another to cleave each strand of the RNA duplex, yielding products that have a characteristic 2-bp overhang at the 3’ end (56, 113). Although cleavage at a reduced rate has been observed *in vitro* for substrates as short as 11 bp (83, 129), biological substrates typically span a minimum of two turns of an RNA helix or ~20 bp in length (137). Consequently, most natural stem-loop structures are too short to be targeted by RNase III *in vivo*. No consensus sequence has been identified for RNase III cleavage sites, but certain sequence features in and around that site appear to influence the ease with which an RNA duplex is cut(129, 162). RNase III is also able to target certain double-stranded RNAs that contain an internal loop, sometimes cleaving only one of the two strands(19).

RNase III has a more limited role in gene regulation than RNase E and RNase Y. Tiling array studies in *E. coli* and *B. subtilis* show a small but significant portion of the transcriptome to be affected, either directly or indirectly, by the absence of RNase III (44, 146). Consistent with its limited regulatory influence, RNase III is not generally essential for viability, except in *B. subtilis*, where it serves as part of a defense mechanism against chromosomally encoded toxins (45).

Minor endonucleases—Other endoribonucleases that function primarily in tRNA biogenesis have also been implicated in the decay of certain mRNAs. For example, RNase P, a ribonucleoprotein complex critical for the maturation of tRNA 5’ ends, targets noncoding regions within some messages(93). RNase Z (RNase BN), which removes aberrant tRNA 3’ ends in *E. coli*and appears to have both endonuclease and 3’ exonuclease activity, has also been implicated in the decay of a few mRNAs(47, 130).

Exoribonucleases

To complement the activity of cellular endonucleases, bacteria rely on a panel of exoribonucleases to rapidly degrade decay intermediates that lack protection at one or the other terminus. For the most part, these exonucleases act processively with little or no sequence specificity.

Phosphorolytic 3’ exonucleases—Bacterial 3’ exoribonucleases function by one of two mechanisms, either hydrolytically and irreversibly to yieldnucleoside monophosphate products or phosphorolytically (i.e., using orthophosphate as a nucleophile) to produce nucleoside diphosphates in a reversible reaction.

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To date, all known phosphorolytic 3’ exonucleases are members of the PDX family of enzymes (163). Prototypical representatives of this family are polynucleotide phosphorylase (PNPase) and RNase PH. The former is heavily involved in the turnover of mRNA, whereas the latter has principally been studied in the context of tRNA maturation and appears to have only a minor role in mRNA decay (41, 73).

True to the nature of the reversible phosphorolytic reaction it catalyzes, PNPase has both degradative and synthetic capabilities. *In vitro*, it can degrade RNA from 3’ to 5’ as well as add a heteropolymeric tail to the 3’ end(61). *In vivo*, both of these activities contribute to mRNA degradation. As an exonuclease, PNPase preferentially degrades RNAs with a single-stranded 3’ end (26, 156). As a polymerase, PNPase is capable of adding single-stranded adenine-rich tails that can facilitate the 3’-exonucleolytic degradation of structured regions of RNA(156) (see section IV below).

Our understanding of how PNPase degrades RNA exonucleolytically is shaped by a combination of biochemical, structural, and genetic studies. The enzyme is a trimer of identical subunits, each of which consists of two PH domains, a KH domain, and an S1 domain (Figure 1). The trimer forms a ring-shaped structure with the KH and S1 domains, which are critical for substrate binding, surrounding one end of the central channel(148, 150). The PH domains, though homologous to one another, are not identical, and in each subunit only one such domain (the second) is catalytically active (150). Because the active sites are located inside the channel, the 3’ end of RNA must thread partway through the channel to reach them. PNPase degrades RNA processively from the 3’ end until it encounters a base-paired structure of significant thermodynamic stability(26), whereupon it dissociates several nucleotides downstream of the stem-loop, likely due to the inability of the stem-loop to enter the narrow channel (145, 150). In *E. coli*, PNPase functions in association with the ATP-dependent RNA helicase RhlB, which can assist PNPase by unwinding internal stem-loops that are encountered (132). When unimpeded, PNPase degrades RNA almost completely, releasing a 5’-terminal dinucleotide as its final product (29).

Hydrolytic 3’ exonucleases—The principal hydrolytic 3’ exoribonucleases in bacterial cells are members of the RNR super family. As catalysts of an irreversible reaction, they function exclusively as degradative enzymes. Like most other Gammaproteobacteria, *E. coli* contains two such exonucleases, RNase II and RNase R. It tolerates the absence of either of these enzymes or of PNPase individually, but paired mutations that eliminate PNPase in combination with either RNase II or RNase R are synthetically lethal (30, 42).

RNase II resembles PNPase in terms of its intrinsic substrate selectivity. A single-stranded 3’ end is required for RNase II to engage and degrade its target(145). The enzyme stalls upon encountering a stable stem-loop (145). However, whereas PNPase is able to slowly navigate through such structural impediments with the aid of its associated helicase (95, 132), RNase II cannot do so and dissociates a few nucleotides downstream of the stem-loop (145).

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RNase II is a monomeric enzyme comprising one catalytic RNB domain flanked on both sides by RNA-binding domains (two cold shock domains and one S1 domain) (Figure 1) (54). To reach the catalytic center, the 3’ end of RNA substrates threads through a narrow channel, where five 3’-terminal nucleotides make intimate contact with the enzyme(54), thereby explaining why unimpeded digestion by RNase II requires an unpaired 3’ end and generates a 5’-terminal oligonucleotide as the final reaction product (28). Additional nucleotides further upstream associate with the three RNA-binding domains, which function as an anchoring region where sustained contact with the RNA ensures degradative processivity with substrates ≥10 nucleotides long (2, 54).

The other RNR family member, RNase R, shares many structural and catalytic properties with RNase II (28). However, a key distinguishing characteristic of RNase R is its intrinsic ability to unwind double-stranded RNA, which enables it to degrade highly structured RNAs nearly to completion without the aid of a helicase or an external source of energy such as ATP, provided that a single-stranded 3’ end is initially available for binding (6, 29). This property of RNase R has been attributed to unique features of its catalytic domain, S1 domain, and carboxy-terminal tail(105, 154).

