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1 Regulation of RNA processing and degradation 2 in bacteria

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1 Abstract

2 Messenger RNA processing and decay is a key mechanism to control gene expression
3 at the post-transcriptional level in response to ever-changing environmental conditions. In this
4 review chapter, we discuss the main ribonucleases involved in these processes in bacteria,
5 with a particular but non-exclusive emphasis on the two best-studied paradigms of Gram-
6 negative and Gram-positive bacteria, *E. coli* and *B. subtilis*, respectively. We provide examples
7 of how the activity and specificity of these enzymes can be modulated at the protein level, by
8 co-factor binding and by post-translational modifications, and how they can be influenced by
9 specific properties of their mRNA substrates, such as 5' protective 'caps', nucleotide
10 modifications, secondary structures and translation.

11

1 Introduction

2 Bacteria need to modulate their gene expression to adapt rapidly to ever changing
3 environmental conditions. Post-transcriptional regulation allows adaptation of gene
4 expression in a timely manner by altering mRNA stability and/or translation. Ribonucleases
5 (RNases), enzymes that break phosphodiester bonds in the RNA chain, are key players in these
6 processes. RNases are divided into two main classes: exoribonucleases, which attack RNA
7 from either its 5' or 3' end and endoribonucleases, which cleave the RNA internally.
8 Exoribonucleases can be either processive, remaining attached to the same RNA molecule for
9 many rounds of catalysis, or distributive, releasing the substrate with each nucleotide (nt)
10 removed. Most RNases are hydrolytic, consuming a molecule of water upon the breaking of
11 each phosphodiester bond, but some are phosphorolytic, using inorganic phosphate instead.

12 Historically, bacterial RNA maturation and decay pathways were first studied principally in
13 *Escherichia coli* and later, in the Gram-positive model organism *Bacillus subtilis*. Little by little,
14 these studies have expanded to include many other bacteria, including some important
15 pathogens. Nonetheless, all but a couple of the 40 bacterial RNases identified thus far were
16 first identified in either *B. subtilis* or *E. coli* [1]. Studies in these organisms, which serve as
17 paradigms for the Enterobacteria and Firmicutes, respectively, will therefore provide the main
18 focus for this review. Only nine of the 40 RNases identified are found in both *E. coli* and *B.*
19 *subtilis*, emphasizing the extent to which the strategies employed for RNA maturation and
20 decay in those two bacteria differ.

21 1. General principles of messenger RNA processing and decay

22 Modulation of mRNA stability is fundamentally important to control gene expression at
23 the post-transcriptional level in bacteria [2]. Messenger RNA instability permits the rapid
24 remodeling of the transcriptome when cells encounter changes in the environment and
25 ensures the recycling of the cell's ribonucleotide pool. In *E. coli*, the median mRNA half-life is
26 around 3 minutes in optimal growth conditions [3]. In *B. subtilis* the median half-life is similar
27 [4]. Indeed, bacterial mRNA stabilities vary from a few seconds to around one hour, but
28 mRNAs half-lives are generally significantly shorter than the cell's doubling time. In contrast,
29 rRNAs and tRNAs are designated as "stable RNAs" since – once processed to their mature form

1 – they can remain intact and functional for several generations [5]. Cleavage in between
2 cistrons encoded by the same mRNA, followed by differential modulation of the stability of
3 the cleavage products also allows the production of different stoichiometric amounts of
4 different products from the same operon [6]. A good example is the regulation of the
5 bicistronic *cggR-gapA* operon in *B. subtilis* that encodes CggR, a transcriptional repressor
6 specific for its own operon and a glycolytic enzyme GapA. An endonucleolytic processing event
7 between two stem-loop structures in the intragenic spacer produces two transcripts with
8 different stabilities: the upstream *cggR* portion of the transcript is very unstable whereas the
9 downstream *gapA* portion is stabilized by a 5' end stem-loop structure. This event leads to a
10 differential expression of the two genes that fit the cellular needs, with an abundant
11 production of the glycolytic enzyme and a much lower level of the CggR repressor [6]. By
12 definition, processing/maturation and decay/degradation events permit the modulation of
13 RNA half-lives by increasing or decreasing their stabilities, respectively.

14

15 **1.1 Endonucleolytic pathways of mRNA decay: comparison of RNase E and RNase Y**

16

17 *E. coli* contains several endoribonucleases and 3'-exoribonucleases, but so far lacks an
18 identified 5'-exoribonuclease capable of degrading RNA from the 5' terminus. Moreover, the
19 presence of a stem-loop structure corresponding to the Rho-independent transcription
20 terminator at 3' end of most mRNAs impedes degradation by 3'-exoribonucleases (see below).
21 Consequently, mRNA decay is generally initiated by endoribonucleolytic cleavage at one or
22 more internal sites, in most cases by RNase E in *E. coli* and RNase Y in *B. subtilis*. In contrast to
23 RNase E in *E. coli* which is essential, RNase Y is non-essential in *B. subtilis*. However, depletion
24 of this enzyme in *B. subtilis* has numerous strong phenotypes and impacts the levels of at least
25 26% of mRNAs [7,8](Fig. 1A). These low-specificity endoribonucleases cleave RNAs in single-
26 stranded AU-rich regions, typically with a key U-residue located two nts downstream of
27 cleavage site for RNase E [9] and a G-residue immediately upstream of the cleavage site for
28 RNase Y [10]. Both enzymes are localized at the bacterial membrane, either through an
29 amphipathic helix located in non-catalytic C-terminal half of the protein for RNase E [11] or as
30 an N-terminal trans-membrane helix in the case of RNase Y [12]. Dissociation of RNase E from
31 the membrane has a strong (positive) impact on global mRNA half-life [13], while dissociation
32 of RNase Y from the membrane in *B. subtilis* is lethal [12], showing that, in both cases,
33 membrane localization of these key RNases is important for correct bacterial cell function.

1 Both RNase E and RNase Y form complexes called degradosomes, or degradosome-like
2 networks, with similar partners. RNase E forms a stable stoichiometric complex with a 3'-
3 exoribonuclease polynucleotide phosphorylase (PNPase), a DEAD-box RNA helicase (RhlB) and
4 the glycolytic enzyme enolase, through locally structured micro-domains in its otherwise
5 unstructured ~500-amino acid (aa) C-terminal domain [14]. Interestingly, RNase E and its
6 degradosome partners are located at the membrane, allowing a compartmentalization of RNA
7 degradation [11,15,16]. In related organisms, other partners are associated with the RNase E-
8 based degradosome, including the hydrolytic 3'-exoribonuclease RNase R in *Pseudomonas* or
9 the transcription termination factor Rho in *Rhodobacter* [14]. The degradosome also shows
10 some plasticity even in *E. coli*, where sub-stoichiometric associations with other proteins have
11 been identified (e.g. with Hfq bound to small regulatory RNAs, polyA polymerase (PAP1)), or
12 where the RhlB helicase has been replaced by the cold shock DEAD-box RNA helicase CsdA at
13 low temperatures. Indeed, up to 18 'non-canonical' interactions with RNase E have been
14 described in the Proteobacteria [14]. The core degradosome association of an
15 endoribonuclease, a 3'-exoribonuclease, an RNA helicase and a glycolytic enzyme has been
16 shown to improve the efficiency of mRNA decay, especially that of structured RNA [17,18],
17 and is thought to tie this process into the metabolic state of the cell, although how this
18 function is achieved is still unclear.

