Review

mRNA degradation and maturation in prokaryotes: the global players

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Abstract

The degradation of messenger RNA is of universal importance for controlling gene expression. It directly affects protein synthesis by modulating the amount of mRNA available for translation. Regulation of mRNA decay provides an efficient means to produce just the proteins needed and to rapidly alter patterns of protein synthesis. In bacteria, the half-lives of individual mRNAs can differ by as much as two orders of magnitude, ranging from seconds to an hour. Most of what we know today about the diverse mechanisms of mRNA decay and maturation in prokaryotes comes from studies of the two model organisms *Escherichia coli* and *Bacillus subtilis*. Their evolutionary distance provided a large picture of potential pathways and enzymes involved in mRNA turnover. Among them are three ribonucleases, two of which have been discovered only recently, which have a truly general role in the initiating events of mRNA degradation: RNase E, RNase J and RNase Y. Their enzymatic characteristics probably determine the strategies of mRNA metabolism in the organism in which they are present. These ribonucleases are coded, alone or in various combinations, in all prokaryotic genomes, thus reflecting how mRNA turnover has been adapted to different ecological niches throughout evolution.

Keywords: mRNA degradation; prokaryote; RNase E; RNase J; RNase Y.

Introduction

Degradation of mRNA is a vital process that enables bacteria to rapidly alter patterns of protein synthesis in an ever-changing environment. The instability of mRNA is also fundamental to permit transcriptional control of gene expression. In exponentially growing prokaryotic cells, mRNA half-lives range from seconds to over an hour. Controlling the lifetime of an mRNA can thus be an efficient way to control gene expression.

Ribonuclease cleavage is not always synonymous to degradation. Some mRNA transcripts can also be productively cleaved (a process normally called processing), so that the major translated species *in vivo* is not the primary transcript. In this case, endonucleolytic cleavage can occur between open reading frames of a polycistronic mRNA and generate transcripts with very different half-lives. As a consequence, a regulatory and a structural protein, for example, can be produced in the very different stoichiometric amounts needed by the bacteria (1, 2).

For more than 30 years, mRNA degradation in bacteria was essentially studied in *Escherichia coli (E. coli)*. Our view of mRNA decay was thus entirely based on the types of RNases present in this organism. The model that emerged from these studies concluded that degradation of mRNA begins with endonucleolytic cleavage at one or more internal sites to produce short-lived decay intermediates. The endonuclease most important for initiating mRNA turnover in *E. coli* is RNase E (3–8). Minor roles are attributed to other more specialized endonucleases, such as RNase G (an RNase E paralog), RNase III, RNase Z and RNase P. Several 3′ exonucleases, notably polynucleotide phosphorylase (PNPase), RNase II, RNase R and oligoribonuclease, then act as scavengers of the initial cleavage products (9–11). Despite the predominant role of RNase E in initiating mRNA decay, alternate pathways should not be neglected. Notably the transient addition of poly(A) tails to *E. coli* RNAs is crucial for the 3′ exonucleolytic degradation of stem-loop structures in decay intermediates, but it is not yet clear what proportion of mRNA degradation is initiated by 3′polyadenylation (12–15).

Despite its central role in mRNA metabolism in *E. coli*, RNase E is absent from many bacterial species, including many firmicutes, such as *Bacillus subtilis* (*B. subtilis)* and even some proteobacteria (16). In *B. subtilis*, just as in *E. coli,* the 5′ end is a major determinant of mRNA stability. However, the stabilizing effects of a 5′ « roadblock », like a stalled ribosome, are far more impressive in *Bacilli,* where the entire downstream segment (but not the upstream segment) can be protected from degradation, even in the absence of translation. Recent advances in the study of mRNA metabolism in organisms devoid of RNase E included the discovery of the paralogous RNases J1 (*rnjA*) and J2 (*rnjB*) in *B. subtilis*, initially characterized as endoribonucleases with RNase E-like cleavage specificity (17). Subsequently, RNases J1/J2 were shown to also possess 5′-3′ exonuclease activity (18), an activity previously unprecedented in bacteria. RNase J1 was actually the first ribonuclease identified capable to perform both types of activity, endo- and exonu-

cleolytic, using a single catalytic site (19). Notably, the 5′ exonucleolytic activity, which strongly prefers a monophosphorylated RNA 5′ end (18, 19), was welcome as it could best explain the strong 5′ dependence of mRNA stability observed in *Bacilli*. RNase J1 was thus assumed to be the key enzyme initiating RNA turnover in *B. subtilis*. However, the recent identification of RNase Y as a 5′ end sensitive endoribonuclease with a cleavage site preference similar to RNase E and RNase J1/J2 (20, 21) clearly requires a re-evaluation of current models on RNA decay in *B. subtilis*.

RNases E, J and Y are represented throughout prokaryotic phylogeny, alone or in diverse combinations. Their inherent enzymatic characteristics and global impact on RNA metabolism are likely to shape, to a very significant extent, the strategies of initiation of mRNA degradation employed by various organisms. Here, we will focus on these three key ribonucleases and how they fit into our current understanding of mRNA processing and degradation in bacteria. In particular, the recently discovered RNases J and Y will be compared to RNase E, the paradigm for enzymes initiating RNA decay. Only certain relevant aspects of RNase E will be described here; a recent comprehensive review on this enzyme can be found elsewhere (22).

Global effects on mRNA metabolism

In *E. coli,* under equilibrium growth conditions the principal pathway for mRNA degradation is mediated by RNase E. The early availability of thermosensitive RNase E mutations allowed researchers to appreciate the full impact of this enzyme on mRNA decay. Thermal inactivation of RNase E increases the chemical stability of global mRNA up to sixfold, from about 2.5 min to over 10 min (4, 7). Macro- and tiling array studies of an *rne* deletion mutant, viable due to overexpression of the paralogous RNase G (which has only very few mRNA substrates), showed that about 40% of all mRNAs increased at least 1.5-fold (23, 24) but about 25% of all mRNAs actually decreased under these conditions (24). It is difficult to estimate potential indirect effects associated with the removal of an important ribonuclease, but it is clear that the post-transcriptional regulation of gene expression in *E. coli* is far more complex than previously envisioned.

In *B. subtilis,* depletion of RNase J1 in a strain also lacking RNase J2 only modestly increased global mRNA stability from 2.6 to 3.6 min and single mutants showed no effect (17). In agreement, a transcriptome and proteome study found only a minor effect of depleting cells for RNase J1 alone or inactivating RNase J2. In the double mutant, the levels of more than 650 transcripts were altered and the amount of, more than 200 proteins, from about 1000 proteins visible on 2D-gels, were significantly changed. These changes concerned roughly equal numbers of up- and downregulated transcripts/proteins (25). For four specific transcripts (*cspC, spoVG, tagD* and *yweA*) a significant increase in mRNA half-life was confirmed by Northern blot analysis. This clearly indicated a general role of RNases J1 and J2 in mRNA turnover. Among the transcripts showing increased

expression in the double mutant, more than one fourth belonged to large regulons controlled by specialized sigma factors or regulatory proteins whose mRNAs were also more abundant (e.g., SigmaD, SigmaW and ComK dependent competence regulons). Ten percent to fifteen percent of the wild type RNase J1 level is sufficient for normal growth, but more RNase J1 is needed in the double mutant to compensate for the loss of RNase J2 (25). With about 2500 molecules per wild type cell (26), RNase J1 is thus produced in significant excess over the cell's requirements. Interestingly, 10%–20% of RNase E produced in a wild type strain is also sufficient to ensure normal growth of *E. coli* and under these conditions mRNAs known to decay in an RNase E-dependent fashion start to show increased half-lives (27–29). Presumably, some targets of RNase J1/J2 may only be detected under more severe depletion conditions. Among the many mRNAs with reduced abundance in the RNase J1/J2 mutant strain, a significant proportion is probably not stabilized by the action of the nucleases. Instead, this decrease in mRNA levels is probably a secondary effect that can be associated with, for example, a reduced growth rate and/or changes in the steady state levels of small regulatory RNAs or other unidentified regulatory factors. In addition, mutants that retain only a single activity, endo- or 5′ exonucleolytic, would be useful to attribute an effect to one or the other activity.

The group A *Streptococci* (GAS) also have two RNase J orthologs and, interestingly, both are essential (30). In this organism, differential mRNA stability is an important mechanism for the regulation of virulence factors. Indeed, two classes of transcripts have been described in this organism. Class I mRNAs decay rapidly in all growth phases, while class II transcripts remain stable for >10 min in the exponential phase before their decay is initiated and are very stable in the stationary phase. The degradation of both classes of mRNAs is affected in RNase J1 and J2 conditional mutants. It has been suggested that degradation of class II transcripts only occurs once RNases J1 and J2 are released from their primary task, degrading preferred class I mRNAs (30).

RNase J orthologs have recently also been characterized as 5′ exoribonucleases in the eury- and crenarcheal clades of the archea (31, 32). Their importance in global mRNA turnover is not yet well established. However, in *S. solfataricus*, the γ subunit of translation initiation factor a/eIF2 was shown to specifically bind to the 5′ PPP moiety of mRNA and to protect it from 5′ exonucleolytic decay (33). This protection could be particularly important because mRNAs in this organism are generally leaderless (34).

The third nuclease with global effects is RNase Y. This novel and essential endoribonuclease co-exists with RNase J and/or RNase E in many bacterial species (20, 21) (see below). *Bacillus subtilis* RNase Y has striking functional similarities with *E. coli* RNase E and its depletion increases the half-life of bulk mRNA more than two-fold (20). Preliminary analysis of a tiling array study of an RNase Y depleted strain identified more than 1200 potential substrates and it supports the initial finding on the global effect of RNase Y on mRNA turnover (our unpublished results). In *S. aureus*

and *S. pyogenes,* orthologs of RNase Y have been implicated in the regulation of virulence gene expression (35, 36). The *S. pyogenes* enzyme is not essential and has not formally been shown to be an RNase. However, its deletion altered the expression of up to 30% of the transcriptome in the stationary phase, including multiple virulence genes (36).

Endonucleolytic cleavage specificity

Escherichia coli RNase E and *B. subtilis* RNases J1/J2 and Y are very diverse enzymes that have no sequence similarity. However, despite their apparent disparity, they share certain common features. The most significant is probably their similar endonucleolytic cleavage specificity. RNase E does not depend on a particular nucleotide sequence for cleavage (37, 38) but a certain preference for AU-rich single-stranded sequences and other parameters has been established (39–41). In *B. subtilis,* the existence of an RNase E-like activity was suggested 15 years ago by the observation that cleavage of the leader region of the *thrS* gene (encoding threonyl-tRNA synthetase) occurred at a similar site upstream of a terminator structure in *B. subtilis* and *E. coli.* In *E. coli,* the processing was shown to be RNase E-dependent (42). The genome sequence published shortly thereafter, unequivocally showed that *B. subtilis* has no authentic RNase E ortholog. This initiated a search for an enzyme in *B. subtilis* capable of cleaving the *thrS* leader RNA and led to the discovery of the RNases J1 and J2 *via* a biochemical approach (17). RNase E and RNases J1 and J2 can cleave the *thrS* mRNA *in vitro* at the same AU-rich site immediately upstream of the leader terminator (Figure 1B). Evidence that RNase J1/J2 can cleave similar sequences *in vivo* was obtained from studies on the *thrZ* gene, encoding a second threonyl-tRNA synthetase, and which contains a leader RNA configuration very similar to that of the *thrS* gene (17). In both cases, this processing event is not a decay initiating step but rather protects (and stabilizes) the downstream mRNA. The cleavage leaves a secondary structure (the terminator) at the 5′ end of the mRNA, which probably protects against the 5′ exonucleolytic activity of RNase J1.