5’ exonucleases—The longstanding belief that 5’ exoribonucleases do not exist in bacteria was overturned by the discovery that RNase J is able to remove nucleotides sequentially from the 5’ end of RNA, with a strong preference for 5’ monophosphorylated substrates (103, 134). Absent from *E. coli* and initially identified in *B. subtilis* as an endonuclease(50), this enzyme is a dimer of dimers in which every subunit contains a bipartite metallo-β-lactamase domain, a β-CASP domain, and a carboxy-terminal domain (Figure 1). At each dimer interface, an RNA-binding channel leads deep inside the protein to a catalytic active site, where a monophosphorylated but not a triphosphorylated 5’ end can bind so as to position the 5’-terminal nucleotide for hydrolytic removal (43, 91). The channel continues past the catalytic center and emerges on the other side of the enzyme, thus explaining the ability of RNase J to act not only as a 5’ exonuclease but also as an endonuclease.

The impact of RNase J on global mRNA decay has been best studied in *B. subtilis*, which encodes two paralogs (J1 and J2) that assemble to form a heterotetramer *in vivo* (104). Of the two, only RNase J1 has significant 5’ exonuclease activity, and its absence markedly slows *B. subtilis* cell growth (52, 104). Severely depleting RNase J1 affects a large portion the *B. subtilis* transcriptome, suggesting that this enzyme plays a major role in *B. subtilis* mRNA degradation (44). The presence of two RNase J paralogs is common in Firmicutes, but in many other species only a single RNase J ortholog is present(18).

Oligoribonucleases

A hydrolytic 3’ exoribonuclease, oligoribonuclease differs from other bacterial exonucleases in one fundamental aspect: this enzyme displays a marked preference for RNA substrates no more than 5 nucleotides long(33). It plays a vital role in RNA degradation. Because the structures and mechanisms of PNPase, RNase II, and RNase R prevent them from completely degrading their substrates, they generate 5’-terminal oligonucleotides ranging

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from 2 to 5 nucleotides in length as reaction products (28, 29). Oligoribonuclease converts these remnants into mononucleotides, thus replenishing the cellular pool of RNA precursors (58) while also preventing the misincorporation of these oligonucleotides at the 5’ end of new transcripts (59).

Oligoribonuclease is essential in *E. coli* (58), where it is the only ribonuclease that can efficiently degrade oligonucleotides, but a sequence homolog of the *E. coli* enzyme (Orn) is not present in all bacterial species. Some species that lack this enzyme have been shown to contain a distinct ribonuclease (NrnA/B or NrnC) with similar properties (51, 96, 111). Other species may contain as yet unidentified ribonucleases that can perform this function.

RNA degradosomes

Presumably to enhance their degradative efficiency, enzymes important for mRNA decay often assemble to form a multimeric complex called an RNA degradosome. These degradosomes commonly contain one or more ribonuclease(s) and an RNA helicase.

The degradosome studied most extensively is that of *E. coli*, where PNPase, RhlB, and the glycolytic enzyme enolase bind to discrete sites in the noncatalytic carboxy-terminal half of RNase E (23, 132, 153). The association of PNPase with RNase E may facilitate the exonucleolytic degradation of decay intermediates produced by endonucleolytic cleavage. Likewise, the ability of the RNA helicase RhlB to disrupt RNA base pairing can both expose internal sites to RNase E cleavage and aid PNPase when significant 3’-terminal structure is encountered (79, 132). Less clear is the role of enolase in the RNA degradosome, where it may play a role in sensing the metabolic state of cells(116). Two-hybrid and co-immunoprecipitation studies suggest that similar degradosome complexes may be present in a number of other Proteobacteria(1, 49, 67, 72). Although the formation of degradosomes is not essential, an *E. coli* strain harboring a truncated form of RNase E that cannot nucleate degradosome assembly grows more slowly and degrades many mRNAs less swiftly than its wild-type counterpart (13, 89). Thus, the ability of components of the ribonucleolytic machinery to associate with one another is of no small consequence.

In bacterial species lacking RNase E, ribonucleolytic counterparts may associate with each other in a similar fashion. Notably, two-hybrid studies in *B. subtilis* and *S. aureus* have detected the interaction of RNase J, PNPase, the RNA helicase CshA, and other proteins(32, 87, 139). In *B. subtilis*, one of those other proteins is RNase Y, which may serve as the scaffold for assembly of a complex(86). However, unlike the RNA degradosome of *E. coli*, a heteromultimer containing stoichiometric amounts of each of these proteins has yet to be verified by purification from cells.

Phylogenetic distribution of ribonucleases

As noted above, no universal set of mRNA-degrading enzymes is present in all bacteria. However, some unifying principles are evident upon examining the phylogenetic distribution of ribonucleases (Table 1). Two ribonucleases, RNase III and PNPase, are encoded by almost all bacterial genomes annotated to date. Other ribonucleases, such as RNase E/G, RNase Y, RNase J, and RNase II/R, are conserved in many species but notably

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absent in a number of others. All told, nearly all bacteria (>90%) contain a low-specificity endonuclease that cuts single-stranded RNA (RNase E/G and/or RNase Y), an endonuclease specific for double-stranded RNA (RNase III), one or more 3’ exonucleases (PNPase, RNase II, and/or RNase R), and an oligoribonuclease (Orn, NrnA/B, and/or NrnC), and more than half also contain a 5’ exonuclease (RNase J). Most species (>75%) contain both PNPase and one or more hydrolytic 3’ exonucleases, and a significant number (~20%) contain both RNase E/G and RNase Y. The fact that very few species other than Spirochaetales lack both RNase E/G and RNase J, two 5’-monophosphate-stimulated ribonucleases, suggests that a 5’-end-dependent degradation pathway may be nearly universal in bacteria.

IV. mRNA DEGRADATION PATHWAYS

Despite the diverse sets of ribonucleases found in bacteria, the basic pathways of mRNA degradation are remarkably similar across species. There appear to be two mechanisms for initiating mRNA decay. In one (direct access) degradation begins with ribonuclease attack, while in the other (5’-end-dependent access) the 5’-terminal triphosphate is first converted to a monophosphate.

Direct-access pathway

The first degradative event in the direct-access pathway is internal cleavage by an endonuclease (Figure 2). In *E. coli* and related species, this step is usually catalyzed by RNase E (7, 112, 119, 126, 151), but for some mRNAs it has been shown that other endonucleases initiate decay(75, 93, 106, 146). By contrast, in species like *B. subtilis* that lack RNase E, degradation often begins instead with internal cleavage by RNase Y (44, 82, 88, 159). Regardless of the endonuclease, this initial cleavage produces 5’- and 3’-terminal mRNA fragments, each of which is typically shorter lived than the full-length transcript.