19 The RNase Y-based degradosome-like network is a much more transitory association
20 of similar proteins to the RNase E-based model. Proposed interacting partners include PNPase,
21 the DEAD-box helicase CshA and enolase. Although the importance of these interactions has
22 yet to be demonstrated in terms of an impact on RNA decay *in vivo* (indeed the association
23 between PNPase and RNase Y was shown to have little impact on degradation of certain
24 mRNAs [19]), the functional analogies between RNase E and RNase Y are quite remarkable,
25 given their total lack of homology.

26 RNase E has two modes of action: the so-called **5' end-dependent** and **direct entry**
27 pathways (Fig. 1). In the 5' end-dependent pathway, internal endonucleolytic cleavage by
28 RNase E is triggered by a prior conversion of the 5'-terminal tri-phosphate to a
29 monophosphate by an enzyme called RNA pyrophosphohydrolase (RppH) (see below). This
30 deprotection process is functionally similar to the decapping of eukaryotic mRNAs. The
31 structure of RNase E bound to RNA revealed a pocket close the catalytic site that allows it to
32 favorably bind a 5' mono-phosphate group [20]. Interestingly, recent evidence suggests that

1 RNase E, once bound to the 5' mono-phosphate end of mRNAs, can search for cleavage sites
2 by one-dimensional scanning along single-stranded regions of RNA [21]. RNase Y has also been
3 shown to be sensitive to the removal of the 5' triphosphate group in *B. subtilis*, but this has so
4 far only been shown for one mRNA, the *yitJ* 5'-UTR [22]. The structural basis for this preference
5 is not yet known.

6 In the direct entry pathway, RNase E bypasses the 5' end sensing mechanism to cleave
7 RNAs internally [2] (Fig. 1). A binding domain for structured RNA near the C-terminus of the
8 catalytic domain is responsible for the direct entry mechanism. Indeed, individual mutations
9 can be made to selectively knock out the 5'-mono-phosphate binding pocket or the second
10 RNA binding domain that block either the 5'-sensing or direct entry mechanisms [20]. A 5'-
11 sensor mutant was used to show that many, if not most mRNAs, employ the direct entry
12 pathway [23]. Knocking out both pathways, e.g. an *rne-ΔCter ΔrppH* mutant is lethal [24].
13 Equivalent studies have not yet been performed on RNase Y to determine whether it has both
14 modes of function and the relative importance of each.

15 The first endoribonucleolytic mRNA cleavage by RNase E or RNase Y results in the
16 production of two mRNA fragments. The 5' mRNA fragment, lacking a protective terminal 3'
17 stem-loop, can be degraded by 3'-exoribonucleases. In parallel, the 3' mRNA fragments
18 undergo additional cycles of RNase E cleavage followed by exoribonucleolytic digestion (Fig.
19 1). Four 3'-exoribonucleases have been implicated in *E. coli* mRNA decay: the phosphorolytic
20 enzyme PNPase, and the hydrolases RNase II, RNase R and oligoribonuclease [2]. A similar set
21 of enzymes with somewhat redundant 3'-exoribonuclease activity is present in *B. subtilis*,
22 namely PNPase, RNase R, RNase PH and YhaM [25]. PNPase is considered to be the main 3'-
23 exoribonuclease involved in *B. subtilis* mRNA decay [26]. Knowledge about the function of the
24 other 3'-exoribonucleases in Firmicute mRNA degradation remains limited, although a recent
25 study has shown that YhaM shortens many transcripts by 2-3 nts in *Streptococcus pyogenes*
26 [27].

27 Although RNase E and RNase Y are the major players in the initiation of mRNA decay
28 in *E. coli* and *B. subtilis*, respectively, other endoribonucleases can perform the first cleavage
29 reaction in a number of cases. For example, *E. coli* possesses a non-essential paralog of the
30 catalytic domain of RNase E, known as RNase G. RNase G has a similar endoribonucleolytic
31 cleavage specificity to RNase E, with a marked preference for 5' mono-phosphorylated RNAs
32 and for cleavage within AU-rich single stranded regions. RNase G overexpression was found

1 to allow growth of an *rne* deletion strain, indicating that RNase G can perform the essential
2 action(s) of RNase E, even though the functions of those two RNases only partially overlap
3 [28]. It should be noted that RNase Y plays a greater role in mRNA turnover in *B. subtilis* than
4 in *Staphylococcus aureus*, for example, where far fewer substrates of RNase Y have been
5 identified [10]. This suggests that yet other endoribonucleases, perhaps with overlapping
6 specificity with RNase Y and RNase E remain to be identified in the Firmicutes. The double-
7 strand (ds) specific endoribonuclease RNase III also initiates mRNA decay in a number of
8 incidences, both in *E. coli* and *B. subtilis* [29,30].

9 Lastly, many toxin/antitoxin (TA) systems employ endoribonucleases as the toxin
10 component of the module. Under stress conditions, these toxins get activated, usually through
11 selective degradation of the antitoxin, and cleave mRNAs containing their consensus cleavage
12 sites [31]. Some of these toxin RNases cleave mRNAs directly, others enter the A-site of the
13 ribosome for function. In general, these metal-independent toxins generate down-stream
14 fragments with a 5'-hydroxyl residue, in contrast to metal-dependent enzymes that generate
15 downstream cleavage products with 5'-mono-phosphate. Some bacteriophages, like T4,
16 possess kinases capable of phosphorylating 5'-OH RNAs ends to allow degradation by
17 endoribonucleases such as RNase G or RNase E [32]. However, since uninfected *E. coli* cells
18 lack such a kinase activity, toxin-cleaved mRNA fragments can presumably only be degraded
19 by 3'-exoribonucleases.

20

21 **1.2 5' exoribonucleolytic pathway of mRNA decay**

22

23 The major mRNA degradation pathway in eukaryotes is catalyzed by the 5'-
24 exoribonuclease Xrn1, following poly(A) tail shortening and decapping of the 5' end (reviewed
25 in [33]). Although several early experiments suggested that *B. subtilis* might also possess 5'-
26 exoribonuclease activity: for example, a ribosome stalled on a transcript in *B. subtilis* can
27 protect the entire downstream RNA segment (but not the upstream part) from degradation
28 [34,35], the enzyme responsible, RNase J1, was not discovered until much later [36].

29 RNase J1 plays an important role in mRNA turnover in *B. subtilis* (Fig. 1B) [29]. It
30 preferentially degrades RNA substrates with a mono-phosphorylated 5' end [36], generated
31 by dephosphorylation of the 5' end by RppH or other dephosphorylation enzymes [37] (see
32 below). In this case, RNase J1 degrades deprotected full-length mRNAs in a manner very

1 similar to the decapping and Xrn1-dependent pathway in eukaryotes. Many examples of
2 stabilized full-length mRNAs were identified in cells depleted for RNase J1, suggesting this
3 process may be widespread [29]. RNase J1 can also attack mono-phosphorylated RNA
4 degradation intermediates, generated by a prior endoribonucleolytic cleavage event, e.g by
5 RNase Y or RNase III (Fig1A) [29]. Interestingly, RNase J1 is also active on 5'-hydroxylated ends
6 [36], generated by RNase toxins such as EndoA [38], or by autocatalytic cleavage by riboswitch
7 ribozymes, as exemplified by the *glmS* mRNA in *B. subtilis* [39]. Thus, *B. subtilis* does not
8 require prior kinase activity to attack 5'-OH RNAs from the 5' end.