As an endoribonuclease, RNase J1 has since been implicated in the maturation of the small cytoplasmic (sc) RNA, a component of the signal recognition particle for protein export (43) as well as in the turnover of the *B. subtilis trp* leader (Figure 1C). This is important for the recycling of the repressor protein TRAP, bound by this region, to avoid deregulation of other genes controlled by TRAP (44).

RNases J1 and J2 likely exist as a heterooligomeric complex *in vivo* (26). Indeed, both enzymes originally co-purified in stoichiometric quantities, despite the fact that à priori they should exhibit quite different chromatographic behavior, i.e., isoelectric points of 6.1 (J1) and 8.8 (J2) (17). The RNase J1/J2 complex has a somewhat different endonucleolytic cleavage specificity *in vitro* compared to the individual enzymes (26).

Notwithstanding the above examples, RNase J1/J2 endonucleolytic activity *in vivo* is not well documented and it might be rare under physiological conditions. There are several reasons as to why *bone-fide* RNase J endonucleolytic targets are difficult to identify. The action of RNase J as a dual activity enzyme is complicated to analyze. Every RNA endonucleolytically cleaved by RNase J could directly become a substrate for its exonucleolytic activity (see below). In addition, compared to RNase E, high enzyme concentrations, generally in significant excess over the substrate, are required to observe cleavage *in vitro* (17, 45, 46). This does not exclude the fact that RNase J does act as an endonuclease *in vivo*, but could simply reflect the fact that the few substrates tested so far are not present in the optimal conformation, or that we are missing an additional factor required for cleavage. However, new crystallographic data, discussed further on, provide a rational insight into why the endonucleolytic activity of RNase J might be restricted *in vivo* (47, 48). In the *archea*, recently identified RNase J orthologs have a strong 5′ exonucleolytic activity but no significant endonuclease activity (31, 32). In contrast, mycobacterial RNase J has both 5′ exo- and endonucleolytic activity (Taverniti and Putzer, unpublished results).

B. subtilis RNase Y was recently characterized as an endoribonuclease that cleaves in AU rich single-stranded regions close to secondary structure. In addition, the rate of cleavage is enhanced on RNA substrates carrying a 5′ monophosphate group (20). RNase Y initiates the turnover of all ten S-adenosyl-methione (SAM) dependent riboswitches by cleaving immediately up- and downstream of the SAM aptamer (Figure 1E). Cleavage of the upstream site *in vitro* depends on SAM binding to the aptamer RNA. This requirement is probably less specific than it might appear; in fact, SAM binding leads to the formation of a secondary structure immediately downstream of the cleavage site. Such a context appears to be important; the second RNase Y cleavage of the SAM riboswitch also occurs upstream of a secondary structure, the leader terminator (Figure 1E). RNase Y has also been implicated in the processing of the *B. subtilis cggR-gapA-pgk-tpi-pgm-eno* operon *in vivo*, that uncouples the expression of the regulator CggR from that of the glycolytic proteins encoded by the operon (21). By its AU-richness and the presence of a downstream secondary structure, the previously known processing site (Figure 1D) that occurs close to the end of the *cggR* mRNA (2) resembles the RNase Y upstream site in the SAM riboswitch, at present the only substrate whose cleavage site has been verified *in vitro*. RNase Y has also been proposed to initiate the decay of the *rpsO* mRNA, by cleaving near the 3′ end of the mature transcript (49), but the exact cleavage site has not been identified.

The known or suspected RNase Y cleavage sites could easily be considered as RNase E sites, particularly the structured RNA substrates that function as internal entry sites that bypass the usual 5′-end requirements of RNase E (50, 51) (Figure 1A). Interestingly, RNase Y can cleave the *B. subtilis thrS* leader *in vitro* at the same site upstream of the leader terminator (our unpublished results), as already described for RNase E and RNases J1 and J2 (Figure 1B). The fact that even a single substrate can be cleaved by all three enzymes,
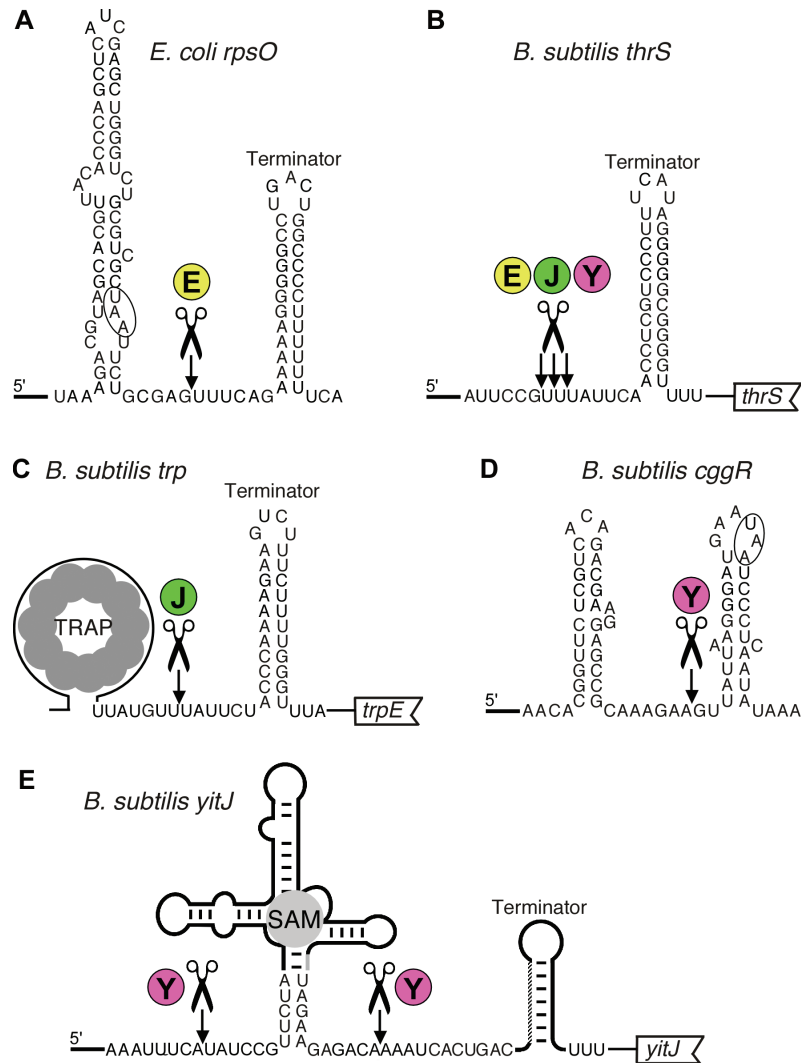
Figure 1 Endonucleolytic cleavage sites of RNases E, J and Y on natural substrates.
(A) Decay initiating internal RNase E cleavage *in vivo* of the 3′ UTR of the *rpsO* mRNA (118, 119). (B) Cleavage of the *B. subtilis thrS* mRNA 5′-UTR upstream of the leader terminator *in vitro* by RNases E, J and Y. Cleavage *in vivo* probably mediated essentially by RNase Y (unpublished observations). (C) Processing of the *B. subtilis trpE* 5′ attenuator region by RNase J1 initiating the recycling of the TRAP regulatory protein (120). (D) *In vivo* cleavage by RNase Y within the *B. subtilis cggR* coding region, uncoupling *cggR* expression from that of the downstream *gapA* gene (21). (E) RNase Y cleavages *in vivo* and *in vitro* of the *B. subtilis yitJ* SAM riboswitch RNA initiating the decay of the SAM bound prematurely terminated leader RNA (20). Encircled triplets represent stop codons of the concerned genes.

suggests a convergent evolutionary pressure towards an enzymatic activity that appears to be important across species boundaries.

It can also be noted that the similar endonucleolytic cleavage specificity of *B. subtilis* RNases J1/J2 and RNase Y complicates the assignment of cleavages to one or other enzyme, and will require a re-evaluation of cleavages attributed solely to RNase J.

Sensitivity to the RNA 5′ end

The 5′ end is a site of critical importance for the fate of many prokaryotic mRNA transcripts, in eubacteria as well as in the *archea*. The accessibility, structure and/or the phos-

phorylation state of the 5′ terminal nucleotide often determines the stability of an mRNA, but by involving different pathways and enzymes in different *phyla*.

The endoribonucleolytic activity of RNase E can be modulated considerably by the nature of the 5′ end of an RNA substrate. This appears to hold true not only for the *E. coli* enzyme, but also for RNase E homologs from other species (52, 53), as well as for the paralogous RNase G (54, 55). For example, replacement of the 5′ UTR of a relatively labile mRNA with that of the long-lived *ompA* mRNA (15–20 min half-life) increased its stability to roughly that of the donor transcript (56). The principal element conferring increased stability was identified as a stem-loop structure no more than 2–4 nucleotides away from the 5′ end (57). Studies on the RNase E dependent decay of the *rpsT* mRNA (encoding

ribosomal protein S20) and investigations using short olig-omeric RNase E substrates revealed the importance of the phosphorylation state of the RNA 5′ end. Indeed, an RNA carrying a 5′ monophosphate was cleaved more efficiently than the same RNA with a 5′ triphosphate or hydroxyl group (55, 58–60). Importantly, even when monophosphorylated, the 5′ end of the RNA has to be in an accessible, i.e., single-stranded, conformation, in order to stimulate RNase E cleavage (58, 61).

The recently discovered 5′ pyrophophatase RppH can convert the 5′ PPP moiety of primary transcripts to a 5′ monophosphate (62, 63), an activity exploited by RNase E. In this novel decay pathway, internal cleavage by RNase E, is thus triggered by a prior event at the 5′ end. The action of RppH and the recognition of the 5′ P end by RNase E both require single-stranded 5′ termini. However, RppH is non-essential and its disruption only affects the stability of about 10% of all *E. coli* transcripts (63). Given the substantially larger proportion of *E. coli* transcripts destabilized by the action of RNase E compared to RppH, it is likely that the rate limiting step in mRNA degradation in many cases is an « internal entry » cleavage by RNase E, modulated by the efficiency of translation of a given transcript (50, 51, 64, 65). Interestingly, a protein with RppH-like activity, was also identified very recently in *B. subtilis* (encoded by the gene *ytkD*). It was shown to initiate the 5′ exonucleolytic degradation of an mRNA following conversion of the 5′ PPP moiety to a 5′P (66). The number of potential substrates of this non-essential pyrophosphohydrolase is not yet known.

Compared to *E. coli*, the 5′ end has been invoked as an even more crucial determinant of mRNA stability in Gram-positive bacteria, notably in *B. subtilis* (61–72). Secondary structure, bound proteins and strong ribosome binding sites near the 5′ end of an mRNA, can stabilize long stretches of downstream RNA, regardless of whether or not it is translated. This protection, at a distance, is a distinguishing feature between *B. subtilis* and *E. coli* RNA degradation (73).

RNases J1 and J2 were initially identified as 5′ end dependent endoribonucleases (17) that can cleave the *B. subtilis thrS* leader at two sites, one of which was only cleaved on a 5′ monophosphorylated transcript. It is now clear that the 5′-P dependent « cleavage » on the *thrS* mRNA is due to an arrest of the 5′ exonucleolytic activity of RNases J1/J2 at a secondary structure (19).