In most cases, the 5’ fragment produced by endonucleolytic cleavage no longer has a protective stem-loop at its 3’end and is therefore susceptible to rapid 3’-exonucleolytic degradation (Figure 2). Such degradation often proceeds to completion despite various obstacles that the 3’exonucleases may encounter. Although thermodynamically robust base pairing typically impedes exonucleolytic degradation, such barriers can eventually be overcome with the aid of an enzyme that appends a single-stranded tail downstream of the impediment (Figure 3). In *E. coli*, tailing is achieved primarily by the action of poly(A) polymerase (PAP), which can polyadenylate the 3’ end of decay intermediates from which a 3’ exonuclease has disengaged(21, 62, 157). In bacterial species that lacka dedicated poly(A) polymerase, A-rich tails can be added by the template-independent polymerase activity of a phosphorolytic exonuclease such as PNPase (114). Successive rounds of poly(A) addition and removal downstream of a base-paired structure provide repeated opportunities for penetration of the barrier by PNPase (with assistance from RhlB) or RNase R, thereby allowing exonucleolytic degradation to proceedpast the structured region. On the other hand, due to its strict specificity for single-stranded 3’ ends, RNase II can impede the exonucleolytic destruction of stem-loop structures by unproductively removing the poly(A) tail on which PNPase and RNase R rely without ever damaging the stem-loop itself (64). Consequently, 3’-exonucleolytic penetration of such structures may often be slower than

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endonucleolytic cleavage upstream, especially when they are thermodynamically robust and located in an untranslated region.

As they degrade 5’-terminal mRNA fragments, 3’ exonucleases may also encounter translating ribosomes that are moving in the opposite direction. To rescue ribosomes stalled at the 3’ end of degradation intermediates that lack a termination codon, a specialized bacterial RNA (tmRNA) that has features of both tRNA and mRNA is recruited together with its protein escort (SmpB)(77). SmpB facilitates ribosome template switching from the truncated mRNA to the tmRNA, which contains a termination codon that allows the ribosome to be released. RNase R subsequently degrades the mRNA fragment from its now exposed 3’ end (136).

Although the 3’ fragment generated by the initial endonucleolytic cleavage ends with a stem-loop that protects it from 3’-exonucleolytic degradation, it too is typically quite labile due to its monophosphorylated 5’ terminus (Figure 2). In bacterial species that contain RNase J, the presence of only one phosphate at that end exposes such intermediates to swift 5’-exonucleolytic degradation(36, 160). In species that lack RNase J, these decay intermediates are rapidly destroyed by RNase E, whose ribonucleolytic potency is greatly enhanced when the 5’ end of a substrate is monophosphorylated(99). Repeated cleavage by this endonuclease yields mRNA fragments susceptible to exonucleolytic degradation from an unprotected 3’ end or, in the case of the 3’-terminal fragment bearing the terminator stem-loop of the original transcript, to degradation by a mechanism involving polyadenylation followed by 3’-exonucleolytic attack (Figure 3)(64, 156, 157).

5’-end-dependent pathway

Though pertinent to the decay of a large percentage of primary transcripts, the direct-access pathway for endonucleolytic initiation does not explain the ability of a 5’-terminal stem-loop to stabilize many transcripts(9, 15, 48, 65, 143). This observation led to the discovery and characterization of a distinct, 5’-end-dependent pathway for mRNA degradation in which endonucleolytic cleavage is not the initial event. Instead, decay by this pathway is triggered by a prior non-nucleolytic event that marks transcripts for rapid turnover: the conversion of the 5’ terminus from a triphosphate to a monophosphate (Figure 4). Catalyzed by the RNA pyrophosphohydrolase RppH, this modification greatly increases the susceptibility of mRNA to degradation by RNase E or RNase J (25, 35, 134), both of which aggressively attack monophosphorylated RNA substrates. In *E. coli*, the steady-state concentration of hundreds of messages increase significantly when the *rppH* gene is deleted, indicating that a significant portion of the transcriptome is degraded via the 5’-end-dependent pathway (98).

The discovery of the mechanism of 5’-end-dependent degradation explained the protective effect of 5’-terminal stem-loops, as RppH, RNase E, and RNase J can only interact with 5’ ends that are single-stranded. Indeed, biochemical studies of RppH from *B. subtilis* and *E. coli* indicate that it requires at least two and preferably three or more unpaired nucleotides at the 5’ end of its substrates (70)(Hsieh and Belasco, unpublished results). In addition, *B. subtilis* RppH, but not *E. coli* RppH, has a strict requirement for guanylate as the second nucleotide. However, 5’-end-dependent mRNA degradation in *B. subtilis* does not rely entirely on the identity of the second nucleotide or even on RppH, apparently due to the

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presence of another, as yet unidentified RNA pyrophosphohydrolase in that species(70, 134). By contrast, there is no evidence for an alternative pyrophosphate-removing enzyme in *E. coli*.

3’-exonucleolytic initiation of decay

mRNA decay in *E. coli* is retarded but not abolished upon inactivation of RNase E, indicating that alternative, RNase E-independent degradation pathways exist. Indeed, several transcripts whose degradation is impeded by RNase E inactivation are further stabilized when cells lack PAP or PNPasein addition to RNase E (62, 64, 125). Taken together, these findings suggest that poly(A)-dependent 3’-exonucleolytic degradation can sometimes initiate mRNA decay. However, the fact that the influence of PAP and PNPase is generally meager when RNase E is present indicates that 3’-exonucleolytic initiation of decay is ordinarily much slower than other degradation mechanisms.

V. mRNA FEATURES THAT GOVERN STABILITY

Because of the low sequence specificity of RNase E and RNase Y, a typical protein-encoding transcriptis likely to possess many potential cleavage sites, no one of which is critical for degradation. Therefore, the diversity of bacterial mRNA lifetimes suggests that the susceptibility of individual transcripts to degradation depends instead on the ease with which RNase Eor RNase Y gains access to those sites, as governed by the sequence and/or structure of each transcript and the cellular factors with which the mRNA interacts.

Ribosome binding and translation

Among the most important non-nucleolytic *trans*-acting factors that influence mRNA stability are ribosomes. In *E. coli*, the lifetime of a monocistronic message can usually be prolonged or abbreviated by increasing or decreasing, respectively, the ribosome-binding affinity of the Shine-Dalgarno element(5, 16, 161). Such effects are observed irrespective of whether the transcript is degraded by a direct-access or 5’-end-dependent mechanism (5, 135). Efficient ribosome binding and translation are thought to stabilize mRNA by sterically masking RNase E cleavage sites within the message. However, several lines of evidence suggest that the mechanism by which ribosomes protect mRNA is more complex, including the relatively modest effect of reducing the frequency of translation initiation by replacing an AUG initiation codon with a less efficient GUG or CUG codon (5)and the variable effect of premature translation termination, which is both transcript- and position-dependent(66, 122). Furthermore, though influenced by ribosome binding, mRNA decay rates appear to be less sensitive to premature translation termination in *B. subtilis* (142), which lacks RNase E but contains another low-specificity endonuclease, RNase Y, and the 5’ exonuclease RNase J.