9 *B. subtilis* contains two RNase J paralogues: RNase J1, which is primarily responsible
10 for cell's 5'-exoribonucleolytic activity and RNase J2, whose main role appears to be the
11 stabilization of RNase J1 by complexing with it [40]. Both enzymes have the capacity to cleave
12 RNA endoribonucleolytically *in vitro*; however, the *in vivo* significance of this activity is unclear,
13 at least in *B. subtilis*. Interestingly, RNase J has been shown to be associated with a DEAD-box
14 RNA helicase in both *Helicobacter pylori* and *S. aureus* [41,42]. Although deletion of the gene
15 encoding RNase J1 (*rnjA*) is viable in *B. subtilis*, the cells are quite sick and unable to sporulate
16 or become competent [7]. They are also hyper-sensitive to the cold and antibiotics. Deletion
17 of the *rnjB* gene, encoding RNase J2, has no obvious phenotype in *B. subtilis*. In *S. aureus*
18 deletion of both the *rnjA* and *rnjB* genes is possible, although growth is restricted to a narrow
19 range around 37°C [40]. In other organisms, such as *Streptococcus pyogenes*, both *rnjA* and
20 *rnjB* are considered essential [43]. Some organisms have a single RNase J homolog, while
21 others have as many as four. These results show that even with a similar set of RNases, the
22 relative importance of each enzyme is variable in different bacteria.

23

24 **1.3 Degradation pathways of non-coding RNAs**

25

26 Like RNases, non-coding RNAs (sRNA and antisense RNA), play a key role in post-
27 transcriptional gene regulation. In *E. coli*, sRNAs require the RNA chaperone Hfq [44] or ProQ
28 [45] to efficiently base-pair with their targets and to protect them from RNases (see next
29 chapter). Although Hfq is conserved in the Firmicutes, its role in sRNA chaperoning and
30 stability is not widely maintained. The maturation/degradation of non-coding-RNAs involves
31 the same ribonucleases as those used for mRNA processing and degradation (see above).
32 Because of its specificity for dsRNA helices, RNase III plays a key role in the degradation of

1 sRNAs and of antisense RNA bound their targets in many organisms [46,47]. Indeed, its
2 essential function in *B. subtilis* is to degrade the antisense RNA component of two type I TA
3 systems encoded by two different prophages in this organism [47]. Numerous sRNAs in *E. coli*
4 and *B. subtilis* are also prone to decay-initiating endoribonucleolytic cleavages by RNase E and
5 RNase Y, respectively [48,49]. Maturation of sRNA by endoribonuclease cleavage may
6 modulate its ability to bind to mRNA. In *B. subtilis*, for example, the *sucCD* mRNA is only bound
7 by a truncated form of the RoxS sRNA, generated by RNase Y, while for the *ppnKB* mRNA, prior
8 processing is not necessary [49].

9 Two 3'-exoribonucleases have also been shown to be involved in the control of the
10 stability of sRNAs in *E. coli* (PNPase and RNase PH) [50]. Interestingly, PNPase is able to
11 associate with sRNAs and protect them from degradation (e.g. RyhB, SgrS, CyaR) during
12 exponential phase of growth while it promotes their degradation during stationary phase.

13

14 2. Modulation of RNase activity

15

16 The RNases described above have a relatively low specificity, and without any control
17 could potentially degrade most RNAs in the cell. To circumvent unwanted degradation of RNAs
18 in some conditions, but promote it in others, the cell uses different strategies to stimulate or
19 inhibit mRNA degradation in a controlled fashion. In this second part of the review,
20 summarized in Fig. 2, we will present the mechanisms used by bacteria to appropriately direct
21 RNA degradation.

22

23 2.1 Co-factors modifying RNase activity

24

25 The activity of RNases involved in RNA degradation can be modified by protein co-factors
26 or by post-translational modification of the RNases themselves. Although numerous proteins,
27 such as helicases and metabolic enzymes, have been shown to interact with RNases to form
28 the degradosome and facilitate RNA degradation (see part 1), under specific conditions
29 RNases can also interact with proteins that are not considered to be part of the core
30 degradosome. Two such proteins, RraA and RraB, were first identified in *E. coli* and bind
31 different regions of the C-terminal domain of RNase E [51,52]. In *E. coli*, besides its interaction

1 with the RNA binding domain of RNase E, RraA can also directly interact with the DEAD box
2 helicase RhlB in the degradosome [53]. RraA is widely distributed in Archaea, Proteobacteria,
3 and is also found in plants. Moreover, there is a good correlation between the presence of the
4 RraA and RNase E in these organisms. In contrast, the RraB protein is found only in the γ -
5 Proteobacteria. The overexpression of RraA and RraB alters the degradosome composition
6 differently, modulating its specificity [52]. Indeed, it was shown that both proteins impact the
7 stability of a different set of target RNAs [52]. Furthermore, RraA is expressed upon entry in
8 stationary phase and in a Sigma S (RpoS)-dependent manner. In contrast, the expression of
9 RraB is not growth phase dependent, but its transcript level increases when the intracellular
10 glucosamine-6-phosphate (GlcN-6-P) concentration decreases. The mechanism underlying
11 this regulation is unknown [54]. RNase E activity has also been found to be regulated by the
12 ϕ KZ phage protein gp37/Dip of *Pseudomonas aeruginosa*. Indeed, this phage protein interacts
13 with two RNA binding domains of RNase E and inhibits its activity [55].

14 Interestingly, RNase Y activity is also modified by co-factor binding. A complex of three
15 proteins, YlbF, YmcA and YaaT (the so-called Y-complex) modulates RNase Y specificity [56].
16 Indeed, in a strain lacking the Y-complex, expression of a specific subset of genes is impacted
17 compared to cells lacking RNase Y, which has a major impact on the transcriptome of *B. subtilis*
18 (Fig. 2) [29]. RNase Y cleavages involved in the maturation of polycistronic operon mRNAs,
19 including the *cggR-gapA* and *glnR-glnA* operon transcripts require formation of the Y-
20 complex. In contrast, RNase Y cleavages that initiate *hbs* mRNA degradation or that promote
21 the maturation of stable non-coding RNA, like the RNA component of RNase P or the RNA
22 component of the signal recognition particle (scrRNA) still occur in absence of the Y-complex.
23 While the Y-complex is in part necessary for the maturation of the RnaC sRNA, it is
24 indispensable for the degradation of two antisense RNAs and most riboswitches [6]. The YaaT
25 protein has been shown to localize to the membrane in a manner dependent on RNase Y and
26 YlbF. The Y-complex is highly conserved in Firmicutes and targets homologous RNAs in *S.*
27 *aureus* and *B. subtilis*, which suggests that the role of this complex is conserved in related
28 bacteria. The impact of these proteins on the composition of the degradosome-like network
29 in Firmicutes has not been tested.

30 The activity of the double-strand specific RNase III can be modulated by co-factor binding.
31 In *E. coli*, the YmdB protein, whose expression is RpoS-dependent, was shown to bind the
32 catalytic domain of RNase III and inhibit its activity under conditions of cold-shock (Fig. 2) [57].

1 YmdB is not found in *B. subtilis* and it is not currently known whether RNase III activity can be
2 regulated by a different co-factor in the Firmicutes.

3 Lastly, type II TA systems are obvious examples of RNase modulation by a cofactor, where
4 the antitoxin binds and completely inhibits toxic RNase activity (reviewed in [58]). An
5 interesting variant of this theme occurs in the type III TA systems, where the antitoxin is
6 actually an RNA. The ToxIN TA module in *Pectrobacterium atrosepticum* codes for a
7 ribonuclease (ToxN) and the antitoxin (ToxI) is a small RNA carrying a large number of repeats
8 recognized by ToxN that inhibit its activity. Although the ToxN mRNA targets are unknown,
9 the expression of the toxin leads to abortive phage infection [59].

