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Regulation of Bacterial Ribonucleases

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Abstract

Ribonucleases (RNases) are essential for almost every aspect of RNA metabolism. However, despite their important metabolic roles, RNases can also be destructive enzymes. As a consequence, cells must carefully regulate the amount, the activity, and the localization of RNases to avoid the inappropriate degradation of essential RNA molecules. In addition, bacterial cells often must adjust RNase levels as environmental situations demand, also requiring careful regulation of these critical enzymes. As the need for strict control of RNases has become more evident, multiple mechanisms for this regulation have been identified and studied, and these are described in this review. The major conclusion that emerges is that no common regulatory mechanism applies to all RNases, or even to a family of RNases; rather, a wide variety of processes have evolved that act on these enzymes, and in some cases, multiple regulatory mechanisms can even act on a single RNase.

Contents

INTRODUCTION

As our understanding of RNA metabolism has increased in recent years, it has become evident that ribonucleases (RNases) are critical mediators for most processes involving RNA. RNases are essential for the degradative reactions that normally turn over mRNAs and that remove defective RNAs for quality control. Additionally, RNases are required to process stable RNA precursors to their mature, functional forms and to differentially control expression of individual regions of polycistronic mRNAs. RNases also participate in many reactions that either process or degrade small regulatory RNAs, and therefore, they have the potential to affect both transcription and translation. In light of their involvement in so many aspects of RNA metabolism and gene regulation, it is not at all surprising that RNases themselves are often regulated. The mechanisms by which this regulation occurs are the subject of this review.

WHY REGULATE RNases?

Since RNases participate in so many aspects of RNA metabolism, it is essential that they act on the correct RNA substrate and do so at the appropriate time. Many RNases have quite broad specificity, with the potential to inappropriately destroy important RNA molecules unless their action is strictly regulated. Therefore, a variety of strategies to keep RNase action under control have evolved. First and foremost are protections built into the RNA substrates themselves. These include secondary and tertiary structural features that provide resistance to certain RNases, blockage of RNA termini to shield against the action of exoribonucleases, and association with protein or RNA molecules that occlude possible cleavage sites. Such built-in protection for RNAs has been examined in detail (20, 82) and is not discussed here.

The second broad group of regulatory strategies are ones that directly affect the RNase and its activity. Included among this group is the catalytic specificity of the RNase. This enables an RNase to act effectively on only a subset of RNA substrates while generally avoiding and bypassing other RNAs, thereby protecting the latter from unwanted RNase action. Secondly, accessibility of specific RNases to potential RNA substrates may be limited by compartmentalizing them to prevent their interaction with inappropriate RNAs. This mechanism could also be employed in a regulatory manner, releasing the sequestered RNase if cellular conditions warrant. Additionally, cells have developed mechanisms that affect the amount or activity of particular RNases. If the

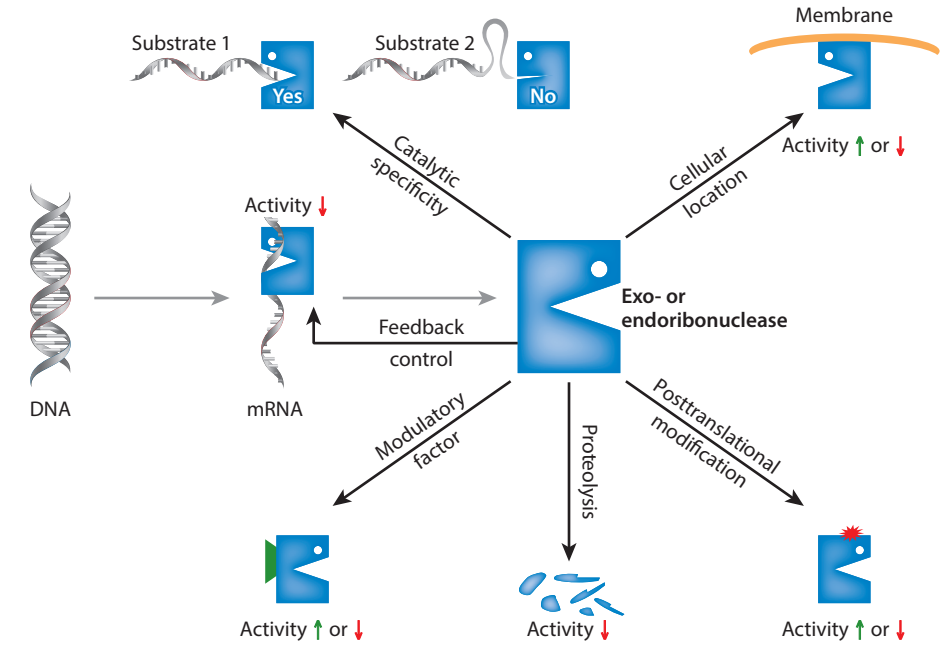

Figure 1

Summary of mechanisms for regulating bacterial ribonucleases. Shown are six known mechanisms that can influence RNase (*blue*) activity, stability, or localization. Substrate 1 is able to enter the active site and be acted upon by the enzyme, as indicated by the word Yes. Substrate 2 is different from Substrate 1 in that it has a stem-loop structure; this structure prevents it from entering the active site and being acted on by the enzyme, as indicated by the word No. More than one regulatory process may affect a single RNase. Regulatory processes can increase or decrease RNase activity.

level of an RNase were to become too elevated, a feedback mechanism could kick in to limit the amount of the RNase and maintain it within useful limits for the current cellular conditions. This mechanism also could be reversed as cellular conditions change.