Rates of mRNA degradation can also be affected by ribosomes that stall during translation elongation or termination due to the sequence of the nascent polypeptide or the scarcity of a required aminoacyl-tRNA. In *E. coli*, such events can trigger cleavage of the mRNA in or adjacent to the ribosomal A-site(68, 92)or upstream of the stalled ribosome(97) by mechanisms that have not yet been fully delineated. Conversely, in *B. subtilis* a stalled

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ribosome can act as a barrier that protects mRNA downstream of the stall site from 5'-exonucleolytic degradation by RNase J(11, 103, 140).

Intramolecular base pairing

Another major influence on bacterial mRNA degradation is RNA structure, which can impact rates of mRNA decay either directly by determining the accessibility of an entire transcript or a segment thereof to ribonuclease attack or indirectly by governing the binding of ribosomes or other non-nucleolytic factors that affect degradation. Some of these structural influences are ubiquitous, such as the stem-loops at the 3' ends of nearly all full-length bacterial transcripts. Present as acomponent of an intrinsic transcription terminator or as a result of exonucleolytic trimming from an unpaired 3' end, these 3'-terminal structures protect mRNAfrom 3'-exonuclease attack and thereby force degradation to begin elsewhere(12, 118). Less common is a stem-loop at the 5' end of mRNA, where it can prevent 5'-end-dependent degradation by inhibiting conversion of the 5'-terminal triphosphate to a monophosphate(35, 134).

Of course, intramolecular base pairing in bacterial mRNAs is not confined to the 5' or 3' end. In a number of cases, an internal stem-loop structure has been shown to play a pivotal role in the differential expression of genes within a polycistronic transcript. Whether such a stem-loop confers greater stability on the upstream or downstream RNA segment depends on the location of the stem-loop relative to the initial site of endonucleolytic cleavage. For example, a large intercistronic stem-loop between the *malE* and *malF* segments of the *E. coli malEFG* transcript protects the upstream *malE* segmentagainst 3'-exonucleolytic propagation of decay from a downstream site of initial endonucleolytic cleavage. As a consequence, a comparatively stable 5'-terminal decay intermediate encompassing only *malE* accumulates, resulting in substantially greater production of maltose-binding protein (MalE) than the membrane-bound subunits of the maltose transporter (MalF and MalG) (120). The large number of *E. coli* operons that contain palindromic sequences in intercistronic regions suggests that stem-loop structures of this kind may have a widespread role in differential gene expression(121, 147). Conversely, the presence of a stem-loop immediately downstream of a site of endonucleolytic cleavage can protect the 3' fragment from 5'-monophosphate-stimulated RNase E cleavage, as observed for the dicistronic *papBA* transcript, which encodes a low-abundance transcription factor (PapB) and a major pilus protein (PapA)in uropathogenic strains of *E. coli*. RNase E cleavage two nucleotides upstream of an intercistronic stem-loop structure contributes to swift 3'-exonucleolytic degradation of the *papB* segment of that transcript without exposing the 5'-monophosphorylated *papA* intermediate to rapid degradation by RNase E (8, 17). An interesting combination of both phenomena is illustrated by the degradation of the *pufQBALMX* photosynthesis transcript of *Rhodobacter capsulatus*, where intercistronic stem-loop structures flanking the internal *pufBA* segment enable this fragment to accumulate as a long-lived decay intermediate that survives the rapid degradation of the surrounding portions of the transcript(69).

Alternatively, intramolecular base pairing can instead act, indirectly or directly, to destabilize a transcript. In *E. coli*, indirect destabilization by such base pairing is usually a

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consequence of an RNA conformation that prevents ribosome binding by sequestering the site of translation initiation(5, 16, 161), whereas direct destabilization by intramolecular base pairing is often attributable to formation of a cleavage site for RNase III, an endonuclease specific for lengthy double-stranded regions of RNA(106, 144). Less frequently, the destabilizing structured element is a metabolite-binding riboswitch that can acceleratemRNA degradation upon undergoing a conformational change in response to an increase or decrease in the concentration of its ligand. It may do soby occluding the ribosome-binding site(123), by unmasking nearby RNase E cleavage sites(22), or, in the case of a catalytic riboswitch, by activating or repressing an intrinsic self-cleavage activity(31).

sRNA binding

Small non-coding RNAs (sRNAs) are among the most common means by which bacteria regulate mRNA abundance post-transcription ally in response to environmental cues. Expressed from regions of the genome that are usually distinct from the genes they regulate, sRNAs bind specific transcripts within segments to which they are partially or fully complementary and in doing so can influence the translation and/or decay rate of those messages(39, 149). Typically, a single sRNA will target multiple transcripts so as to coordinately modulate the production of several proteins.

sRNAs can either destabilize or stabilize a target transcript, depending on the nature of their interaction. Sometimes the mRNA-sRNA duplex itself is cleaved by RNase III (24). More frequently, sRNA binding stimulates mRNA degradation indirectly by interfering with ribosome binding and translation initiation(39). As a result, the repressive effect of the sRNA becomes irreversible. Interestingly, sRNA binding can also stimulate RNase E cleavage by mechanisms apparently unrelated to translation. For example, in *Salmonella enterica*, binding of the sRNA MicC to a site deep inside the coding region of *ompD* mRNA induces RNase E cleavage 4–5 nucleotides downstream of the sRNA-mRNA complex without affecting translation initiation(131). A mechanism has been proposed wherein *ompD* cleavage by RNase E is stimulated in *trans* by a monophosphate at the 5’ end of MicC (10). While demonstrable with purified components *in vitro*, it is unclear whether this mechanism explains the destabilizing effect of MicC in *Salmonella*, where <1% of MicC is monophosphorylated (Foley and Belasco, unpublished results).