10

11 **2.2 Post-translational modification of RNases**

12

13 Another way to control RNase activity in bacteria is *via* their post-translational
14 modification. In *E. coli*, the acetylation of the 3'-exoribonuclease R (RNase R) by the Pka
15 protein on residue Lys544 decreases its stability (Fig. 2) [60]. Indeed, during exponential phase
16 of growth, Lys544 acetylation modifies the secondary structure of RNase R, allowing binding
17 of the *trans*-translation machinery (the tmRNA and the SmpB protein) to the C-terminal part
18 of RNase R, on the one hand, and proteases (Lon and HslUV) to bind to the N-terminal part of
19 the protein to promote its degradation, on the other [60]. RNase R bound to the ribosome is
20 insensitive to proteases, allowing it to play its role in *trans*-translation, while degradation of
21 free RNase R prevents it from non-specifically degrading mRNAs. During stationary phase, the
22 Pka protein is not expressed and RNase R is no longer modified. This results in an increase in
23 its stability and steady state levels, necessary for the turnover of ribosomes when growth is
24 arrested (Fig. 2).

25 The 3'-exoribonuclease RNase II in *E. coli* was also found to be acetylated on Lys501 by Pka
26 (Fig. 2) [61]. Its acetylation increases during slow growth. However, this RNase can be
27 deacetylated by the CobB protein, making this post-translational modification reversible [61].
28 The acetylation of RNase II affects its RNA binding capacities and decreases its catalytic
29 activity. Other acetylations of RNase II have been detected on residues Lys31, 60 and 107 in
30 the N-terminal domain necessary to accommodate single stranded (ss) RNA, suggesting that
31 these modifications may also alter substrate binding [61].

1 After infection of *E. coli*, the bacteriophage T7 synthesizes a protein kinase that
2 phosphorylates several RNases, leading to a modification of their activities [62,63]. Indeed,
3 two proteins of the RNA degradosome, RNase E and the helicase RhlB, are heavily
4 phosphorylated by the T7 protein kinase. These phosphorylation events lead to the
5 stabilization of phage RNAs, although the direct impact of these modifications on RNase E
6 activity has not yet been studied [62]. In contrast, the activity of RNase III is increased by the
7 phosphorylation of Ser33 and Ser34 by the T7 protein kinase [63].

8 Lastly, phosphorylation and acetylation of other RNases have also recently been detected
9 in *B. subtilis* [64]. However, the significance of these modifications has not yet been
10 investigated.

11 12 3. Modulation of RNase access to mRNA

13 14 **3.1 Influence of the status of the 5' nucleotide and internal modifications on mRNA** 15 **decay**

16
17 The main endo- (RNase E and RNase Y) and 5-exo-ribonucleases (RNase J) involved in
18 RNA degradation in *E. coli* and *B. subtilis* are sensitive to the status of the 5' end of RNAs with
19 a bias for RNA with a 5' monophosphate end (see part 1). This preference is used by the cells
20 to modulate RNA degradation. The different types of RNA 5'-ends, the enzymes involved in
21 these modifications and their impact on RNA stability will be described in this section and
22 summarized in Fig. 3.

23 24 *3.1.1 Conversion of 5'-triphosphate to 5'-monophosphate end: the role of RppH*

25
26 In balanced growth conditions, it is generally accepted that most primary transcripts
27 generated by bacterial RNA polymerase have a triphosphate group at their 5'-end that helps
28 protect them from degradation by cellular RNases. Ten years ago, the RNA
29 pyrophosphohydrolase RppH was shown to convert triphosphorylated RNA to a 5'
30 monophosphorylated species to accelerate RNA degradation [65]. The 5'
31 monophosphorylated RNA can be efficiently degraded by RNase E in *E. coli* and by RNases J1
32 and Y in *B. subtilis*. Of note, RNase J in *S. aureus* has been shown to be active on

1 triphosphorylated 5'-ends, raising a question about the requirement of a pyrophosphatase
2 enzyme in *S. aureus* and how mRNAs are initially protected from degradation [66].

3 Although RppH was first identified in *E. coli* [65], this enzyme is present in most
4 bacteria and the role of its ortholog has been studied in diverse organisms such as
5 *Pseudomonas syringae*, *Neisseria gonorrhoeae*, *Helicobacter pylori*, and *Azotobacter*
6 *vinelandii* [67–70]. A similar enzyme was also identified in the Gram-positive bacterium *B.*
7 *subtilis*, known as BsRppH [37]. Although all these proteins belong to the Nudix hydrolase
8 family, they share little sequence homology outside their NUDIX motif, rendering their
9 identification difficult. For example, BsRppH has only 23% identity with the enzyme of *E. coli*.
10 A comparison of EcRppH and BsRppH activities has revealed some notable differences:
11 BsRppH releases exclusively orthophosphate from triphosphorylated RNA, whereas EcRppH
12 releases mainly pyrophosphate. The specificity of both enzymes is also different. BsRppH has
13 a strong preference for mRNAs with a guanosine at the 2nd position of its substrate and
14 additionally slightly favors substrates with an A at the first position and a purine at the 3rd
15 position [71]. EcRppH is more tolerant to sequence variation, with only a modest preference
16 for A in the first position and for a purine in the second position [72]. Both enzymes require at
17 least 2 unpaired nucleotides at the 5' end for activity but are more efficient on longer
18 substrates. Several transcriptome studies have been performed in different bacteria to
19 identify RppH targets. In *B. subtilis*, about 13% of transcripts (>580 RNAs) are enriched in a
20 $\Delta rppH$ strain and 80% of them are linked to the general stress response [73]. In *E. coli*, deletion
21 of EcRppH affects hundreds of transcripts, including the *osmY* mRNA involved in the resistance
22 of *E. coli* to high osmolarity, and the *csrA* mRNA encoding a translational regulator.
23 Interestingly, the mRNA encoding the CsrA homolog in *Azotobacter vinelandii* (RsmA) is also a
24 target of AvRppH, indicating a conservation of this regulation in different bacterial species. In
25 *H. pylori*, HpRppH has 63 potential targets (mRNA or sRNA) and in *N. gonorrhoeae*, numerous
26 sRNAs are targeted by NgRppH [68,69].

27 In eukaryotes, the decapping enzyme Dcp2, which also belongs to the NUDIX family,
28 requires several cofactors to be active [74]. In a screen designed to identify potential partners
29 of EcRppH, the DapF protein was found to bind and stimulate EcRppH activity [75]. Several
30 known targets of RppH, for example the *rpsT* and *yeiP* mRNAs, are destabilized by the
31 overexpression of RppH or DapF. Their overexpression in *E. coli* also causes a greater
32 sensitivity to high osmolarity, and interestingly, co-overexpression of the RppH-targeted *osmY*

1 RNA (see above) reverses this hypersensitivity [75]. These results indicate that a proportion
2 of RppH activity is linked to DapF *in vivo*. The primary function of DapF is as a diaminopimelate
3 (DAP) epimerase that plays a role in the biosynthesis of lysine and peptidoglycan. However,
4 enzyme activity is not required to stimulate the RppH activity, revealing a moonlighting
5 function of this protein in RNA deprotection [75]. Structural studies have shown that DapF and
6 RppH interact to form a heterotetramer which probably promotes the binding and/or release
7 of the RNA substrate [76]. Although DapF is conserved in other bacteria including *B. subtilis*,
8 it remains to be investigated whether it plays a role in mRNA decay in other organisms. Like
9 Dcp2, other co-factors could additionally stimulate RppH activity and therefore expand its
10 regulon by modifying its specificity in response to different environmental conditions.

11 Another intriguing property of EcRppH is its capacity to remove one phosphate from a
12 diphosphorylated RNA 10-fold more efficiently than a triphosphorylated RNA [77].
13 Remarkably, diphosphorylated RNAs are over-represented in *E. coli* compared to their
14 triphosphorylated counterparts [77]. The enzyme that removes the γ -phosphate from
15 triphosphorylated RNA is unknown. Moreover, extracts of the *B. subtilis* $\Delta rppH$ mutant strain
16 retain about 20% RNA pyrophosphohydrolase activity compared to wild-type cell extracts.
17 These observations suggest that other enzymes capable of dephosphorylating the 5'-ends of
18 RNA remain to be identified.