The 5′ exonucleolytic activity of RNase J1 is most active on 5′ monophosphorylated or 5′ hydroxylated RNA (18) and strongly inhibited by 5′PPP ends of primary transcripts (19, 46). In contrast, available evidence indicates that the endonucleolytic cleavage is independent of the nature of the RNA 5′ end (17, 18).

The 5′ exonucleolytic activity of RNase J2 measured on a short oligomeric RNA is about 200-fold lower than that of RNase J1 (26). We would like to note here that the *in vitro* activity of RNases J1/J2 can be very sensitive to the presence of affinity tags. For example, a His-tag can increase the 5′ exonucleolytic activity up to 10-fold over an enzyme purified by intein technology which produces a native protein. Similarly, the efficiency of RNase J1 mediated endonucleolytic

cleavage, can be greatly attenuated when the protein maintains a tag (our unpublished results). Although this observation does not question the principal conclusions drawn from various *in vitro* experiments, it points out the fact that a tag might have a considerable influence on enzyme activity.

RNase Y does not only share a similar cleavage specificity with RNase E, but also a sensitivity to the phosphorylation state of the substrate. The initial rate of RNase Y cleavage on the SAM riboswitch RNA is 20-fold faster when the RNA carries a 5′-P rather than a 5′-PPP. However, *in vivo*, RNase Y most likely initiates SAM riboswitch degradation through cleavage of the primary tri-phosphorylated transcript (20), similar to the internal entry mode for RNase E. At present, we ignore to what extent tethering to the 5′-P group of an RNA is important for the function of RNase Y *in vivo*.

Protein structure and catalytic mechanism

Despite some clear functional analogies between RNases E, J and Y, the three enzymes have evolved independently. There is no sequence similarity and the proteins belong to different structural families (Figure 2). Recent crystallographic data on the structures of RNase E and RNase J have considerably helped to rationalize the enzymatic behavior of these enzymes, and some surprising structural similarities have also been revealed (see below).

For RNase E, we will only describe the most relevant information in order to highlight the principle differences with respect to RNase J. The RNase E catalytic domain corresponds to the N-terminal half of the 1061 aa protein and the crystal structure of this domain (aa 1–529) bound to small RNA substrates has been solved (74). It is a composite
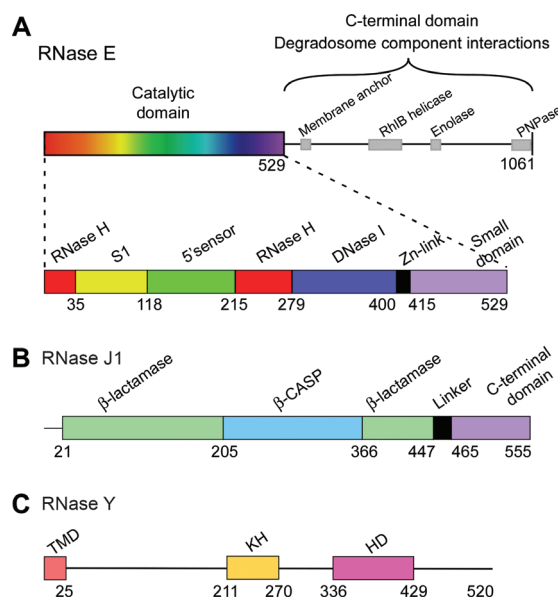
Figure 2 Domain structures of RNases E, J and Y.
For a general description, see text. RNase E protein domains establishing interactions with degradosome components are in gray. TMD=transmembrane domain.

of recurrent structural subdomains that closely resembles the globular folds of RNase H, DNase I and the ubiquitous S1 RNA binding domain (Figure 2A). The enzyme crystallized as a dimer of dimers and each protomer carries a 5′P binding pocket distinct from its active site. A substrate carrying an accessible 5′P can bind to the 5′P binding pocket of one protomer, and can be cleaved in the active site of the other protomer, within the same « principal » dimer. This explains why the enzyme prefers 5′P substrates and why dimeric or higher order complexes are necessary to express this preference (55). Between these two points of interaction, there is room to accommodate long, and even structured, RNA stretches, indicating that the enzyme does not need to scan the RNA to reach downstream cleavage sites. However, this mode of action also imposes constraints concerning the conformation of the substrate. The RNA must be single-stranded at the 5′P and at the cleavage site, and the separation between them should fit the geometry of the enzyme. Enzyme specificity thus probably results from the fact that many RNAs are not able to satisfy all of these requirements simultaneously (74).

The available structures of RNase E, from the holoenzyme as well as the apo-form (75), reveal large conformational changes upon substrate binding. While the apo-form is in an open state, it has been proposed that binding of the 5′P to the 5′ sensor domain causes this domain, together with the neighboring S1 domain, to clampdown on the downstream RNA to organize the catalytic site. However, it is not obvious how binding at the 5′P could by itself induce the conformational switch. Studies with the paralogous RNase G showed that interaction with the 5′ monophosphate contributes to the overall affinity of binding (76). Thus, it can be envisaged that a monophosphorylated 5′ end contributes to RNase E activity by increasing the affinity of the enzyme for the RNA.

As indicated earlier, RNase E can cleave certain substrates directly without any need to tether to the 5′ end (50, 51, 65). It has been proposed that these structured RNA substrates are accommodated in the large cleft seen in the open state structure. In this way, the structured RNA could fill the space by binding to the S1 domain and presenting a single-stranded region correctly to the active site residues without requiring the S1 domain movement.

RNase J is a member of the β-CASP subfamily of zinc-dependent metallo-β-lactamases (Figure 2B), a category taking its name from the proteins C̱PSF-73, A̱rtemis (involved in V(D)J recombination) and the S̱nm1/P̱so2 enzyme family (involved in DNA repair) (77). The structure of *Thermus thermophilus* RNase J, the apo-form as well as a complex with UMP, has been solved to high resolution (19). The enzyme is composed of three major domains (Figure 3A): the β-lactamase domain, the β-CASP domain (which is inserted into the β-lactamase core) and a C-terminal domain connected to the β-lactamase domain by a flexible linker. The enzyme contains a single catalytic site. It is defined by the presence of two Zn ions coordinated in an octahedral environment, which are located deep in the cleft between the β-lactamase and β-CASP domains (Figure 3B). Access to

the active site is limited in the structure that corresponds to a 'closed' conformation. This conformation must open up to allow access and movement of the RNA.

Mutations affecting the coordination of the zinc ions abolish both endo- and 5′ exonucleolytic activity, indicating that a single catalytic site is reponsible for the dual activity of RNase J (19). When soaked into the crystal, UMP binds to a site one nucleotide distant from the catalytic center (Figure 3B), thus representing the post-cleavage state of the RNase J in 5′ exonuclease mode. The 5′ monophosphate group is coordinated by several residues creating a phosphate-binding pocket for the 5′ terminal nucleotide and thus providing a rationale for the enzyme's preference for a 5′P in exonuclease mode. However, a 5′OH group can be accommodated in this site and a nice example of RNase J1 acting on such a substrate *in vivo* is the turnover of the riboswitch-regulated *glmS* mRNA. In the presence of glucosamine-6-phosphate, the riboswitch is activated to become a self-cleaving ribozyme, generating a downstream fragment bearing a 5′OH which is degraded by RNase J1 (78).

In contrast, a 5′ triphosphate bound by the γ-phosphate would place the scissile phosphodiester bond out of phase with the catalytic Zn ions. The one nucleotide separation between the 5′-P binding pocket and the active site suggested that the endo- and exonucleolytic activities of RNase J can be coupled. In fact, the downstream product of endonucleolytic cleavage, bearing a 5′-P, would be perfectly placed to slide into the monophosphate-binding pocket, enabling the enzyme to switch to 5′ exonucleolytic mode on the same RNA molecule. Mutations that reduce the coordination of the phosphate moiety, but add no steric constraints, fully retain both activities. However, replacing a coordinating residue with a bulkier amino acid like leucine, severely inhibits not only the 5′-exo- but also the endonucleolytic activity (19). Thus, as expected, inhibition of 5′-phosphate docking does eliminate the exonuclease activity, but it is less evident why these mutations also have an effect on the endonucleolytic activity (see later in text). Nevertheless, it is clear that the accommodation of the phosphate group (and also of a 5′-OH) does not require optimal coordination, but rather unhindered steric access to the binding pocket that puts no constraints on the correct positioning of the scissile phosphodiester bond.

Bacillus subtilis RNase J1 as an N-terminal 6xHis version and native RNase J1, exist predominantly as a dimer in solution, together with minor quantities of monomers and tetramers (19, 48). By contrast, C-terminally His-tagged RNase J1 primarily forms a tetramer in solution (26). The observed difference in the oligomerization state thus appears to stem from the position of the His-tag. The linker and the C-terminal domains are required for activity and maintaining the dimeric state in solution (19). At present, it is not clear whether it is the dimerization of the enzyme, the C-terminal domain itself, or both, that is important for enzyme function. By comparison, archeal RNase J orthologs, which show only 5′ exonuclease activity (31, 32) have no equivalent C-terminal domain, but their oligomerization status is unknown.

It is noteworthy that the structure of the catalytic domain of RNase E (aa 1–529) shows some surprising similarities
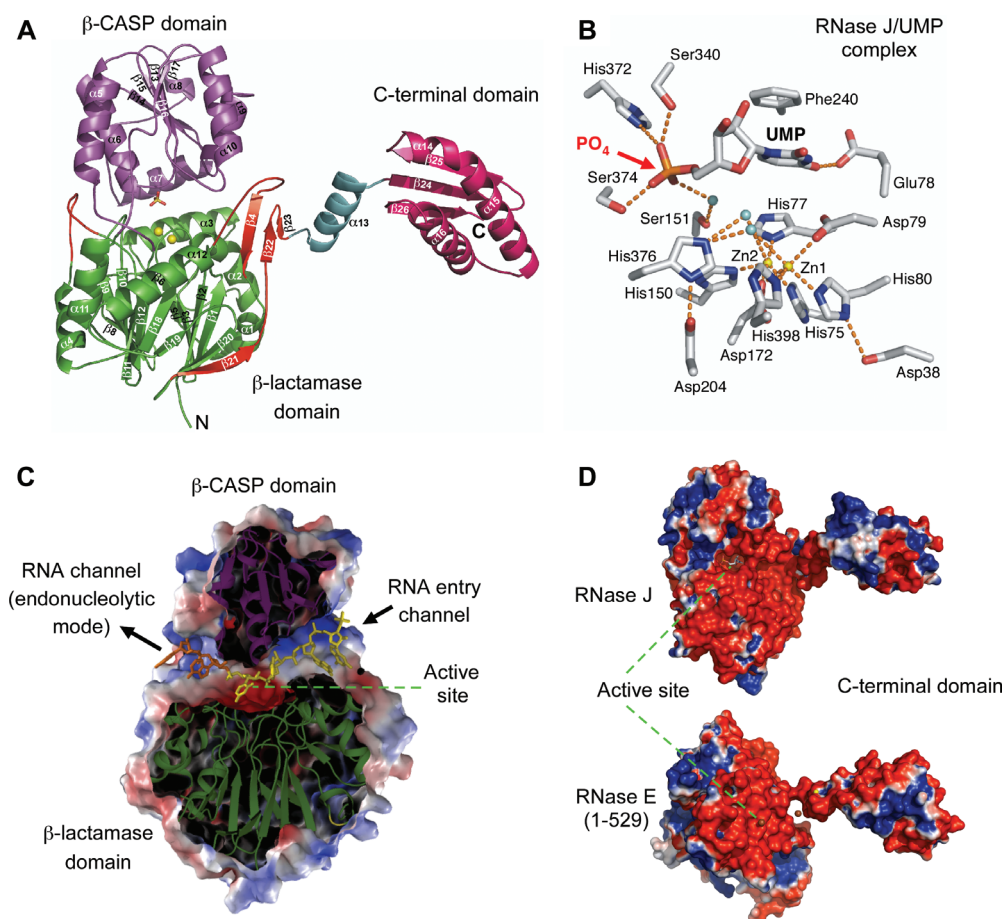
Figure 3 Structure of *T. thermophilus* RNase J and close-up of the active site (20). (A) Structure of the RNase J monomer. The yellow spheres represent the two Zn^{+2} ions in the active site. N and C indicate the N- and C-terminal ends of the protein, respectively. (B) The catalytic center of RNase J complexed with a UMP residue. Dotted orange lines indicate ligand-mediated and hydrogen bond interactions. (C) Slab view of RNA binding channels (47). Positively charged surfaces are shown in blue; negatively charged surfaces are shown in red. The bound 4nt RNA is shown in yellow. Two extra nucleotides (orange) are modeled to show the RNA in endonucleolytic binding mode. (D) Comparison of the electrostatic surfaces of RNase J and the catalytic N-terminal domain of *E. coli* RNase E. Both structures are presented with the active site facing upwards.