In a number of other cases, sRNAs have been shown to upregulate the expression of the messages they target. Often they do so by disrupting an inhibitory stem-loop that would otherwise sequester the Shine-Dalgarno element(107). By exposing the ribosome binding site, the sRNA both facilitates translation initiation and, as a consequence, prolongs the lifetime of the message. In addition, sRNAs sometimes act directly to protect mRNA from degradation by masking RNase E cleavage sites without help from ribosomes(55, 128) or by sequestering the 5’terminus so as to prevent mRNA degradation via a 5’-end-dependent pathway (133).

In many species such as *E. coli*, sRNAs generally act in concert with the RNA chaperone protein Hfq. Hfq has a multifaceted role in sRNA-mediated regulation. It not only protects sRNAs from degradation by cellular ribonucleases (102) but also facilitates sRNA-mRNA

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base pairing (115). Hfq also has been shown to associate directly with RNase E, and this binding may play a role in mRNA degradation by facilitating RNase E recruitment to sRNA-associated transcripts (117). Finally, Hfq can stimulate the activity of poly(A) polymerase, an enzyme important for 3'-exonucleolytic degradation (63).

VI. CONTROL OF mRNA-DEGRADING ENZYMES

Needing at times to alter the abundance of a great manytranscripts simultaneously, bacteria have several ways to coordinate changes in mRNA stability. These include altering the concentration or specific activity of cellular ribonucleases or activating bacterial toxins. In addition, bacteriophage have evolved mechanisms to protect their transcripts from rapid degradation by host enzymes.

Regulation of ribonuclease concentration and activity

Bacteria maintain precise control over the cellular activity of many of the ribonucleases most important for mRNA decay by regulating either their concentration or their specific activity. For instance, to achieve homeostasis, RNase E, RNase III, and PNPase autoregulate their synthesis in *E. coli* by modulating the decay rates of their respective mRNAs as a function of the cellular activity of the corresponding enzymes(74, 75, 106). The concentration of other ribonucleases is growth-phase-dependent. During stationary phase or upon cold shock, RNase R is 3- to 10-fold more abundant in *E. coli* than during unimpeded exponential growth due to its diminished susceptibility to proteolysis(94). *B. subtilis* RNase Y also exhibits growth-phase-dependent changes in abundance by an undetermined mechanism (88).

In addition to concentration changes, the cellular activity of RNase E, RNase III, and PNPase in *E. coli* can also be modulated in response to environmental signals by altering the specific activity of these enzymes. These changes in catalytic potency result from binding either a cellular metabolite or a protein. For example, PNPase activity is inhibited by ATP and citrate, suggesting that RNA degradation may be sensitive to cellular energy levels and to central metabolism(37, 124). RNase III activity is regulated by the protein YmdB, which is expressed upon coldshock or entry into stationary phase and acts by preventing RNase III dimerization (80). Similarily, RNase E activity can be inhibited by the proteins RraA and RraB, which bind to its carboxy-terminal domain and are thought to stabilize distinct sets of mRNAsunder certain stress conditions (57, 60, 85). RraA can also interact directly with the RNA degradosome helicase RhlB and impair its function(60).

TA toxins that degrade mRNA

Bacterial genomes encode several toxin-antitoxin (TA) systems, some of which have an impact on mRNA degradation. A TA system consists of a toxin-antitoxin pair in which the deleterious effect of the toxin protein is neutralized by the presence of its cognate antitoxin. The toxin of many type II or type III TA systems is a ribonuclease that normally is inhibited by the tight binding of a protein or RNA antitoxin(158). When triggered by stress, such as amino acid starvation, DNA damage, or heat shock, the unstable antitoxin is degraded, freeing the more stable toxin to attack cellular RNAs. The endonuclease toxins of these TA

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systems are of two kinds: those that cleave RNA at specific sequences (MazF- and VapC-like toxins) and those that cut ribosome-associated RNAs within the coding region (RelE-like toxins). Because the specificity of MazF-like toxins is defined by a rather short sequence motif (typically 3–5 nt), they degrademRNAsfairly indiscriminately(158), as do RelE-like toxins(71). The consequent reduction in protein synthesis is thought to help cells become dormant for the duration of the stress.

Effects of phage infection

Infecting bacteriophage utilize a variety of mechanisms to manipulate mRNA degradation in host cells to their advantage. For example, the protein product of phage T7 *gene 0.7* phosphorylates RNase E and RhlB, among other *E. coli* proteins, thereby selectively inhibiting endonucleolytic cleavage of nascent T7 transcripts that are transiently ribosome-deficient due to the ability of T7 RNA polymerase to outpace ribosomes (100). Another *E. coli* endonuclease implicated in mRNA degradation in phage-infected cells is RNase LS (RnlA), the toxin component of a TA system (81). Owing to its short lifetime, the cognate antitoxin RnlB is quickly degraded upon global inhibition of host gene expression by phage T4. As a result, RNase LSbecomes activated. To prevent RNase LS from degradingT4 transcripts, the bacteriophage encodes its own antitoxin, Dmd, which neutralizes RNase LS (127). In addition, the ability of T4 polynucleotide kinase to monophosphorylate the 5’-hydroxyl termini of decay intermediates generated by a T4-encoded endonuclease accelerates their degradation by RNase E(46).

VII. CONCLUDING REMARKS

The interaction of bacterial ribonucleases with their mRNA targets and the mechanisms that bacteria use to govern rates of mRNA degradation have profound implications for gene regulation, environmental adaptation, cell growth and survival, and pathogenesis. Although studies to date have provided an informative glimpseof the many strategies utilized by bacteria to control mRNA turnover, our understanding is far from complete. Consequently, the field of bacterial mRNA decay remains an area ripe for further study.