19

20 3.1.2 Alternative mRNA caps

21

22 3.1.2.1 NAD capping

23 Although most bacterial transcripts begin with a 5'-triphosphate, more recently other
24 5' caps have been identified, including nicotinamide adenine dinucleotide (NAD), dinucleoside
25 tetraphosphate (Np4N) and co-enzyme A (CoA). The first detection of NAD at the 5'-end of
26 RNAs was through an LC/MS analysis of digested total RNA in *E. coli* and *Streptomyces*
27 *venezuelae* in 2009 [78]. From this study, it was estimated that approximately 3000 NAD-RNA
28 molecules are present in *E. coli*. More recently, NAD-RNA was also found in *B. subtilis* but in
29 much lower amounts (14-fold less) than in *E. coli* [73]. Ribosomal and transfer RNA was not
30 found to be linked to NAD in either organism. The RNA polymerases from *E. coli* and *B. subtilis*
31 are intrinsically able to incorporate NAD at the +1 position, but with a preference for ATP over
32 NAD for transcription initiation [73,79]. NAD incorporation is higher in stationary phase [73]

1 and the efficiency of NAD incorporation depends on the identity of the nucleotide at the -1
2 position ($G > A \approx T > C$).

3 The presence of the NAD cap on RNA raises the question of how it is removed. In *E.*
4 *coli* removal can be performed by NudC which, like RppH, belongs to the NUDIX family of
5 proteins. The NudC protein was previously shown to convert NAD(H) into NMN(H) and AMP.
6 However, this activity is 1000-fold less efficient on free NAD than on NAD capped RNA. While
7 EcRppH is inactive on NAD-RNA in *E. coli* [80], the BsRppH enzyme can remove the NAD cap
8 with a similar specificity to that observed for triphosphorylated mRNA (see above) [73].

9 A global transcriptomic study in *E. coli* and *B. subtilis* identified NAD-linked RNA by
10 chemo-enzymatic capture, followed by RNAseq (NAD captureSeq) [80]. Interestingly,
11 sequences representing short 5'-fragments of different mRNAs (*gatY*, *pgk*, *hdeD*) and several
12 sRNAs (for example *GadY*, *GcvB*, *ChiX*, *McaS* and *RNAI*) were found to be enriched in *E. coli*. In
13 comparison, longer NAD-mRNAs were enriched in *B. subtilis* and nearly half of the NAD-
14 bearing RNAs identified had a guanine at the +2 position, which fits perfectly with the
15 specificity of BsRppH (see above).

16 The physiological role of NAD capped RNAs is not well understood but it has been
17 proposed that it protects specific RNAs from degradation by RNases in changing growth
18 conditions. In agreement with this hypothesis, the deletion of the NudC protein in *E. coli*,
19 which should reduce NAD-decapping in the cell, shows a global increase in RNA stability [79].
20 The *B. subtilis* NAD-veg RNA is protected *in vitro* against the 5'-exoribonuclease J1 (Fig. 3) [73].
21 In a similar manner, the *E. coli* NAD-RNAI is strongly protected *in vitro* against RNase E
22 compared to the 5'-monophosphorylated RNAI species. However, deletion of *nudC* does not
23 affect the half-life of RNAI *in vivo*, suggesting either that the NAD cap can be removed by other
24 enzymes or that the level of RNAI modified with NAD is too low to have a major impact on its
25 stability. Indeed, only 26% of the full length RNAI contains an NAD cap in a $\Delta nudC$ mutant
26 strain [80]. Finally, the NAD cap could also fulfill different functions. It has been speculated
27 that the NAD cap could be recognized by specific proteins leading to a modification of their
28 structure and/or catalytic properties.

29

30 3.1.2.2 Np4N capping

31 Recently, a study from J. Belasco's lab identified a new type of cap at the 5'-end of
32 mRNA [81]. It was known that during stress conditions, the levels of Np4Ns increase in

1 bacterial cells, in particular Ap4A and Gp4A [82]. As observed for NAD, RNA polymerase can
2 initiate *in vitro* transcription with Ap4A. However, amino-acyl-tRNA synthetases, such as the
3 lysyl-tRNA synthetase that is known to produce Np4As as a bi-product of the aminoacylation
4 reaction, are also able to incorporate Ap4A post-transcriptionally into RNA *in vitro*. The *in vivo*
5 contribution of each pathway(s) to Np4A capping needs to be determined. It was found that
6 *in vivo* the highest levels of Np4A capping are on transcripts initiated with A, C or U.

7 The removal of the Np4A cap from RNAs can be done either by RppH or ApaH, an
8 enzyme which was already known to hydrolyze Np4As (Fig. 3) [83]. However, the decapping
9 activity of ApaH is two-fold faster than RppH and the level of Np4A capped RNA increases in
10 unstressed $\Delta apaH$ cells [81]. ApaH is predicted to generate a diphosphorylated 5'-end that
11 that could subsequently be converted to 5'-monophosphate by RppH.

12 The capping of RNAs with Np4A is much higher during oxidative stress induced by
13 cadmium or diamide. It has been proposed that the ApaH enzyme is likely to be inactivated
14 by the crosslinking of its cysteines during disulfide stress. This inhibition would block the
15 dephosphorylation of Np4A capped RNAs and allow their stabilization. One example is the
16 *trxB* mRNA, encoding a thioredoxin reductase involved in the resistance to oxidative stress in
17 *E. coli* [84].

18

19 3.1.2.3 Coenzyme A capping

20 NAD and Np4A are not the only non-conventional nucleotides which can be used to
21 initiate transcription. Indeed, it was also shown that RNAs can bear CoA at their 5' end [85]
22 and, like NAD, this incorporation can be done by bacterial RNA polymerase. However, the
23 impact of this modification on mRNA stability has not been studied.

24 This large spectrum of 5'-end modifications of RNAs allows post-transcriptional
25 regulation of gene expression in response to stress. Interestingly, NAD, CoA and Np4N are all
26 involved in sensing and/or defense against oxidative stress, suggesting that RNA degradation
27 is highly regulated in these conditions [86,87].

28

29 3.2 Methylation of RNA

1 In eukaryotes, N⁶-methyladenosine (m⁶A) is a common modification found in mRNAs,
2 with an impact on mRNA stability. The human protein YTH-domain family 2 (YTHDF2)
3 recognizes m⁶A RNAs and promotes their degradation [88]. In prokaryotes, m⁶A RNA
4 modification has been observed in *E. coli*, *B. subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas*
5 *syringae*, *S. aureus*, *Anabaena sp. PCC 7120* and *Synechocystis sp. PCC 6803* [89]. However,
6 the m⁶A modification is less frequent in Gram-positive bacteria like *B. subtilis* (m⁶A/A ratio <
7 0.08 %) than in Gram-negative bacteria like *E. coli* and *P. aeruginosa* (m⁶A/A ratio > 0.2 %).
8 Messenger RNAs encoding proteins involved in energy metabolism and sRNAs, for example
9 RsmY and RsmZ in *P. aeruginosa* and ChiX, FnrS or MicF in *E. coli*, are preferentially
10 methylated. The role of the m⁶A modification in prokaryotes and its impact on mRNA
11 degradation is still awaiting investigation. It should be noted that it has recently been shown
12 that hypo-modified tRNA is degraded by the RNA degradosome in *V. cholerae*, suggesting that
13 modifications can also stabilize RNA, perhaps through an indirect effect on tRNA structure in
14 this case [5].