with that of RNase J. In addition, to a similar overall shape (Figure 3D), the C-terminal domain of RNase J shares the same architecture with an equivalent domain of the catalytic part of RNase E (Figure 2) (19, 74). The significance of this observation, if any, remains to be analyzed.

New information on how RNase J functions, comes from two recent studies that show the RNase J structure in an open conformation: one describes the structure of *T. thermophilus* RNase J bound to a short 4-nucleotide RNA (47); the other that of *B. subtilis* RNase J1 in the apo-form including a model for the enzyme/RNA complex (48). In the open structures, the angle between the β-lactamase and β-CASP domains is widened to create a channel for the RNA. This channel is only wide enough for a single-stranded RNA, which explains the enzyme's substrate preference. Most of the amino acids contact the RNA through the sugar-phosphate backbone, as expected for an exonuclease. The catalytic mechanism is anticipated to be identical to that of other β-lactamases. For *T. thermophilus* RNase J, this would involve an aspartate residue (Asp79, Figure 3B) to act as a general base, taking

a proton from a nearby water molecule. The resulting hydroxyl ion then attacks the scissile phosphodiester bond, polarized by the Zn ions.

B. subtilis RNase J1 is a processive 5′ exonuclease, which implies that it must have at least two points of attachment for RNA, to not lose contact with the substrate during the translocation step. RNase J1 becomes distributive once the substrate is shorter than 5 nucleotides (47). The first binding site obviously is the 5′ phosphate-binding pocket while the second site of attachment is thought to be a path of positively charged residues at the surface of the β-CASP domain. In accordance with the biochemical data, the 5th residue would be the first to exit the catalytic channel and bind to the positively charged residues (47).

Euryarcheal RNase J is also a processive 5′ exonuclease (31), but its crenarcheal counterpart appears to be distributive (32).

Three active site residues, equivalent to Asp79, His80 and His376 in *T. thermophilus* RNase J (also present in *B. subtilis* RNase J1), all of which co-ordinate to one of the catalytic

Zn ions, are not conserved in *B. subtilis* RNase J2 (Glu78, Asn79 and Asn390). This suggests that RNase J2 cannot bind both Zn ions, probably causing a change in the geometry of the catalytic site. These differences might explain the weaker 5′ exonuclease activity of RNase J2 compared to that of RNase J1 (26, 48). Surprisingly, RNase J2 retains equivalent endonuclease activity to RNase J1 (17, 26).

Interestingly, the RNase J open conformation structures show that the RNA channel continues past the active site and the 5′ phosphate-binding pocket, providing clues as to how RNase J might function in endonucleolytic mode (47, 48) (Figure 3C). A bound single-stranded RNA could stretch across the entire catalytic cleft (about 8 to 9 nucleotides) and exit to the solvent on both sites, at least in the dimeric conformation. In this way, much longer RNAs would be acceptable for cleavage. It is noteworthy that these requirements are met by all known or suspected RNase J endonucleolytic cleavage sites (Figure 1B,C), that is, a single stranded region of 10–15 nucleotides generally located between two structured regions.

Modeling of extra nucleotides extending into this channel predicts that the contacts between the phosphate of the nucleotide at position -1 and the phosphate-binding pocket are the same in both endo- and exonucleolytic cleavage mode. This would explain why mutational inactivation of the phosphate-binding pocket abolishes both endo- and exonuclease activity. Nonetheless, while the RNA can position itself to lie across the catalytic site in the monomer, direct access is blocked by the symmetric subunit in the dimeric conformation. The enzyme must thus first dissociate into monomers to directly accommodate an RNA for endonucleolytic cleavage. Since the monomeric form of RNase J1 or J2 preparations rarely exceeds a few percent compared to the predominant dimeric form (19, 48), large quantities of enzyme might be necessary to provide enough monomers capable of internal endonucleolytic cleavage *in vitro*.

Alternatively, the RNA could be threaded through the entry channel as in 5′ exonucleolytic mode, but continue its progression past the active site until it reaches the internal site of cleavage. However, there is no clue from the available structures that could explain why the active site would stay inactive during the threading process. In addition, for a substrate like the *thrZ* leader (17), the enzyme would have to scan through 800 nucleotides of extensively structured RNA to reach the cleavage site. Similar constraints are encountered in the case of the *trp* leader (46). We thus believe that 5′ scanning is a very unlikely mode of action for most known RNase J cleavage sites that are far from the 5′-end. Clearly, more work is required to appreciate the extent of RNase J endonuclease activity and to understand its molecular mechanism.

Structural data for RNase Y are not available at present. This enzyme is the first member of a new class of ribonucleases that constitute a subgroup within the HD protein superfamily. They combine the metal-chelating HD domain (79) with the RNA-binding KH domain (80) (Figure 2C). The conserved histidine and aspartate residues within members of this family indicate that coordination of divalent cat-ions is essential for their activity (79). Mutations in the highly conserved HD motif strongly reduce the endonucleolytic activity of RNase Y, indicating that the catalytic activity resides within the HD domain (20). This also suggests that it is the impaired ribonucleolytic activity that causes previously observed defects of an RNase Y mutation (formerly *ymdA*) in cell and chromosome morphology (81).

mRNA turnover: endo- versus exonucleolytic activity

The degradation of an mRNA can theoretically be initiated by endonucleolytic cleavage in the body of the molecule, or by exonucleolytic attack of the extremities, 3′ and/or 5′. All known bacteria have 3′ exoribonucleases, but they are probably not used to degrade mRNA from the 3′-end on a large scale. This would be biologically inefficient and lead to the wasteful accumulation of incomplete polypeptides from truncated mRNAs. The 3′ end of bacterial mRNAs is usually protected from attack by 3′ exonucleases by secondary structure, generally an intrinsic transcription terminator. Nonetheless, in *E. coli*, a degradation route *via* polyadenylation followed by 3′ exonucleolytic digestion plays a role in maintaining the steady state levels of a significant number of transcripts and there is some evidence that endonucleolytic cleavage by RNase E might provide a signal for polyadenylation (82, 83). In *B. subtilis,* 3′ exonucleases appear to be involved primarily in the degradation of decay intermediates and a mutant devoid of all four known 3′ exonucleases (PNPase, RNase PH, RNase R and YhaM) is sick but viable (84).

Thus, in *E. coli* and *B. subtilis*, the only organisms for which we have enough data to allow a comparison, an overall 5′–3′ direction of decay seems to be the rule. The observed 5′–3′ directionality of decay in *E. coli* probably reflects the vectorial nature of transcription and translation, which both proceed in the 5′–3′ direction. Since the 5′ region is transcribed first, it should also be cleaved first, providing that susceptibility to RNase E is uniform. The principal mRNA decay pathway in *E. coli* following an initial cleavage by RNase E, is briefly outlined in Figure 4A.

In *B. subtilis*, which has a very different set of RNases, the recent discovery of novel ribonucleases requires a significant re-evaluation of RNA decay models. The often dramatic effect of 5′ stabilizers in this organism provided a strong argument in favor of the biological importance of the 5′ exonucleolytic activity of RNase J over its RNase E-like endonucleolytic acitvity. However, the novel RNase Y has been shown to play an even more important role in global mRNA turnover and stability and can, in many aspects, be considered a better functional homolog of RNase E, than RNase J (20). Thus, it has become clear that endonucleolytic cleavage has a major function in mRNA decay and gene regulation in *B. subtilis*. In fact, all ten functional SAM riboswitches in *B. subtilis* are substrates for RNase Y and the enzyme can also cleave a *thrS* leader transcript upstream of the leader terminator *in vitro* (Figure 1B). There are clear
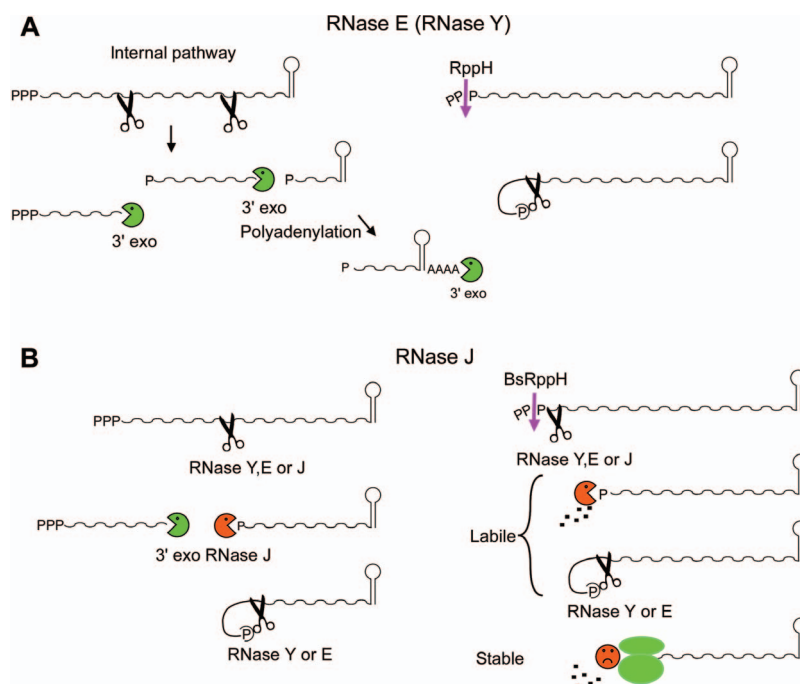
Figure 4 Bacterial mRNA degradation pathways.
(A) RNA decay in organisms that contain the endonuclease RNase E or a homolog and/or RNase Y, a functional analog of RNase E. In *E. coli*, one or several internal cleavages by RNase E generate RNA fragments, which are susceptible to attack by 3′ exonucleases (PNPase, RNase II, RNase R, oligoribonuclease). Fragments protected by 3′ secondary structure (e.g., a transcription terminator) against exonuclease digestion are polyadenylated by polyA polymerase. This enables the 3′ exonucleases to re-engage; reiteration of this process provides many opportunities to eventually succeed complete degradation. A second 5′ end dependent pathway involves pyrophosphate removal by RppH, which generates a 5′ terminal monophosphate that is recognized by a dedicated binding pocket of RNase E. Tethering of RNase E to the 5′ end facilitates mRNA cleavage at a downstream site. This mechanism can also be used on degradation intermediates carrying a 5′P group. (B) mRNA decay in bacteria that contain the 5′ exonuclease RNase J. Internal cleavage by an endonuclease (e.g., RNase E, RNase Y or RNase J in endonucleolytic mode) creates a 5′ monophosphorylated fragment that is susceptible to 5′ exonuclease digestion by RNase J. If present, RNase E or Y can also be tethered to this 5′P and induce new downstream cleavages. The upstream fragment, carrying a 5′PPP resistant to 5′ exonuclease digestion, is degraded by 3′ exonucleases. As an alternative, notably on mRNAs that are not susceptible to internal cleavage and have an accessible 5′ end, an RppH analog or endonuclease cleavage very close to the 5′ end can trigger 5′ exonuclease attack from the 5′ end. This pathway is notably compatible with the observation that, e.g., a 5′ proximal stalled ribosome can stabilize the entire downstream RNA. Organisms having an RNase J do a priori not require a polyadenylation assisted decay pathway as the 5′ exonuclease activity can efficiently degrade 3′ structured RNA fragments.