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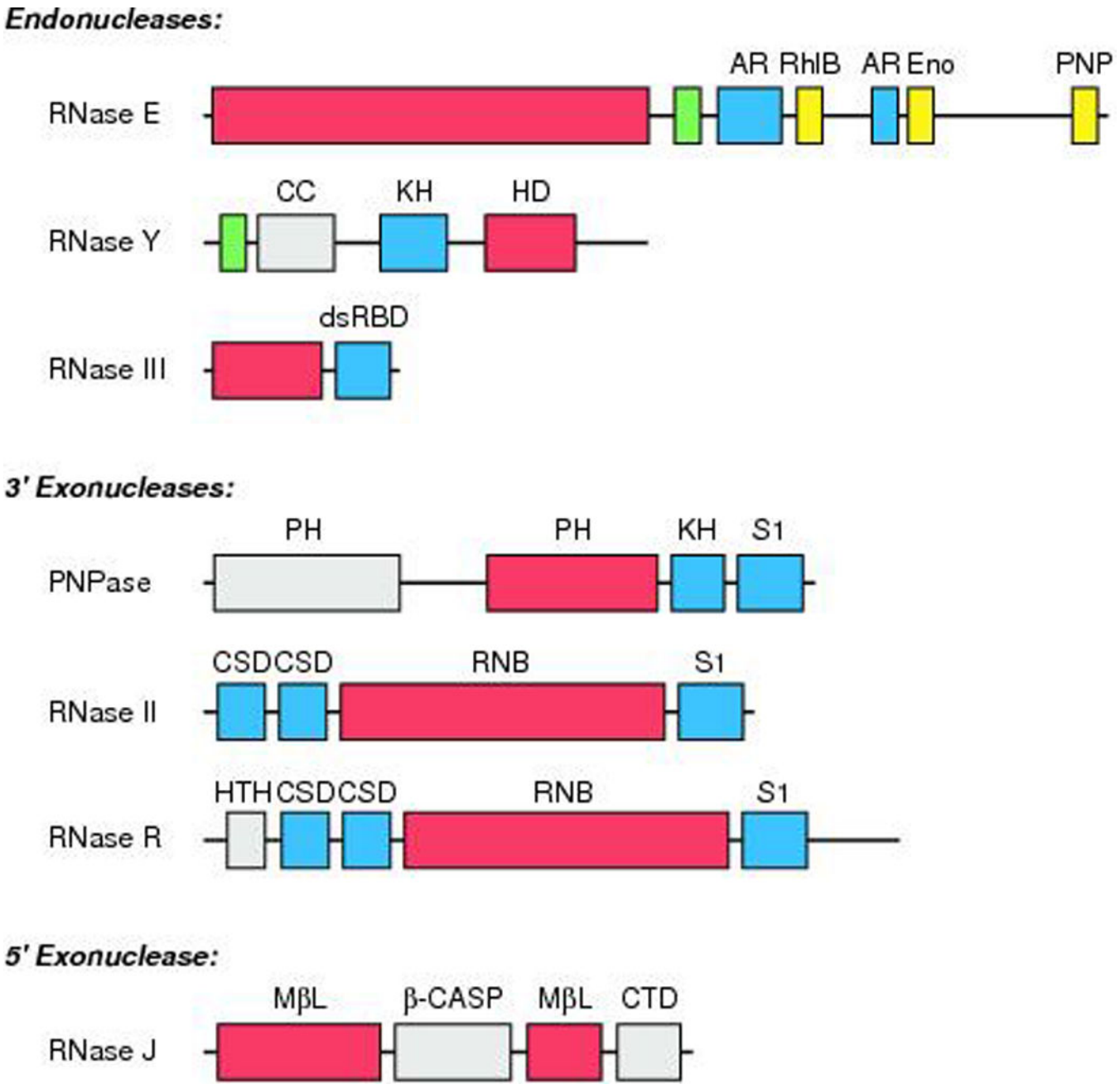

Figure 1. Constituent domains of mRNA-degrading ribonucleases

The domain composition of representative ribonucleases from *E. coli* (RNase E, RNase III, PNPase, RNase II, and RNase R) and *B. subtilis* (RNase Y and RNase J) is shown. Structural domains are depicted as colored rectangles: red, catalytic domain; blue, RNA-binding domain; yellow, protein-binding domain; green, membrane-binding domain; gray, miscellaneous domain. The sites where RhlB, enolase (Eno), and PNPase (PNP) bind to RNase E are marked, as are its two arginine-rich RNA-binding domains (AR). Of the two PH domains in PNPase, only the second is catalytically active. The single metallo-β-lactamase domain (MβL) of RNase J comprises two noncontiguous segments of the

polypeptide. CC, coiled coil domain; dsRBD, double-stranded RNA-binding domain; CSD, cold shock domain; HTH, helix-turn-helix domain; CTD, carboxy-terminal domain. The catalytic domains of RNase E and RNase III have not been named.

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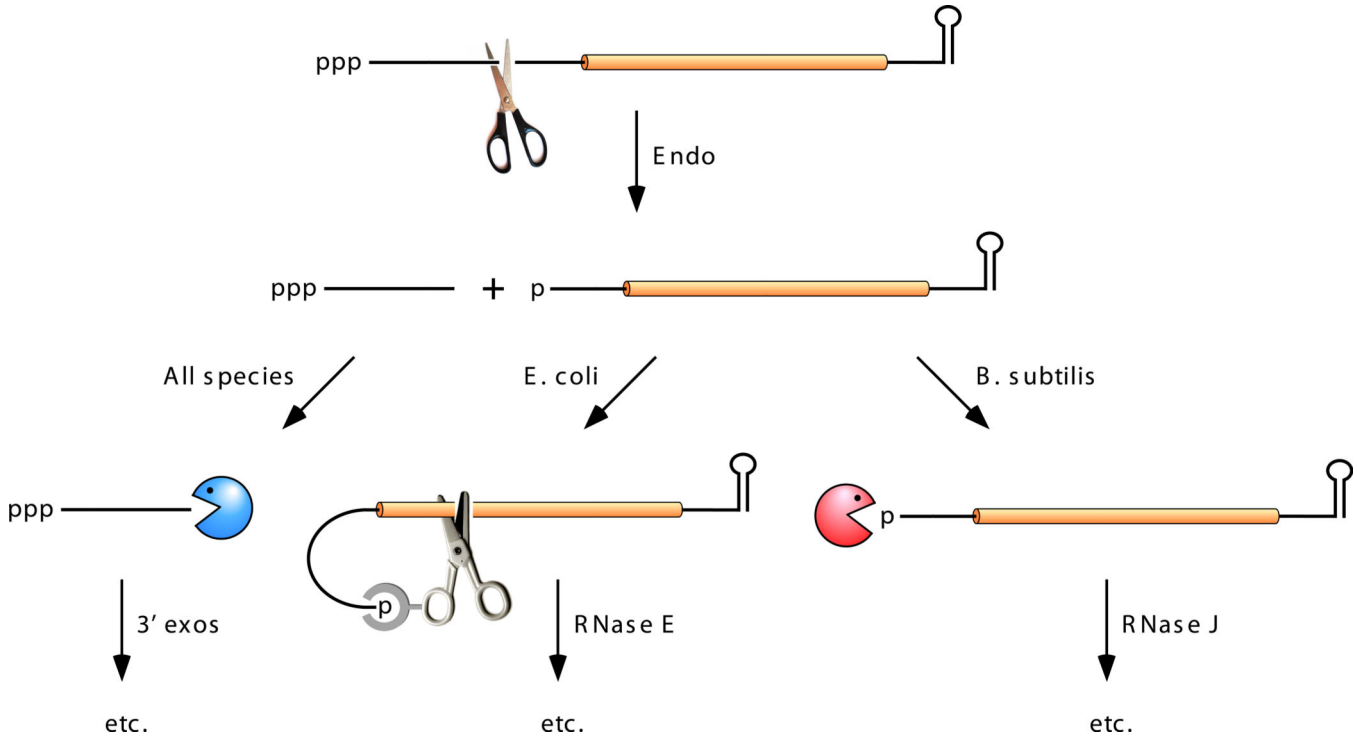