15

16 **3.3 Influence of secondary structure on mRNA stability**

17

18 RNA secondary structures can impact RNase activities in different ways. While highly
19 structured RNAs generally have greater stability, indicating that they can impair RNase
20 activity, there are also many examples where RNA secondary structures are recognized by
21 RNases and result in RNA destabilization.

22 In most mRNAs, the hairpin corresponding to the transcriptional terminator either
23 slows down or blocks the 3'-exoribonucleases that processively degrade RNA from the 3'
24 extremity. Thus, degradation by the 3'-exoribonucleases often requires a prior
25 endoribonuclease cleavage to eliminate this secondary structure (see above). Degradation of
26 structured RNA from the 3' extremity by PNPase may be facilitated by its association with an
27 RNA helicase (RhIB in *E. coli* or CshA in *B. subtilis*), either in degradosome-like complexes, or
28 through direct interaction [90,91]. A mutation of RhIB that attenuates its ability to interact
29 with PNPase, without affecting its interaction with RNase E, increases the stability of the
30 mRNA encoding the central regulator CysB, linking the RhIB-PNPase complex to cysteine
31 biosynthesis [92]. While a degradosome-like network is also thought to exist in *S. aureus*, no
32 direct interaction between PNPase and CshA was detected [93].

1 In *E. coli*, the accessibility of the 3' extremity can be improved by the synthesis of a
2 poly(A) tail which is used as an on-ramp by the 3'-exoribonucleases [94]. Polyadenylation of
3 mRNA is mainly catalyzed by PAP I (poly(A) polymerase I) in *E. coli*, where it is encoded by the
4 *pcnB* gene. Polyadenylated mRNAs are more abundant in *pnp rnb* double mutants than in
5 either *pnp* or *rnb* single mutants, indicating that both PNPase and RNase II degrade poly(A)
6 tails in *E. coli* [94]. However, while poly(A) tails allow PNPase to go through secondary
7 structures and to efficiently degrade RNAs, RNase II remains blocked by secondary structures
8 [94]. The removal of the poly(A) tail by RNase II without degrading the stem loop impedes the
9 ability of other RNases, such as PNPase, to attack these RNAs. This explains why inactivation
10 of RNase II can, paradoxically, lead to the destabilization of some mRNAs [94]. Moreover, the
11 RNA chaperone Hfq also protects RNAs from 3' exoribonucleolytic degradation by binding to
12 poly(A) tails [94]. Poly(A) tails, shorter than those found in *E. coli*, have also been detected in
13 *B. subtilis* even though it is devoid of an identifiable PAP I homolog. PNPase, which
14 predominantly functions as a 3'-exoribonuclease, can also act as a polynucleotide or poly(A)
15 polymerase in the reverse reaction and is thought to be the most likely enzyme responsible
16 for polyadenylation events in *B. subtilis* [95]. Whether it acts as a polymerase or
17 exoribonuclease most likely depends on local concentrations of NDPs or inorganic phosphate,
18 respectively. Other enzymes with polyadenylation activity may exist, as a *B. subtilis* PNPase
19 mutant still possesses some poly(A) tails [95].

20 In many cases, the presence of a 5'-terminal stem-loop also protects RNA from
21 degradation, because it inhibits pyrophosphate removal by RppH [37,65], as well as 5'-
22 monophosphate-assisted cleavage by RNase E [96]. The 5'-terminal structure can also inhibit
23 the 5' exoribonucleolytic degradation by RNase J1 since this enzyme requires at least four
24 unpaired 5' terminal nucleotides to initiate the exonucleolytic RNA degradation from the 5'
25 extremity [97]. Several studies illustrate the important role of structure at the 5'-end of mRNA.
26 Deletion of the 5' secondary structure of the *ompA* mRNA in *E. coli* (encoding the outer
27 membrane protein A), or at the extreme 5' end of the *aprE* mRNA leader in *B. subtilis*
28 (encoding an extracellular protease produced in stationary phase), severely destabilized these
29 transcripts [98,99]. The half-life of the 2.2-kb transcript of the *B. subtilis yhxA-glpP* operon,
30 which encodes a regulator of the synthesis of glycerol-3-phosphate dehydrogenase, can be
31 prolonged by adding a 5'-terminal stem loop [37]. In order to degrade these protected RNAs,
32 the 5' stem loop can be by-passed by endoribonucleolytic cleavages downstream, generating

1 a 5' monophosphorylated end that can be attacked by RNase E in *E. coli* and RNase J1 in *B.*
2 *subtilis*. This is the case for the RoxS sRNA and the *hbs* mRNA in *B. subtilis*, for example. Indeed,
3 both RNAs have protective stem-loops at their extreme 5'-ends which are removed by an
4 internal cleavage by RNase Y, followed by exoribonucleolytic degradation of the downstream
5 fragment by RNase J1 [49,100]. In some organisms, RNase J is believed to interact with an RNA
6 helicase, similarly to PNPase, to promote RNA unwinding and exoribonucleolytic degradation
7 of secondary structures. *In vitro* experiments have shown that the *H. pylori* DEAD box RNA
8 helicase RhpA allows RNase J to degrade double-stranded RNA [41]. However, it is unclear
9 whether the proposed interaction between CshA DEAD box RNA helicase and RNase J in *B.*
10 *subtilis* and *S. aureus* is direct and whether it improves RNA degradation by RNase J *in vivo*.

11 Secondary structures surrounding RNase E or RNase Y cutting sites were originally
12 proposed to help maintain single-stranded regions thus favoring nearby cleavages. In
13 agreement, a transcriptome analysis revealed that in the majority of RNase E substrates, a
14 hairpin is present upstream of the cutting site [101]. Interestingly, however, it was recently
15 shown that RNase E activity is favored by its recognition of RNA secondary structure [20]. The
16 crystal structure of the complex between RNase E and the sRNA RprA revealed that the
17 enzyme interacts with a stem-loop structure and that this interaction plays an important role
18 in internal cleavage of RprA by RNase E. Moreover, when RNase E is in 5'-end dependent mode
19 and scans the RNA from the 5' end (see above), the enzyme can also by-pass secondary
20 structures at their base [21].

21 In *S. aureus*, the RNase Y cleavage of the *saePQRS* operon, which controls the
22 expression of major virulence factors and encodes a histidine kinase, is located 6 nts upstream
23 of a 24-bp double stranded RNA structure necessary for enzyme activity [102]. This suggests
24 that like RNase E, RNase Y may also interact with secondary structures to cleave mRNA. The
25 crystal structure of RNase Y has not yet been solved and therefore, the precise molecular
26 mechanisms used by RNase Y to cleave mRNA is still under investigation.

27 An intriguing example of how RNA secondary structure modulates RNase E cleavage
28 comes from the *lysC* riboswitch. Riboswitches are highly structured *cis*-acting regulatory
29 elements found in 5'UTRs. They directly sense cellular metabolites leading to a modification
30 of riboswitch structure, thereby impacting expression of the downstream gene at the
31 transcriptional, post-transcriptional or translational level. Upon lysine binding to the *lysC*
32 5'UTR, the riboswitch adopts a conformation that promotes RNase E cleavage and

1 destabilization of the *lysC* mRNA, while in absence of lysine, the riboswitch folds into an
2 alternative conformation that sequesters the RNase E cleavage site into a secondary structure
3 [103].