indications that RNase Y can also cleave in the body of mRNAs, e.g., the *cggR* transcript in the *gapA* operon (21) and the *rpsO* mRNA (49). RNase Y cleavage within the leader sequence of the *infC-rpmI-rplT* operon is responsible for uncoupling the expression of translation factor IF3 from that of the ribosomal proteins L35 and L20 (85). A preliminary evaluation of a tiling array of an RNase Y depleted strain actually reveals more than 1200 potential substrates, including many transcriptional attenuators like the T-box regulated genes and confirms a potential role of RNase Y in the cleavage of all the substrates cited above (our unpublished results).

Even though RNase Y activity is stimulated by a 5′ monophosphorylated substrate *in vitro*, there appears to be no strict requirement for a 5′ tethering mechanism. *In vivo*, the upstream fragment generated by RNase Y cleavage of the SAM riboswitch RNA accumulates to very high levels in the absence of 3′ exonucleases, indicating that no significant conversion of the original 5′ PPP end to 5′ P or 5′ OH occurs,

that would allow RNase J to degrade the native transcript exonucleolytically from the 5′ end (20).

The emerging data thus clearly point to the possibility that mRNA processing and degradation are more similar between *B. subtilis* and *E. coli* than presently assumed, with an endonucleolytic cleavage being the crucial step in initiating mRNA decay in both organisms.

So, how can we reconcile the impressive examples of protection at a distance with a globally acting endoribonuclease that apparently does not even need a 5′-P to work efficiently? First, we might consider that protection at a distance has only been shown for a handful of mRNAs that, by chance, may lack RNase Y cleavage sites. Indeed, the *sacA, epr, sacB* and *penP* mRNAs that were more stable *in vivo* when fused to a 5′ stabilizer (the *ermC* ribosome stall sequence) (86), are not significantly stabilized in a tiling array analysis of an RNase Y depleted strain (unpublished results). Thus, protection at a distance will maybe prove less impressive on a genomic scale. RNase Y cleavage sites, characterized by an

AU rich sequence flanked by a rather stable secondary structure, appear to occur quite frequently within 5′ untranslated leader sequences containing transcriptional attenuators. Similar configurations are also encountered in coding regions, e.g., the *cggR* mRNA where cleavage, most likely by RNase Y, occurs in an AU rich sequence between two well-defined secondary structures, of which the downstream hairpin is required *in vivo* for cleavage to occur (2) (Figure 1). We clearly need more data on how RNase Y (and RNase E) recognizes complex substrates.

While RNase Y is the enzyme that has the most important effect on global mRNA stability in *B. subtilis,* there are many mRNAs, whose half-lives appear to be dependent largely on RNase J1/J2 (25). For several mRNAs studied (*ermC, rpsO, cryIIIA, hbs*), the generation of shorter, often more stable mRNA transcripts is thought to be due to RNase J1 exonuclease activity, which trims a longer precursor before it is partially halted, generally by a bound or stalled ribosome (18, 87–90). In some cases (*ermC, rpsO*), it has been suggested that RNase J1 might act as an endonuclease, but the corresponding upstream RNA fragments have never been detected (91).

Since RNase J1 does not work well in exonuclease mode on native 5′ triphosphorylated transcripts or RNAs protected by a structure at the 5′ end, the important question that subsists is what creates the entry site. It is possible that the initiating step is still a cleavage by RNase Y, which occurs, for example, in the 5′ proximal region of a transcript. Following depletion of RNase J1, the downstream portion of the processed transcript is not further degraded exonucleolytically from the 5′ end, and its steady state level is increased. A good example for such a scenario is the *tagD* mRNA. Its half-life is strongly increased (from not measurable to 2.5 min) in an *rnjA/rnjB* double mutant (RNase J1 was depleted) compared to the wild type strain. Under these conditions, the *tagD* mRNA accumulates six-fold but, interestingly, as a shorter processed transcript (25). Since the *tagD* mRNA also accumulates strongly in the tiling array of an RNase Y depleted strain, it is likely that RNase Y initiates mRNA degradation in this case.

By contrast, the *yweA* mRNA steady state level is not affected in the RNase Y depletion tiling array, but the full-length transcript increases 13-fold in the RNase J1/J2 mutant (25). The *yweA* transcript is thus likely a good example to study the role of RNases J1 and J2 in the initiation of mRNA decay that should occur at or very close to the 5′ end. In this case, the entry site for the RNase J1 5′ exonuclease activity could be provided by the recently identified equivalent to the *E. coli* RNA phosphohydrolase RppH (66) which removes the 5′ tri-phosphate group (Figure 4B) or by endonucleolytic cleavage, for example, by RNase J itself, close to the 5′ end.

We envisage that RNase J 5′ exonuclease activity may have evolved rather in organisms like *B. subtilis* that, in contrast to *E. coli,* do not have an efficient polyadenylation pathway to degrade structured RNA (e.g., transcription terminators) exonucleolytically from the 3′ end. These RNAs can thus be efficiently degraded from the 5′ side by RNase J.

Another parameter that probably deserves more attention, if we want to fully understand mRNA decay pathways, concerns subcellular localization of the major macro-molecular machineries in the cell (see below).

Multiprotein complexes and cellular localization

Available evidence indicates that, in *E. coli,* most if not all of the RNase E in the cell is part of the degradosome (92, 93). The carboxy-terminal half (CTH) of RNase E provides the scaffold for this large multienzyme complex that also contains the 3′ exonuclease PNPase, the ATP-dependent helicase RhlB and the glycolytic enzyme enolase (94, 95) (Figure 2). The CTH of RNase E is not essential for catalytic activity and its removal has only a moderate effect on global mRNA decay (96, 97). However, the CTH is important for the rapid degradation of many untranslated mRNA (98) and a microarray study shows that the assembled degradosome regulates the abundance of certain metabolic pathways in *E. coli* (99). The composition of the degradosome can be modified depending on conditions of growth or stress (100–103). Functional and structural aspects of the *E. coli* degradosome have been extensively reviewed elsewhere (104).

Variations of RNase E based degradosomes exist in other bacteria. The psychrotrophic bacterium *Pseudomonas syringae* contains RNase R (105), a 3′ exonuclease which is also found in *E. coli,* where its expression is induced by cold and stress (106). *Streptomyces* has an RNase E where the catalytic domain is flanked by two scaffold domains which interact with PNPase (107). The *Rhodobacter capsulatus* degradosome contains the transcription factor Rho and two DEAD-box helicases (108).

A degradosome complex assembling glycolytic enzymes, together with RNases, has also been proposed to exist in *B. subtilis,* with RNase Y being the coordinating partner. The components identified by *in vivo* cross-linking and bacterial two-hybrid screening were enolase, phosphofructokinase, the RNA helicase CshA, PNPase and RNases J1/J2 (21, 109). The observation that key nucleases, other than RNase E, can also form a degradosome, underlines the general importance of protein complexes for RNA maturation and decay. However, in contrast to the *E. coli* degradosome, it has not yet been possible to isolate the proposed *B. subtilis* degradosome without cross-linking, for example, by a pull-down experiment. The functional importance of such a complex remains to be analyzed.

An interesting feature shared between *E. coli* RNase E and *B. subtilis* RNase Y is their localization at the membrane. RNase E and other degradosome components are associated with the inner surface of the cell (110), interact with the bacterial cytoskeleton (111, 112) and the inner membrane (113). RNase Y has a transmembrane domain at its N-terminus and has been localized at the membrane *in vivo* (81). This sublocalization might have important consequences for the fate of an mRNA. In *B. subtilis,* ribosomes are preferentially localized at the cell periphery and poles, and RNA polymerase in the nucleoid, indicating that transcription and translation occur predominantly in separate functional domains (114). By contrast, in *Caulobacter crescentus* and

E. coli, chromosomally expressed mRNAs remain close to their site of transcription during their lifetime. In addition, in *C. crescentus,* RNase E co-locates significantly with DNA. Thus, bacteria can spatially organize translation and, potentially, mRNA decay by using the chromosome layout as a template (115). Moreover, certain mRNAs in *E. coli* can be targeted to the future destination of their encoded proteins, cytoplasm, poles, or inner membrane in a translation-independent manner (116).

Based on these emerging data, a comparison of mRNA decay strategies between *B. subtilis* and *E. coli,* based exclusively on the biochemical characteristics of the major nucleases, might not give the full picture. Maybe the access of the relevant ribonucleases to mRNA being transcribed and/or translated is variable due to a differential spatial organization of these processes in different bacterial species.

Occurrence

For the first time now, we can conclude that all prokaryotic *phyla* whose genomes have been sequenced contain at least one of the three RNases (E/G, J and Y) discussed here. The results of a BLAST analysis, using the NCBI server, are shown in Table 1. There is no recognizable pattern that suggests a prevalence of certain combinations of enzymes occurring in a single organism, or hint at an incompatibility of enzymes. Many species apparently survive with a single

Table 1   Occurrence of RNases E, J and Y in procaryotes.

	RNase		
	E/G	J	Y
Archaea			
Crenarchaeota	NO	26/26	NO
Euryarchaeota	NO	63/63	NO
Nanoarchaeota	NO	1/1	NO
Bacteria			
Actinobacteria	171/182	182/182	38/182
Bacteroidetes	146/147	1/147	145/147
Chlamydiae	11-Nov	NO	3/11
Cyanobacteria	48/49	48/49	NO
Firmicutes			
Bacillales	37/77	76/77	76/77
Clostridia	98/153	152/153	153/153
Lactobacillales	NO	90/90	89/90
Others	16/37	37/37	37/37
Mollicutes	NO	30/30	16/30
Others	82/126	71/126	114/126
Proteobacteria			
Alpha	185/192	189/192	NO
Beta	115/115	NO	NO
Delta	48/48	39/48	41/48
Epsilon	NO	38/38	38/38
Gamma	298/304	21/304	NO
Spirochatales	NO	3/32	29/32

The number of bacterial species containing a given RNase with respect to the total number of organisms within a *phylum* are given in the boxes. White boxes indicate that none or <10% of the organisms carry the respective RNase, this value is all or >90% for the black boxes and intermediate for the grey boxes.

member. This is the case for the *archea,* which only contain RNase J orthologs, the β- and γ-proteobacteria, which have almost exclusively RNase E/G type enzymes, and the *Spirochaetales,* which rely essentially on RNase Y.