Figure 2. Direct-access pathway for mRNA degradation

An endonuclease (black-handled scissors), usually but not always RNase E or RNase Y, cleaves the primary transcript internally to generate two fragments. Unprotected at its 3' end, the 5' fragment is quickly attacked by 3' exonucleases (blue Pac-Man) in all bacterial species. The fate of the monophosphorylated 3' fragment depends on the ribonucleases present in the cell. In some species, like *E. coli*, this fragment undergoes further endonucleolytic cleavage by RNase E (gray scissors), which rapidly degrades such intermediates by selectively binding the monophosphorylated 5' terminus in a discrete pocket on the surface of the catalytic domain and cutting downstream. In others, like *B. subtilis*, it undergoes rapid 5'-exonucleolytic digestion by RNase J (red Pac-Man), whose exonuclease activity aggressively degrades RNAs bearing a single phosphate at the 5' end.

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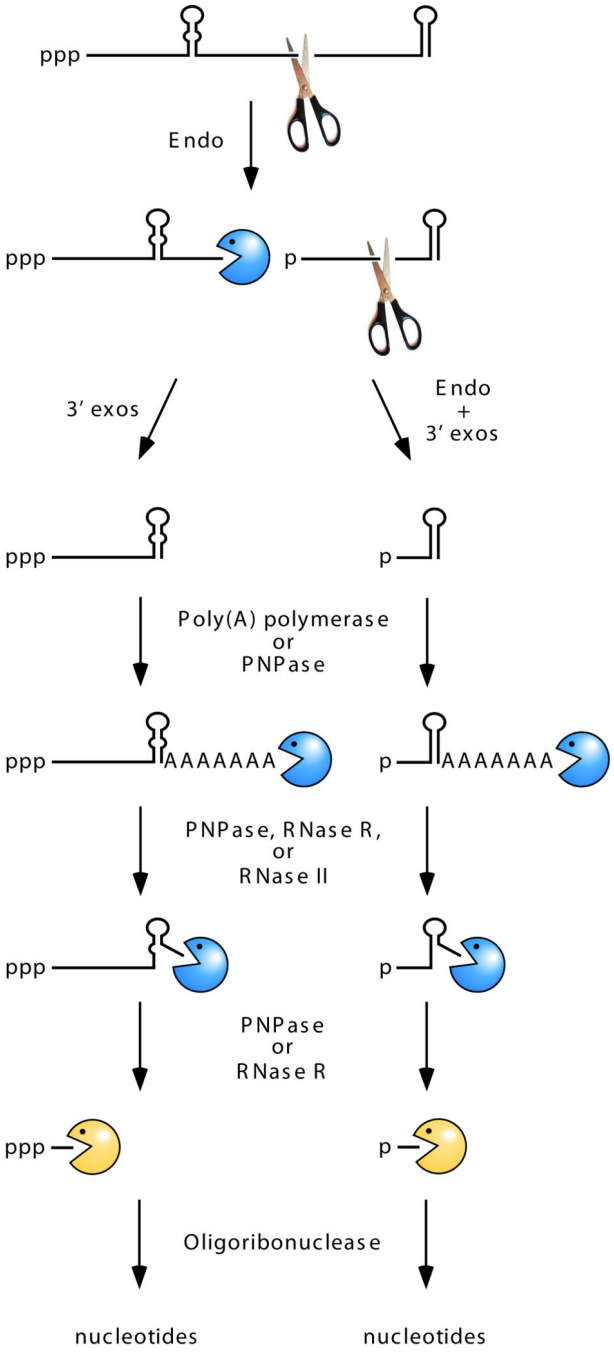

Figure 3. 3’-exonucleolytic degradation of decay intermediates

Endonucleolytic cleavage of mRNA generates a 5’-terminal fragment whose single-stranded 3’ end is trimmed exonucleolytically until a structural barrier is encountered, as well as a 3’-terminal fragment whose 3’ end is protected from exonucleolytic digestion by a terminator stem-loop. Subsequent polyadenylation by poly(A) polymerase or PNPase provides the 3’ exonucleases PNPase (operating with help from the RNA helicase RhlB) and RNase R (operating alone) an opportunity to overcome the barriers by creating a single-stranded binding site from which they can launch an attack. The process of poly(A) addition and

removal is repeated until the structural barriers are breached. By contrast, RNase II can degrade poly(A) and other unpaired 3’ ends but not structured 3’ ends. Degradation by these 3’ exonucleases (blue Pac-Man) eventually generates fragments that are too small for them to shorten further and are instead degraded by an oligoribonuclease (yellow Pac-Man).