4 In contrast to RNase Y and RNase E, RNase III cleaves within double stranded regions
5 of RNA. The main substrates for RNase III in numerous bacteria are the rRNA transcripts [1].
6 In *E. coli*, RNase III, encoded by the *rnc* gene, regulates its own expression by cutting a stem-
7 loop structure located just upstream of the coding region [104]. An initial cleavage by RNase
8 III is also required for the autoregulation of PNPase expression [105]. Moreover, RNase III
9 targets duplexes formed by sRNAs paired with their mRNA targets, leading to an irreversible
10 inactivation of these RNAs. For example, RNase III is involved in the degradation of the *ppnkB*
11 mRNA, encoding an NAD⁺/NADH kinase, paired with the sRNA RoxS in *B. subtilis* [49], as well
12 as in the degradation of the *spa* mRNA, coding for the surface protein A, bound by the
13 regulatory RNAIII in *S. aureus* [106].

14

15 **3.4 Translation and mRNA stability**

16

17 In bacteria, transcription and translation are coupled, meaning that mRNAs are
18 translated as soon as they are synthesized. Decoupling transcription and translation in *E. coli*,
19 using the T7 RNA polymerase to transcribe *lacZ* mRNA much faster than the host RNA
20 polymerase, leads to a dramatic destabilization of the mRNA [107]. This is reversed upon
21 inactivation of RNase E, indicating that ribosomes protect *lacZ* mRNA from RNase E cleavages
22 by masking their potential cleavage sites. In agreement with this model, ribosomes reaching
23 the stop codon of the *rpsO* mRNA in *E. coli* (encoding the ribosomal protein S15) protect it
24 from degradation by covering the decay-initiating RNase E cleavage site located only 10 nts
25 downstream [108]. Increasing the spacing between the RNase E site and the stop codon was
26 sufficient to promote *rpsO* mRNA destabilization.

27 Ribosomes may not only protect mRNA by masking cleavage sites within coding
28 sequences. A recent study showed that increasing the strength of the Shine-Dalgarno (SD) to
29 increase the efficiency of ribosome binding inhibited RNase E cleavages located in the 3' UTR
30 at a distance from the stop codon [21]. Since no ribosomes are present in the 3'UTR to cover
31 the RNase E cleavage site, it was proposed that ribosomes can impede RNase E scanning from
32 the 5' monophosphate extremity.

1 In Gram-positive bacteria, it has been found that a stalled ribosome blocks 5'-
2 exoribonucleolytic degradation mediated by RNase J1 [34,35]. A well-studied example is the
3 *ermC* mRNA, which encodes a ribosomal RNA methylase conferring erythromycin (Em)
4 resistance [34]. Em promotes ribosome stalling in a small ORF in the leader region upstream
5 of the *ermC* coding sequence in a way that modifies RNA folding and permits translation of
6 *ermC* mRNA. The stalled ribosome protects the transcript from exoribonucleolytic
7 degradation by RNase J1 regardless of whether or not the downstream *ermC* mRNA is
8 translated. A similar example of ribosome protection of the 5' end of an mRNA comes from
9 the gene encoding the CryIIIA toxin of *Bacillus thuringiensis*. The *cryIIIA* mRNA possesses a
10 strong SD sequence (GAAAGGAGGGAU) with 9 out of 12 nts complementarity to the anti-SD
11 sequence of the 16S rRNA, located 129 nt upstream of the start codon [35]. This SD-like
12 sequence (Stab-SD) is not involved in translation initiation, but rather promotes mRNA
13 stabilization. Indeed, the major transcript detected in cells does not correspond to the full-
14 length transcript but to a transcript whose 5' end starts 8 nts upstream of the first G-residue
15 of the GGAGG sequence. This transcript is produced by 5'-exoribonucleolytic degradation by
16 RNase J1 stopped by a ribosome bound to the exceptionally strong Stab-SD sequence [36].
17 The *hbs* mRNA encoding a histone-like protein in *B. subtilis* possesses a strong SD sequence
18 (GGAGGGAGGUGA) consistent with the observation that Hbs is one of the most abundant
19 proteins, with an estimated 60,000 molecules per cell. Strikingly, the major *hbs* transcript
20 observed in wild-type *B. subtilis* cells corresponds to a matured transcript whose 5' end starts
21 8 nts upstream of the first G-residue of the GGAGG sequence, as observed for *cryIIIA* [109].
22 Increasing the strength of the SD sequence of *hbs* mRNA led to an accumulation of the
23 matured transcript while weakening it led to its disappearance [100]. The stability of the whole
24 messenger promoted by a sole ribosome bound to a strong SD, suggested that the
25 degradation is driven from the 5' end by the 5'-exoribonuclease RNase J1. However, recent
26 studies showed that the initiating ribosome also covers a key RNase Y site; while a second site
27 in the middle of the *hbs* ORF had little impact on mRNA stability [100]. These observations
28 point to an apparent difference between the role of ribosomes in *B. subtilis* and *E. coli* mRNA
29 stability. In *B. subtilis*, initiating or stalled ribosomes can protect mRNAs from degradation,
30 while in *E. coli*, the greatest impact on stability generally comes from ribosomes tracking over
31 the coding sequence.

1 In some circumstances, stalled ribosomes can actually promote mRNA degradation.
2 Under stress conditions, the RelE toxin of *E. coli*, which is no longer neutralized by the antitoxin
3 RelB, binds to stalled ribosomes and cleaves mRNA in the ribosomal A site [110]. RelE cleavage
4 is highly codon dependent; it cleaves mRNAs at second position of stop codons (UAG > UAA >
5 UGA) and some sense codons (CAG and UCG) [110]. After RelE-mediated cleavage of the
6 mRNA, the blocked ribosome is recycled by the tmRNA rescue system [110]. It was recently
7 shown that in *B. subtilis*, which does not possess a RelE homolog, the r_ribosome _associated
8 _endoribonuclease Rae1 cleaves mRNAs in a translation-dependent manner [111]. Unlike RelE,
9 Rae1 is related to the NYN/PIN family of ribonucleases and does not belong to a TA system.
10 Rae1 binds to the ribosome and its cleavages are dependent on the reading frame of its
11 substrates. It is not yet understood whether Rae1 cleavage is induced by ribosome stalling and
12 whether the tmRNA rescue system is involved. Rae1 has no endoribonuclease activity without
13 ribosomes, making it a good candidate for a ribosome acceptor (A)-site ribonuclease. In
14 agreement, the Rae1 crystal structure can be docked into the A-site of the ribosome,
15 positioned to cleave between the P-site and A-site codons [111]. These data suggest that Rae1
16 may play a role in mRNA surveillance similar to the CUE2 endoribonuclease in yeast, which
17 was recently found to be involved in resolving problems occurring during translation by
18 cleaving mRNAs in the A site of collided ribosomes during No Go Decay [112].

19 In summary, mRNA stability is tightly coupled to translation, since RNases and
20 ribosomes compete for access to the transcript. A recent analysis using a multi-parameter
21 computational modeling of results to dissect the mRNA sequence features controlling protein
22 expression in *E. coli*, shows that inefficiently translated mRNAs are rapidly degraded and
23 suggest that codon content directly modulates both translation efficiency and mRNA stability
24 [113].

25

26 In conclusion, bacteria use several mechanisms to control RNase activity
27 (autoregulation, post-translational modifications, protein cofactors) and specificity (RNA
28 secondary structure, translation and modification), showing the importance of controlling the
29 RNA degradation machinery. This tight control prevents unwanted RNA degradation and
30 places RNases at the center of post-transcriptional regulation of gene expression. The access
31 of RNases to their target RNAs can also be controlled by non-coding RNAs (antisense RNA,

1 sRNA) and can even lead to direct recruitment of RNases. The diverse mechanisms used by
2 non-coding RNAs to regulate gene expression are presented in the next chapter.