All possible combinations of the three RNases are found in the remaining eubacterial species (Table 1). Interestingly, several *phyla* have all three RNase types. This is notably the case for many *Bacilli, Clostridia* and the δ-proteobacteria. It also raises interesting questions as to the partitioning of functions between the three nucleases.

There are two organisms, *Leptolyngbya* and *Pasteuria,* for which we found no orthologue for RNases E/G, J or Y. Nevertheless, we suspect that this absence rather reflects annotation problems or sequence errors, as other organisms, e.g., *Sulcia muelleri,* previously thought to not contain any of the three RNases, now clearly give positive hits.

RNases E/G, J and Y are typically prokaryotic proteins with potentially similar functions in the species where they are present. Nevertheless, RNase J belongs to the same family of enzymes as the eukaryotic cleavage and polyadenylation factor CPSF-73 which also has both activities, 5′ exo- and endonucleolytic (117).

Moreover, RNase E/G type enzymes appear to exist in several eukaryotic species, like *Strongyloides ratti, Bombus impatiens, Xenopus tropicalis, Onchocerca, Aedes* and *Ixodes*. Potential RNase J orthologs occur in *Caenorhabditis, Lama pacos, Ixodes, Phytophthora* and *Rhodnius prolixus.* The three *arthropoda Anopheles darlingi*, *Drosophila willistoni* and *Lepeophtheirus salmoni* have an RNase Y like enzyme. The latter actually has potential orthologs for all three RNases.

Expert opinion

The study of *E. coli* and *B. subtilis* has been very informative in terms of understanding the strategies available in prokaryotes to regulate mRNA processing and decay. The large evolutionary distance between the two model organisms is reflected in the very diverse repertoire of ribonucleases at their disposal. This diversity has several advantages for the molecular biologist: we can study enzymes that are completely unrelated, highlighting different pathways of mRNA decay that are possible and thinkable. Indeed, taken together, the ribonucleases present in *E. coli* and *B. subtilis* represent practically all known enzymes of this type in eubacteria and *archea*. The three global players that we discussed are present in all prokaryotes, alone or in various combinations. It is not unlikely, that we actually now have a quite complete picture of the major prokaryotic ribonucleases.

The presence of a given set of nucleases is probably also related to other global phenomena in a given species. For example, in contrast to *E. coli, B. subtilis* regulates a significant proportion of global gene expression *via* transcriptional attenuators. These small and stable RNAs need to be efficiently disposed of, in order to avoid, for example, deleterious titration of regulatory factors. RNase Y seems to be very good at initiating the turnover of these non-coding

RNAs. The RNase E-like activity of RNase Y indicates that endonucleolytic cleavage plays an important role in mRNA degradation in *B. subtilis*. This changes our perception of mRNA metabolism in this organism, which was centered on the 5′ exonucleolytic pathway. The 5′ exonuclease activity of RNase J is very useful to get rid of RNAs with structured 3′ ends (i.e., transcription terminators), especially in bacteria, like *B. subtilis,* that have no polyadenylation assisted pathway to degrade them exonucleolytically from the 3′ end. It is also noteworthy that, in contrast to *B. subtilis*, many *Bacilli* have an RNase E ortholog in addition to RNase Y and RNase J. This illustrates that there exists no dogmatic barrier or incompatibility between these globally acting enzymes.

However, we need more data on how RNases E, J and Y act on natural substrates, including X-ray structures of the enzyme complexed with structured RNAs. There is a lack of understanding of what enables RNase J to exonucleolytically degrade a native mRNA from the 5′ end, in cases where decay is not initiated by RNase Y or another endonuclease. Is there an absolute requirement for a pyrophosphatase, or can RNase J, under certain conditions, attack a 5′PPP end on its own? The potential of RNase Y to form degradosome complexes requires more investigation, notably to identify which interactions are relevant and how such a complex adds value to the degradation machinery. We believe it is worthwhile to study model organisms like *E. coli* and *B. subtilis* for some time to come. As we have seen, there are still fundamental aspects related to mRNA decay and gene regulation that need to be understood. Given the widespread presence of RNases E, J and Y in prokaryotes, many of the results obtained can probably be extrapolated to a large number of species.

A major new field that has probably been neglected up to now, concerns the notion that transcription, translation and mRNA decay may be compartmentalized in prokaryotes to an extent that we did not anticipate. The small size of a bacterial cell is a special challenge to obtain meaningful results, but live observation of the major macromolecular machineries will add a completely new dimension, also for the mRNA degradation field, for many years to come.

Outlook

At present, most if not all major ribonucleases in prokaryotes have probably been identified. In a few years, we should have a detailed understanding of all of these enzymes at the atomic level. This requires, particularly, information about their recognition of complex substrates and their interaction with other partners. From the study of individual genes, we should get a good picture of how the different nucleases participate in controlling gene expression. Global approaches, like tiling arrays, will have identified many potential candidates that are worth looking at in detail. The model organisms *E. coli* and *B. subtilis* will continue to provide valuable insights into RNA metabolism for many years to come. However, the study of other bacteria will become increasingly important and contribute to our understanding

of the presumably important role of mRNA processing/degradation in pathogenicity. We suspect that a number of new proteins will be identified that can modulate and adapt the activity of the RNA degradation machinery to specific environmental conditions. We also anticipate that spatial organization of gene expression in bacteria will become an important field of investigation, and this will be aided by new technological advances in microscopy. This aspect is probably crucial to fully comprehend the role and impact of RNA processing/degradation on gene expression and its relation to transcription and translation.

Highlights

- mRNA decay is a crucial parameter for controlling prokaryotic gene expression.
- Endoribonuclease E is the major enzyme initiating mRNA degradation in *E. coli*. It can cleave its substrate, either when tethered to a monophosphorylated RNA 5′ end, or by internal entry.
- RNase J is an endo- and 5′ exonuclease. The latter activity is not functional on a native 5′PPP transcript, but requires a 5′P or 5′OH extremity, potentially provided by endonucleolytic cleavage.
- In *B. subtilis,* the often-observed strong 5′-3′ directionality of mRNA degradation is attributed to the presumed dominant role of the 5′ exonuclease activity of RNase J.
- The 5′ exonuclease activity of RNase J is required to degrade 3′ structured RNAs that, in the absence of an efficient polyadenylation pathway in *B. subtilis*, are resistant to 3′ exonuclease attack.
- The endonuclease RNase Y, a functional analog of RNase E in *B. subtilis,* affects global mRNA stability and demonstrates the important role for endonucleolytic cleavage in mRNA decay in *B. subtilis.*
- All prokaryotic *phyla* contain at least one of the three RNases (E/G, J and Y). They can occur in all possible combinations and some organisms contain all three enzymes.
- RNase Y can probably coordinate formation of a multiprotein complex, similar to RNase E and the degradosome in *E. coli*.
- In eubacteria, endonucleolytic cleavage might be globally more important than 5′ exonuclease activity for initiating mRNA degradation, but the relative contributions remain to be established.
- Spatial organization of transcription, translation and mRNA degradation/processing could play an important role in how RNA decay affects gene expression.

Acknowledgments

References

1. Burton ZF, Gross CA, Watanabe KK, Burgess RR. The operon that encodes the sigma subunit of RNA polymerase also encodes ribosomal protein S21 and DNA primase in E. coli K12. Cell 1983; 32: 335–49.

2. Meinken C, Blencke HM, Ludwig H, Stulke J. Expression of the glycolytic gapA operon in Bacillus subtilis: differential syntheses of proteins encoded by the operon. Microbiology 2003; 149: 751–61.

3. Apirion D, Lassar AB. A conditional lethal mutant of Escherichia coli which affects processing of ribosomal RNA. J Biol Chem 1978; 253: 1738–42.

4. Babitzke P, Kushner SR. The Ams (altered mRNA stability) protein and ribonuclease E are encoded by the same structural gene of Escherichia coli. Proc Natl Acad Sci USA 1991; 88: 1–5.

5. Melefors O, von Gabain A. Genetic studies of cleavage-initiated mRNA decay and processing of ribosomal 9S RNA show that the Escherichia coli ams and rne loci are the same. Mol Microbiol 1991; 5: 857–64.

6. Mudd EA, Carpousis AJ, Krisch HM. Escherichia coli RNAse E has a role in the decay of bacteriophage T4 mRNA. Genes Dev 1990; 4: 873–81.

7. Ono M, Kuwano M. A conditional lethal mutation in an E. coli strain with a longer chemical lifetime of messenger RNA. J Mol Biol 1979; 129: 343–57.

8. Taraseviciene L, Miczak A, Apirion D. The gene specifying RNase E (rne) and a gene affecting mRNA stability (ams) are the same gene. Mol Microbiol 1991; 5: 851–5.

9. Donovan WP, Kushner SR. Polynucleotide phosphorylase and ribonuclease II are required for cell viability and mRNA turnover in Escherichia coli K-12. Proc Natl Acad Sci USA 1986; 83: 120–4.

10. Ghosh S, Deutscher MP. Oligoribonuclease is an essential component of the mRNA decay pathway. Proc Natl Acad Sci USA 1999; 96: 4372–7.

11. Cheng ZF, Deutscher MP. An important role for RNase R in mRNA decay. Mol Cell 2005; 17: 313–8.

12. Xu F, Cohen SN. RNA degradation in Escherichia coli regulated by 3′ adenylation and 5′ phosphorylation. Nature 1995; 374: 180–3.

13. Hajnsdorf E, Braun F, Haugel-Nielsen J, Regnier P. Polyadenylylation destabilizes the rpsO mRNA of Escherichia coli. Proc Natl Acad Sci USA 1995; 92: 3973–7.

14. Haugel-Nielsen J, Hajnsdorf E, Regnier P. The rpsO mRNA of Escherichia coli is polyadenylated at multiple sites resulting from endonucleolytic processing and exonucleolytic degradation. EMBO J 1996; 15: 3144–52.

15. Andrade JM, Hajnsdorf E, Regnier P, Arraiano CM. The poly(A)-dependent degradation pathway of rpsO mRNA is primarily mediated by RNase R. RNA 2009; 15: 316–26.

16. Condon C, Putzer H. The phylogenetic distribution of bacterial ribonucleases. Nucleic Acids Res 2002; 30: 5339–46.

17. Even S, Pellegrini O, Zig L, Labas V, Vinh J, Brechemmier-Baey D, Putzer H. Ribonucleases J1 and J2: two novel endoribonucleases in B. subtilis with functional homology to E. coli RNase E. Nucleic Acids Res 2005; 33: 2141–52.

18. Mathy N, Benard L, Pellegrini O, Daou R, Wen T, Condon C. 5′-to-3′ Exoribonuclease activity in bacteria: role of RNase J1 in rRNA maturation and 5′ stability of mRNA. Cell 2007; 129: 681–92.

19. Li de la Sierra-Gallay I, Zig L, Jamalli A, Putzer H. Structural insights into the dual activity of RNase J. Nat Struct Mol Biol 2008; 15: 206–12.

20. Shahbabian K, Jamalli A, Zig L, Putzer H. RNase Y. A novel endoribonuclease, initiates riboswitch turnover in Bacillus subtilis. EMBO J 2009; 28: 3523–33.