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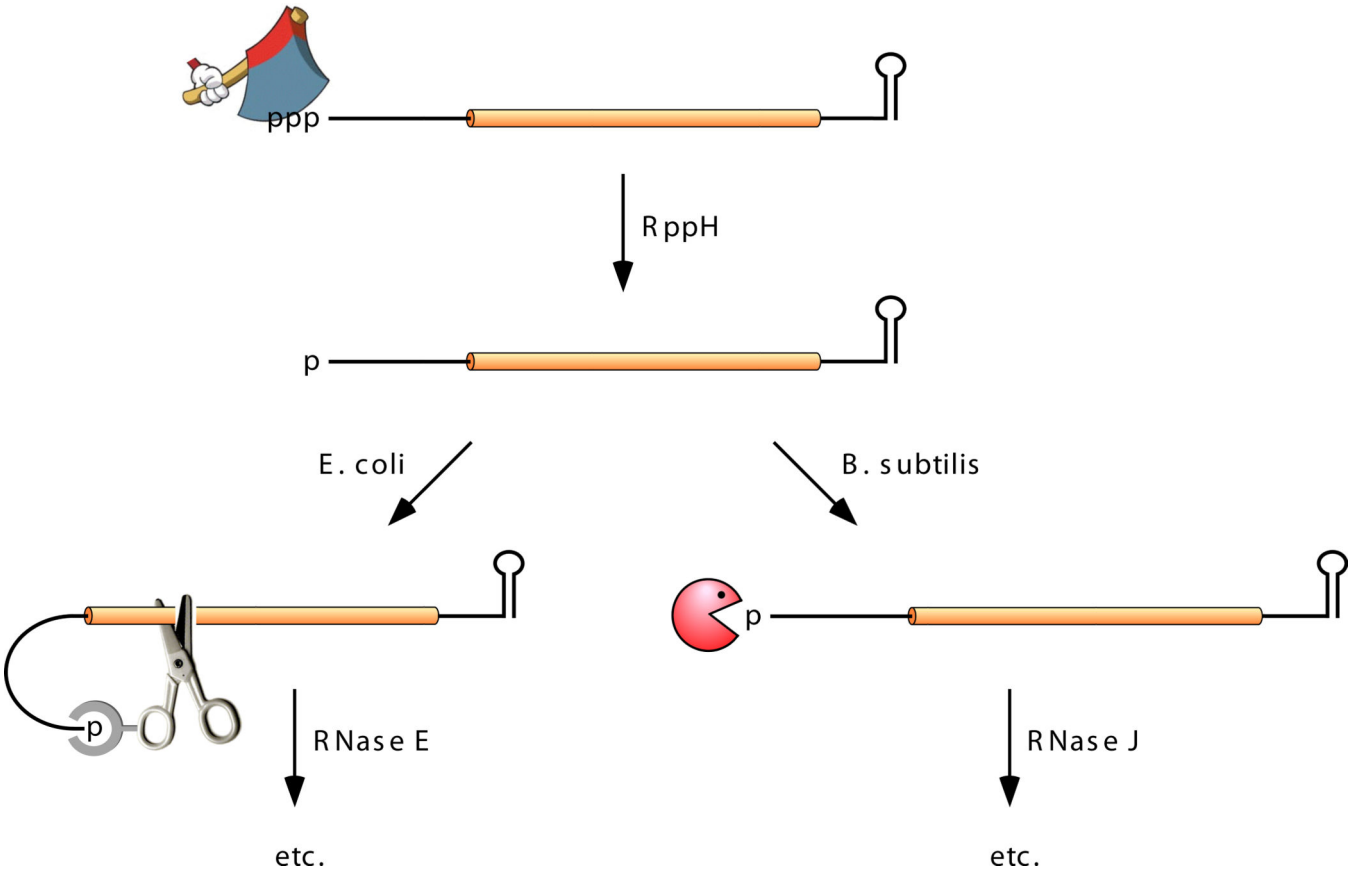

Figure 4. 5’-end-dependent pathway for initiating mRNA degradation

The RNA pyrophosphohydrolase RppH (hatchet) converts the 5’-terminal triphosphate of the primary transcript to a monophosphate. The resulting full-length decay intermediate is then rapidly degraded by either RNase E (scissors) or RNase J (Pac-Man), depending on which of these enzymes is present in the host species.

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Table 1

Phylogenetic distribution of major bacterial mRNA-degrading ribonucleases[a]

Taxon	Endos			5' Exo	3' Exos		Oligoribonucleases		
	RNase E/G	RNase Y	RNase III	RNase J	PNPase	RNase II/R	Orn	NrnA/B	NrnC
Proteobacteria									
Alpha	91/91	0/91	91/91	91/91	91/91	64/91	0/91	0/91	91/91
Beta	53/53	0/53	53/53	0/53	53/53	53/53	52/53	0/53	0/53
Gamma	122/122	0/122	122/122	0/122	122/122	118/122	122/122	0/122	0/122
Delta	29/29	24/29	29/29	25/29	29/29	28/29	5/29	20/29	0/29
Epsilon	0/16	15/16	16/16	16/16	16/16	16/16	0/16	16/16	0/16
Firmicutes									
Bacilli									
Bacillales	14/32	32/32	32/32	32/32	32/32	32/32	0/32	32/32	0/32
Lactobacillales	0/30	29/30	30/30	30/30	13/30	30/30	0/30	30/30	0/30
Clostridia	34/37	37/37	36/37	37/37	37/37	31/37	0/37	37/37	1/37
Actinobacteria	65/70	18/70	70/70	69/70	70/70	32/70	65/70	35/70	0/70
Cyanobacteria	25/25	0/25	24/25	25/25	25/25	25/25	0/25	0/25	25/25
Bacteroidetes & Chlorobi	31/31	29/31	31/31	0/31	31/31	31/31	0/31	31/31	0/31
Tenericutes	0/22	14/22	22/22	22/22	5/22	18/22	0/22	22/22	0/22
Chlamydiae & Verrucomicrobia	10/10	2/10	10/10	0/10	10/10	9/10	0/10	3/10	0/10
Spirochaetales	0/13	10/13	13/13	1/13	13/13	6/13	0/13	10/13	3/13
Chloroflexi	0/12	12/12	12/12	12/12	12/12	0/12	0/12	8/12	0/12
Thermotogae	11/11	11/11	11/11	0/11	11/11	11/11	0/11	11/11	0/11
Deinococcus-Thermus	0/4	4/4	0/4	4/4	4/4	4/4	0/4	4/4	0/4
Aquificae	5/5	5/5	5/5	0/5	5/5	5/5	0/5	5/5	0/5
Fusobacteria	1/4	4/4	4/4	4/4	4/4	4/4	0/4	4/4	0/4
Fibrobacteres	4/4	0/4	4/4	3/4	4/4	4/4	1/4	3/4	0/4

0-10%	10-50%	50-90%	90-100%

[a]Numbers (colors) correspond to the fraction (approximate percentage) of analyzed species in each taxon that contain a particular ribonuclease. Homologs of major bacterial ribonucleases were identified in the sequenced and annotated genomes of 621 distinct bacterial species by using the program STRING v9.1 (53). Initial hits were defined as those having a minimum bit score of 60. Individual sequence alignments with bit scores above 120 were accepted as homologs, and those with bit scores between 60 and 120 were re-analyzed with BLAST (Basic Local Alignment Search Tool) to identify true homologs with the proper protein length, a good sequence alignment, and an E value below 1×10^{-8}. The distribution of ribonucleases was then mapped by using iTOL (Interactive Tree of Life) (90) and tallied as a fraction of the number of analyzed species in each taxonomic clade. Phyla are listed in boldface type, classes in regular type, and orders in italics. Abbreviations: Endo, endonuclease; Exo, exonuclease.