3

4

5

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1 Figure 1: A schematic view of the pathways involved in RNA degradation in bacteria. A)
2 Endonucleolytic pathways of mRNA decay: primary mRNA transcripts in bacteria are protected
3 at their 5'-end by a tri-phosphate group. Initiation of mRNA degradation can involve a direct
4 endoribonuclease cut (RNase Y, RNase E or RNase III) as the rate-limiting step. In some cases,
5 dephosphorylation by the RNA pyrophosphohydrolase (e.g. RppH) is required prior to the first
6 endoribonucleolytic cut. The cleavage generates a downstream product with a 5'
7 monophosphate extremity, which can be attacked by the 5'-exoribonuclease RNase J (in
8 burgundy) or submitted to further endoribonucleases cleavages stimulated by this new 5'
9 monophosphate end. The 3'-end of the upstream cleavage product is degraded by 3'-5'
10 exoribonucleases (in green). B) In the alternative degradation pathway, the 5' tri-phosphate
11 of the mRNA is converted to a 5'-monophosphate by RppH or similar enzyme. After removal
12 of the tri-phosphate, the mRNA can be degraded directly by the 5'-3' exoribonuclease RNase
13 J.

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15 Figure 2: Schematic of the different ways to modulate RNase activity.

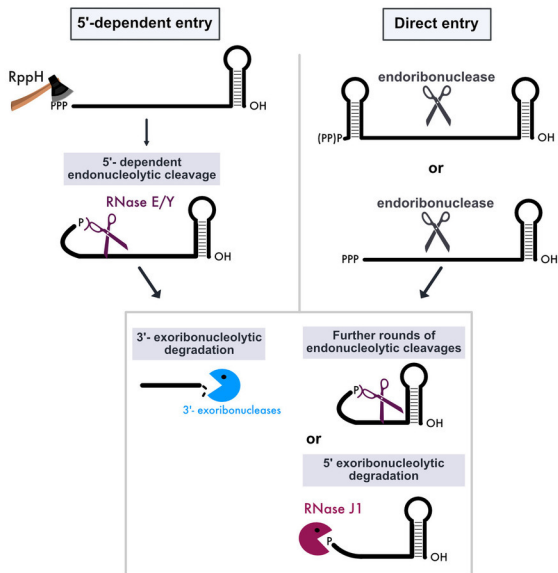
16 **1st row:** Some cofactors change RNase specificity. In the case of RNase Y, its association with
17 the Y-complex promotes degradation of some RNA targets (e.g. RNA substrate 1 in the
18 schematic), while others (e.g. RNA substrate 2) do not require the Y-complex. **2nd row:**
19 Cofactors can also modify RNase activity. An example is the inhibition of RNase III the YmdB
20 protein produced during cold shock. **3rd row:** Modifications, such as acetylation can alter
21 RNase stability. For example, acetylation by Pka targets RNase R for proteolytic degradation
22 during exponential phase of growth. However, Pka is absent during stationary phase, when
23 RNase R is required for ribosome turnover. **4th row:** Modifications can also modify RNase
24 affinity. RNase II, like RNase R, is acetylated by the Pka enzyme reducing RNase II affinity for
25 its RNA substrate. This modification can be removed by the CobB protein.

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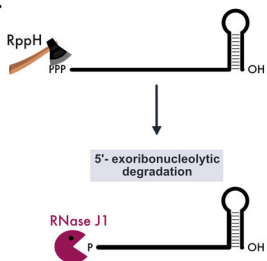
27 Figure 3: Schematic of the different ways to modulate RNase access to RNA.

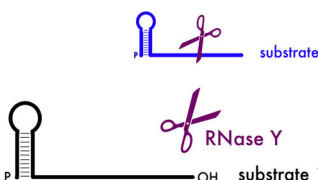
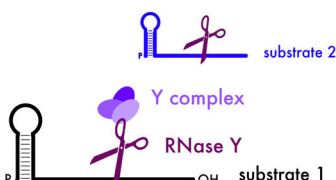
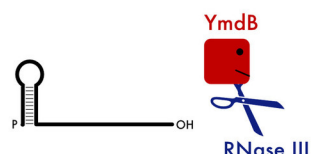
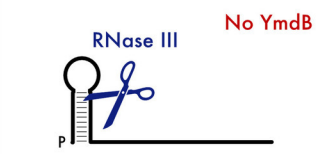
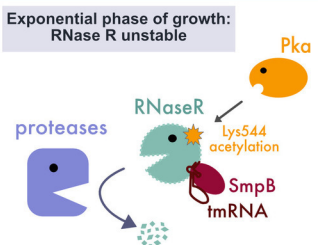
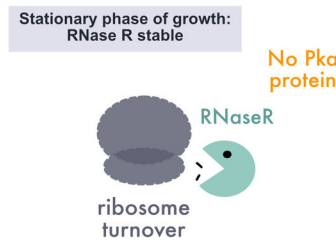
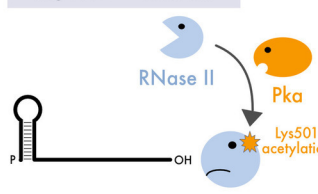
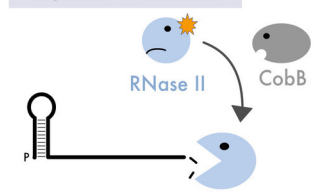
28 **1st row:** The presence of a cap at the 5' end impairs 5'-dependent ribonucleolytic degradation.
29 These modifications can be removed by ApaH and RppH proteins. **2nd row:** Presence of
30 structures at the 5' end 3' end prevent access to exoribonucleases. Nearby stem-loop
31 structures can recruit RNase E to single-stranded cleavage sites (pink). **3rd row:** Translating
32 ribosomes can block both endo- and exoribonuclease access.

A.



B.



	RNA stabilizing events	RNA destabilizing events
Effect on RNase specificity by cofactors	<p>RNase Y alone doesn't degrade RNA substrate 1</p> 	<p>Y-complex-associated RNase Y degrades RNA substrate 1</p> 
Effect on RNase activity by cofactors	<p>Cold-shock : inactive RNase III</p> 	<p>Active RNase III</p> 
Effect on RNase stability by modification	<p>Exponential phase of growth: RNase R unstable</p> 	<p>Stationary phase of growth: RNase R stable</p> 
Effect on RNase affinity by modification	<p>Acetylated RNase II doesn't degrade RNA substrate</p> 	<p>Unmodified RNase II degrades RNA substrate</p> 

	RNA stabilizing events	RNA destabilizing events
RNA 5' CAPS	<p>Inhibition of 5'-dependent ribonucleolytic degradation</p> <p>RNase E</p> <p>RNase J1</p> <p>NAD</p> <p>Ap4N</p>	<p>5'-dependent endo- or exoribonucleolytic degradation after cap removal by ApaH/RppH</p> <p>RNase J1</p> <p>P</p> <p>OH</p> <p>or</p> <p>RNase E/Y</p> <p>P</p> <p>OH</p>
RNA STRUCTURES	<p>Blocking of endo- or exoribonuclease access</p> <p>endo-ribonuclease</p> <p>endoribonuclease cleavage site</p> <p>P</p> <p>OH</p> <p>RNase J1</p> <p>3' exo-ribonucleases</p>	<p>Endo- or exoribonucleolytic degradation</p> <p>RNase E</p> <p>OH</p>
TRANSLATION	<p>Blocking of endo- or exoribonuclease access</p> <p>endo-ribonuclease</p> <p>translating ribosomes</p> <p>P</p> <p>OH</p> <p>RNase J1</p>	<p>Endo- or exoribonucleolytic degradation</p> <p>endoribonucleases</p> <p>RNase J1</p> <p>P</p> <p>OH</p>