21. Commichau FM, Rothe FM, Herzberg C, Wagner E, Hellwig D, Lehnik-Habrink M, Hammer E, Völker U, Stülke J. Novel activities of glycolytic enzymes in Bacillus subtilis: interactions with essential proteins involved in mRNA processing. Mol Cell Proteomics 2009; 8: 1350–60.

22. Carpousis AJ, Luisi BF, McDowall KJ. Endonucleolytic initiation of mRNA decay in Escherichia coli. Prog Mol Biol Transl Sci 2009; 85: 91–135.

23. Lee K, Bernstein JA, Cohen SN. RNase G complementation of rne null mutation identifies functional interrelationships with RNase E in Escherichia coli. Mol Microbiol 2002; 43: 1445–56.

24. Stead MB, Marshburn S, Mohanty BK, Mitra J, Castillo LP, Ray D, van Bakel H, Hughes TR, Kushner SR. Analysis of Escherichia coli RNase E and RNase III activity in vivo using tiling microarrays. Nucleic Acids Res 2011; 39: 3188–203.

25. Mäder U, Zig L, Kretschmer J, Homuth G, Putzer H. mRNA processing by RNases J1 and J2 affects Bacillus subtilis gene expression on a global scale. Mol Microbiol 2008; 70: 183–96.

26. Mathy N, Hébert A, Mervelet P, Bénard L, Dorléans A, Li de la Sierra-Gallay I, Noirot P, Putzer H, Condon C. Bacillus subtilis ribonucleases J1 and J2 form a complex with altered enzyme behaviour. Mol Microbiol 2010; 75: 489–98.

27. Sousa S, Marchand I, Dreyfus M. Autoregulation allows Escherichia coli RNase E to adjust continuously its synthesis to that of its substrates. Mol Microbiol 2001; 42: 867–78.

28. Jain C, Deana A, Belasco JG. Consequences of RNase E scarcity in Escherichia coli. Mol Microbiol 2002; 43: 1053–64.

29. Ow MC, Liu Q, Mohanty BK, Andrew ME, Maples VF, Kushner SR. RNase E levels in Escherichia coli are controlled by a complex regulatory system that involves transcription of the rne gene from three promoters. Mol Microbiol 2002; 43: 159–71.

30. Bugrysheva JV, Scott JR. The ribonucleases J1 and J2 are essential for growth and have independent roles in mRNA decay in Streptococcus pyogenes. Mol Microbiol 2010; 75: 731–43.

31. Clouet-d'Orval B, Rinaldi D, Quentin Y, Carpousis AJ. Euryarchaeal β-CASP proteins with homology to bacterial RNase J Have 5′- to 3′-exoribonuclease activity. J Biol Chem 2010; 285: 17574–83.

32. Hasenohrl D, Konrat R, Blasi U. Identification of an RNase J ortholog in Sulfolobus solfataricus: implications for 5′-to-3′ directional decay and 5′-end protection of mRNA in Crenarchaeota. RNA 2011; 17: 99–107.

33. Hasenohrl D, Lombo T, Kaberdin V, Londei P, Blasi U. Translation initiation factor a/eIF2(-gamma) counteracts 5′ to 3′ mRNA decay in the archaeon Sulfolobus solfataricus. Proc Natl Acad Sci USA 2008; 105: 2146–50.

34. She Q, Singh RK, Confalonieri F, Zivanovic Y, Allard G, Awayez MJ, Chan-Weiher CC-Y, Clausen IBG, Curtis BA, De Moors A, Erauso G, Fletcher C, Gordon PMK, Heikamp-de Jong I, Jeffries AC, Kozera CJ, Medina N, Peng X, Thi-Ngoc HP, Redder P, Schenk ME, Theriault C, Tolstrup N, Charlebois RL, Doolittle WF, Duguet M, Gaasterland T, Garrett RA, Ragan MA, Sensen CW, Van der Oost J. The complete genome of the crenarchaeon Sulfolobus solfataricus P2. Proc Natl Acad Sci USA 2001; 98: 7835–40.

35. Nagata M, Kaito C, Sekimizu K. Phosphodiesterase activity of CvfA is required for virulence in Staphylococcus aureus. J Biol Chem 2008; 283: 2176–84.

36. Kang SO, Caparon MG, Cho KH. Virulence gene regulation by CvfA, a putative RNase: the CvfA-enolase complex in Streptococcus pyogenes links nutritional stress, growth-phase control, and virulence gene expression. Infect Immun 2010; 78: 2754–67.

37. Lin-Chao S, Wong TT, McDowall KJ, Cohen SN. Effects of nucleotide sequence on the specificity of rne-dependent and RNase E-mediated cleavages of RNA I encoded by the pBR322 plasmid. J Biol Chem 1994; 269: 10797–803.

38. McDowall KJ, Lin-Chao S, Cohen SN. A+U content rather than a particular nucleotide order determines the specificity of RNase E cleavage. J Biol Chem 1994; 269: 10790–6.

39. Ehretsmann C, Carpousis AJ, Krisch HM. Specificity of Escherichia coli endoribonuclease RNAse E: in vivo and in vitro analysis of mutants in a bacteriophage T4 mRNA processing site. Genes Dev 1992; 6: 149–59.

40. Redko Y, Tock MR, Adams CJ, Kaberdin VR, Grasby JA, McDowall KJ. Determination of the catalytic parameters of the N-terminal half of Escherichia coli ribonuclease E and the identification of critical functional groups in RNA substrates. J Biol Chem 2003; 278: 44001–8.

41. Kaberdin VR. Probing the substrate specificity of Escherichia coli RNase E using a novel oligonucleotide-based assay. Nucleic Acids Res 2003; 31: 4710–6.

42. Condon C, Putzer H, Luo D, Grunberg-Manago M. Processing of the Bacillus subtilis thrS leader mRNA is RNase E-dependent in Escherichia coli. J Mol Biol 1997; 268: 235–42.

43. Yao S, Blaustein JB, Bechhofer DH. Processing of Bacillus subtilis small cytoplasmic RNA: evidence for an additional endonuclease cleavage site. Nucleic Acids Res 2007; 35: 4464–73.

44. Deikus G, Babitzke P, Bechhofer DH. Recycling of a regulatory protein by degradation of the RNA to which it binds. Proc Natl Acad Sci USA 2004; 101: 2747–51.

45. Daou-Chabo R, Condon C. RNase J1 endonuclease activity as a probe of RNA secondary structure. RNA 2009; 15: 1417–25.

46. Deikus G, Condon C, Bechhofer DH. Role of Bacillus subtilis RNase J1 endonuclease and 5′-exonuclease activities in trp leader RNA turnover. J Biol Chem 2008; 283: 17158–67.

47. Dorléans A, Li de la Sierra Gallay I, Piton J, Zig L, Winieski L, Putzer H, Condon C. Molecular basis for the recognition and cleavage of RNA by the bifunctional 5′-3′ exo/endoribonuclease RNase J. Structure 2011; 19: 1252–61.

48. Newman JA, Hewitt L, Rodrigues C, Solovyova A, Harwood CR, Lewis RJ. Unusual, dual endo- and exo-nuclease activity in the degradosome explained by crystal structure analysis of RNase J1. Structure 2011; 19: 1241–51.

49. Yao S, Bechhofer DH. Initiation of decay of Bacillus subtilis rpsO mRNA by endoribonuclease RNase Y. J Bacteriol 2010; 192: 3279–86.

50. Baker KE, Mackie GA. Ectopic RNase E sites promote bypass of 5′-end-dependent mRNA decay in Escherichia coli. Mol Microbiol 2003; 47: 75–88.

51. Hankins JS, Zappavigna C, Prud'homme-Genereux A, Mackie GA. Role of RNA structure and susceptibility to RNase E in regulation of a cold shock mRNA, cspA mRNA. J Bacteriol 2007; 189: 4353–8.

52. Zeller ME, Csanadi A, Miczak A, Rose T, Bizebard T, Kaberdin VR. Quaternary structure and biochemical properties of mycobacterial RNase E/G. Biochem J 2007; 403: 207–15.

53. Kaberdin VR, Bizebard T. Characterization of Aquifex aeolicus RNase E/G. Biochem Biophys Res Commun 2005; 327: 382–92.

54. Tock MR, Walsh AP, Carroll G, McDowall KJ. The CafA protein required for the 5′-maturation of 16 S rRNA is a 5′-end-dependent ribonuclease that has context-dependent broad sequence specificity. J Biol Chem 2000; 275: 8726–32.

55. Jiang X, Belasco JG. Catalytic activation of multimeric RNase E and RNase G by 5′-monophosphorylated RNA. Proc Natl Acad Sci USA 2004; 101: 9211–6.

56. Belasco JG, Nilsson G, von Gabain A, Cohen SN. The stability of E. coli gene transcripts is dependent on determinants localized to specific mRNA segments. Cell 1986; 46: 245–51.

57. Emory SA, Bouvet P, Belasco JG. A 5′-terminal stem-loop structure can stabilize mRNA in Escherichia coli. Genes Dev 1992; 6: 135–48.

58. Mackie GA. Ribonuclease E is a 5′-end-dependent endonuclease. Nature 1998; 395: 720–3.

59. Walsh AP, Tock MR, Mallen MH, Kaberdin VR, Gabain Av, McDowall KJ. Cleavage of poly(A) tails on the 3′-end of RNA by ribonuclease E of Escherichia coli. Nucleic Acids Res 2001; 29: 1864–71.

60. Feng Y, Vickers TA, Cohen SN. The catalytic domain of RNase E shows inherent 3′ to 5′ directionality in cleavage site selection. Proc Natl Acad Sci USA 2002; 99: 14746–51.

61. Mackie GA. Stabilization of circular rpsT mRNA demonstrates the 5′-end dependence of RNase E action in vivo. J Biol Chem 2000; 275: 25069–72.

62. Celesnik H, Deana A, Belasco JG. Initiation of RNA decay in Escherichia coli by 5′ pyrophosphate removal. Mol Cell 2007; 27: 79–90.

63. Deana A, Celesnik H, Belasco JG. The bacterial enzyme RppH triggers messenger RNA degradation by 5′ pyrophosphate removal. Nature 2008; 451: 355–8.

64. Kime L, Jourdan SS, Stead JA, Hidalgo-Sastre A, McDowall KJ. Rapid cleavage of RNA by RNase E in the absence of 5′ monophosphate stimulation. Mol Microbiol 2010; 76: 590–604.

65. Marchand I, Nicholson AW, Dreyfus M. Bacteriophage T7 protein kinase phosphorylates RNase E and stabilizes mRNAs synthesized by T7 RNA polymerase. Mol Microbiol 2001; 42: 767–76.

66. Richards J, Liu Q, Pellegrini O, Celesnik H, Yao S, Bechhofer DH, Condon C, Belasco JG. An RNA pyrophosphohydrolase triggers 5′-exonucleolytic degradation of mRNA in Bacillus subtilis. Mol Cell 2011; 43: DOI 10.1016/j.molcel.2011.07.023.

67. Agaisse H, Lereclus D. STAB−SD: a Shine-Dalgarno sequence in the 5′ untranslated region is a determinant of mRNA stability. Mol Microbiol 1996; 20: 633–43.

68. Bechhofer DH, Dubnau D. Induced mRNA stability in Bacillus subtilis. Proc Natl Acad Sci USA 1987; 84: 498–502.

69. Hambraeus G, Karhumaa K, Rutberg B. A 5′ stem-loop and ribosome binding site but not translation are important for the stability of Bacillus subtilis aprE leader mRNA. Microbiology 2002; 148: 1795–1803.

70. Hue KK, Cohen SD, Bechhofer DH. A polypurine sequence that acts as a 5′ mRNA stabilizer in Bacillus subtilis. J Bacteriol 1995; 177: 3465–71.

71. Sandler P, Weisblum B. Erythromycin-induced ribosome stall in the ermA leader: a barricade to 5′-to-3′ nucleolytic cleavage of the ermA transcript. J Bacteriol 1989; 171: 6680–8.

72. Sharp JS, Bechhofer DH Effect of translational signals on mRNA decay in Bacillus subtilis. J Bacteriol 2003; 185: 5372–9.

73. Dreyfus M. Killer and protective ribosomes. Prog Mol Biol Transl Sci 2009; 85: 423–66.

74. Callaghan AJ, Marcaida MJ, Stead JA, McDowall KJ, Scott WG, Luisi BF. Structure of Escherichia coli RNase E catalytic domain and implications for RNA turnover. Nature 2005; 437: 1187–91.

75. Koslover DJ, Callaghan AJ, Marcaida MJ, Garman EF, Martick M, Scott WG, Luisi BF. The crystal structure of the Escherichia coli RNase E apoprotein and a mechanism for RNA degradation. Structure 2008; 16: 1238–44.

76. Jourdan SS, McDowall KJ. Sensing of 5′ monophosphate by Escherichia coli RNase G can significantly enhance association with RNA and stimulate the decay of functional mRNA transcripts in vivo. Mol Microbiol 2008; 67: 102–15.

77. Callebaut I, Moshous D, Mornon JP, de Villartay JP. Metallo-β-lactamase fold within nucleic acids processing enzymes: the β-CASP family. Nucleic Acids Res 2002; 30: 3592–601.

78. Collins JA, Irnov I, Baker S, Winkler WC. Mechanism of mRNA destabilization by the glmS ribozyme. Genes Dev 2007; 21: 3356–68.

79. Aravind L, Koonin EV. The HD domain defines a new super-family of metal-dependent phosphohydrolases. Trends Biochem Sci 1998; 23: 469–72.

80. Grishin NV. KH domain: one motif, two-folds. Nucleic Acids Res 2001; 29: 638–43.

81. Hunt A, Rawlins JP, Thomaides HB, Errington J. Functional analysis of 11 putative essential genes in Bacillus subtilis. Microbiology 2006; 152: 2895–907.

82. Mohanty BK, Kushner SR. The majority of Escherichia coli mRNAs undergo post-transcriptional modification in exponentially growing cells. Nucleic Acids Res 2006; 34: 5695–704.

83. Regnier P, Hajnsdorf E. Poly(A)-assisted RNA decay and modulators of RNA stability. Prog Mol Biol Transl Sci 2009; 85: 137–85.

84. Oussenko IA, Abe T, Ujiie H, Muto A, Bechhofer DH. Participation of 3′-to-5′ exoribonucleases in the turnover of Bacillus subtilis mRNA. J Bacteriol 2005; 187: 2758–67.

85. Bruscella P, Shahbabian K, Laalami S, Putzer H. RNase Y is responsible for uncoupling the expression of translation factor IF3 from that of the ribosomal proteins L35 and L20 in Bacillus subtilis. Mol Microbiol 2011; DOI 10.1111/j.1365-2958.2011.07793.x.

86. Bechhofer DH. Messenger RNA decay and maturation in Bacillus subtilis. Prog Mol Biol Transl Sci 2009; 85: 231–73.

87. Yao S, Bechhofer DH. Processing and stability of inducibly expressed rpsO mRNA derivatives in Bacillus subtilis. J Bacteriol 2009; 191: 5680–9.

88. Yao S, Blaustein JB, Bechhofer DH. Erythromycin-induced ribosome stalling and RNase J1-mediated mRNA processing in Bacillus subtilis. Mol Microbiol 2008; 69: 1439–49.

89. Yao S, Sharp JS, Bechhofer DH. Bacillus subtilis RNase J1 endonuclease and 5′ exonuclease activities in the turnover of DeltaermC mRNA. RNA 2009; 15: 2331–9.

90. Daou-Chabo R, Mathy N, Benard L, Condon C. Ribosomes initiating translation of the hbs mRNA protect it from 5′-to-3′ exoribonucleolytic degradation by RNase J1. Mol Microbiol 2009; 71: 1538–50.

91. Drider D, DiChiara JM, Wei J, Sharp JS, Bechhofer DH. Endonuclease cleavage of messenger RNA in Bacillus subtilis. Mol Microbiol 2002; 43: 1319–29.

92. Carpousis AJ, Van Houwe G, Ehretsmann C, Krisch HM. Copurification of E. coli RNAse E and PNPase: evidence for a specific association between two enzymes important in mRNA processing and degradation. Cell 1994; 76: 889–900.

93. Py B, Causton H, Mudd EA, Higgins CF. A protein complex mediating mRNA degradation in Escherichia coli. Mol Microbiol 1994; 14: 717–29.

94. Miczak A, Kaberdin VR, Wei C-L, Lin-Chao S. Proteins associated with RNAse E in a multicomponent ribonucleolytic complex. Proc Natl Acad Sci USA 1996; 93: 3865–69.

95. Py B, Higgins CF, Krisch HM, Carpousis AJ. A DEAD-box RNA helicase in the Escherichia coli RNA degradosome. Nature 1996; 381: 169–72.

96. Lopez PJ, Marchand I, Joyce SA, Dreyfus M. The C-terminal half of RNase E, which organizes the Escherichia coli degradosome, participates in mRNA degradation but not rRNA processing in vivo. Mol Microbiol 1999; 33: 188–99.

97. Ow MC, Liu Q, Kushner SR. Analysis of mRNA decay and rRNA processing in Escherichia coli in the absence of RNase E-based degradosome assembly. Mol Microbiol 2000; 38: 854–66.

98. Leroy A, Vanzo NF, Sousa S, Dreyfus M, Carpousis AJ. Function in Escherichia coli of the non-catalytic part of RNase E: role in the degradation of ribosome-free mRNA. Mol Microbiol 2002; 45: 1231–43.

99. Bernstein JA, Lin PH, Cohen SN, Lin-Chao S. Global analysis of Escherichia coli RNA degradosome function using DNA microarrays. Proc Natl Acad Sci USA 2004; 101: 2758–63.

100. Khemici V, Toesca I, Poljak L, Vanzo NF, Carpousis AJ. The RNase E of Escherichia coli has at least two binding sites for DEAD-box RNA helicases: functional replacement of RhlB by RhlE. Mol Microbiol 2004; 54: 1422–30.

101. Prud'homme-Genereux A, Beran RK, Iost I, Ramey CS, Mackie GA, Simons RW. Physical and functional interactions among RNase E, polynucleotide phosphorylase and the cold-shock protein, CsdA: evidence for a 'cold shock degradosome'. Mol Microbiol 2004; 54: 1409–21.

102. Morita T, Maki K, Aiba H. RNase E-based ribonucleoprotein complexes: mechanical basis of mRNA destabilization mediated by bacterial non-coding RNAs. Genes Dev 2005; 19: 2176–86.

103. Gao J, Lee K, Zhao M, Qiu J, Zhan X, Saxena A, Moore CJ, Cohen SN. Georgiou G. Differential modulation of E. coli mRNA abundance by inhibitory proteins that alter the composition of the degradosome. Mol Microbiol 2006; 61: 394–406.

104. Carpousis AJ. The RNA degradosome of Escherichia coli: an mRNA-degrading machine assembled on RNase E. Annu Rev Microbiol 2007; 61: 71–87.

105. Purusharth RI, Klein F, Sulthana S, Jager S, Jagannadham MV, Evguenieva-Hackenberg E, Ray MK, Klug G. Exoribonuclease R interacts with endoribonuclease E and an RNA helicase in the psychrotrophic bacterium Pseudomonas syringae Lz4W. J Biol Chem 2005; 280: 14572–8.

106. Chen C, Deutscher MP. Elevation of RNase R in response to multiple stress conditions. J Biol Chem 2005; 280: 34393–6.

107. Lee K, Cohen SN. A Streptomyces coelicolor functional orthologue of Escherichia coli RNase E shows shuffling of catalytic and PNPase-binding domains. Mol Microbiol 2003; 48: 349–60.

108. Jager S, Fuhrmann O, Heck C, Hebermehl M, Schiltz E, Rauhut R, Klug G. An mRNA degrading complex in Rhodobacter capsulatus. Nucleic Acids Res 2001; 29: 4581–8.

109. Lehnik-Habrink M, Pfortner H, Rempeters L, Pietack N, Herzberg C, Stulke J. The RNA degradosome in Bacillus subtilis: identification of CshA as the major RNA helicase in the multiprotein complex. Mol Microbiol 2010; 77: 958–71.

110. Liou GG, Jane WN, Cohen SN, Lin NS, Lin-Chao S. RNA degradosomes exist in vivo in Escherichia coli as multicomponent complexes associated with the cytoplasmic membrane via the N-terminal region of ribonuclease E. Proc Natl Acad Sci USA 2001; 98: 63–8.

111. Taghbalout A, Rothfield L. RNaseE and the other constituents of the RNA degradosome are components of the bacterial cytoskeleton. Proc Natl Acad Sci USA 2007; 104: 1667–72.

112. Taghbalout A, Rothfield L. RNaseE and RNA helicase B play central roles in the cytoskeletal organization of the RNA degradosome. J Biol Chem 2008; 283: 13850–5.

113. Khemici V, Poljak L, Luisi BF, Carpousis AJ. The RNase E of Escherichia coli is a membrane-binding protein. Mol Microbiol 2008; 70: 799–813.

114. Lewis PJ, Thaker SD, Errington J. Compartmentalization of transcription and translation in Bacillus subtilis. EMBO J 2000; 19: 710–8.

115. Montero Llopis P, Jackson AF, Sliusarenko O, Surovtsev I, Heinritz J, Emonet T, Jacobs-Wagner C. Spatial organization of the flow of genetic information in bacteria. Nature 2010; 466: 77–81.

116. Nevo-Dinur K, Nussbaum-Shochat A, Ben-Yehuda S, Amster-Choder O. Translation-independent localization of mRNA in E. coli. Science 2011; 331: 1081–4.

117. Yang XC, Sullivan KD, Marzluff WF, Dominski Z. Studies of the 5′ exonuclease and endonuclease activities of CPSF-73 in histone pre-mRNA processing. Mol Cell Biol 2009; 29: 31–42.

118. Régnier P, Hajnsdorf E. Decay of mRNA encoding ribosomal protein S15 of Escherichia coli is initiated by an RNAse E-dependent endonucleolytic cleavage that removes the 3′ stabilizing stem and loop structure. J Mol Biol 1991; 217, 283–92.

119. Marujo PE, Braun F, Haugel-Nielsen J, Le Derout J, Arraiano CM, Regnier P. Inactivation of the decay pathway initiated at an internal site by RNase E promotes poly(A)-dependent degradation of the rpsO mRNA in Escherichia coli. Mol Microbiol 2003; 50: 1283–94.

120. Deikus G, Bechhofer DH. Bacillus subtilis trp leader RNA: RNase J1 endonuclease cleavage specificity and PNPase processing. J Biol Chem 2009; 284: 26394–401.

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