Microbial cells frequently need to adapt to altered environments, such as occur during change in growth phase, upon bacteriophage infection, or as a result of other stresses. Cells sense and respond to these altered conditions by modifying gene expression, adjusting RNA stability, or changing the level of functioning ribosomes. Each situation might require readjustment of the preexisting set point for a particular RNase. Multiple examples of such fine-tuning of RNases have been identified that affect the synthesis, stability, or activity of RNases. What emerges from this sampling of regulatory processes is that there is no universal mechanism of regulation that applies to all RNases, or even to a single family of RNases. Rather, cells have evolved many mechanisms to keep RNases under control and to adjust their levels to cellular requirements. In fact, in some cases, multiple regulatory mechanisms may act on a single RNase. Our current understanding of the variety of strategies that cells employ to regulate RNases is discussed in more detail below and summarized in **Figure 1**.

SHORT PRIMER ON RNases

RNases cleave RNA molecules. Endoribonucleases cut RNA internally to release RNA fragments that contain either 3′-OH or 3′-PO_4 residues, dependent on which side of the cleaved

Table 1 RNases in model gram-negative (*Escherichia coli*) and gram-positive (*Bacillus subtilis*) organisms[a]

Endoribonucleases			Exoribonucleases		
E. coli* and *B. subtilis	***E. coli***	***B. subtilis***	***E. coli* and *B. subtilis***	***E. coli***	***B. subtilis***
RNase III[b]	RNase I[b]	RNase M5	PNPase[b]	RNase II[b]	nanoRNase A
RNase P[b]	RNase E[b]	RNase Mini-III	RNase PH[b]	RNase D[b]	nanoRNase B
RNase BN/Z[b,c]	RNase G	RNase HIII	RNase R[b]	RNase T[b]	RNase J1/J2[d]
RNase HII	RNase HI	RNase Y[b]		Orn	YhaM
YbeY/YqfG	RNase LS	Rae 1		RNase AM	

[a]Toxins and extracellular RNases are not included.
[b]Presently known to be regulated and discussed herein.
[c]RNase BN/Z also has exoribonuclease activity.
[d]RNases J1/J2 also have endoribonuclease activity.

phosphodiester bond the break has occurred. In contrast, exoribonucleases act at the ends of RNA chains, initiating attack at either the 5′ or 3′ terminus to release mononucleotides. Depending on whether the attacking nucleophile is water or inorganic phosphate, the released product will be a nucleoside monophosphate or a nucleoside diphosphate, respectively.

Phosphorolytic exoribonucleases conserve the energy of the cleaved phosphodiester bond and therefore can act reversibly to synthesize RNA. The action of hydrolytic nucleases is irreversible.

The vast majority of the nearly 40 distinct bacterial RNases currently known were first identified and characterized in either *Escherichia coli* or *Bacillus subtilis*, the model gram-negative and gram-positive organisms, respectively. Detailed reviews describing the properties and functions of these enzymes have been published recently (5). The catalog of RNases in these two organisms is likely nearing completion, as most RNA metabolic processes now have RNases associated with them. Nevertheless, surprises may still await, as was recently found for the involvement of newly discovered RNase AM in stable RNA maturation (33).

Based on the known RNases (an abbreviated list lacking toxins and secreted RNases is presented in **Table 1**), several general points have emerged. (*a*) There is only limited overlap between the RNases of *E. coli* and *B. subtilis*, indicating that despite similar RNA metabolic requirements, they have evolved different proteins to carry out these processes. (*b*) RNases can differ greatly in size and subunit structure, arguing against a universal RNase architecture and implying multiple routes of RNase evolution. Nevertheless, some RNase families with similar catalytic properties and similar structural features are known. (*c*) The same RNase can function both in RNA maturation and in RNA degradation, indicating that RNA structure and/or additional factors influence RNase action. (*d*) An increasing number of RNases are subject to regulation, although most of the known examples involve RNases from *E. coli* (**Table 1**).

MECHANISMS OF RNase REGULATION

Catalytic Specificity

While the catalytic specificity built into an RNase is not yet proven to be a regulatory mechanism, there is no question that it has a profound effect on the spectrum of possible RNA substrates. For example, the action of the endoribonuclease RNase III is limited to double-stranded RNAs or structured regions of RNA molecules (68), while the action of other enzymes such as the 3′ exoribonuclease RNase II is largely limited to single-stranded or unstructured regions (15). Likewise,

the action of oligoribonuclease and nanoRNases is limited to very small RNA molecules (57, 62). Even subtler specificity exists for some other RNases. RNase BN/Z is unable to digest mature tRNA molecules because its action is inhibited by the universal -CCA sequence (23), and the exoribonuclease RNase T is unable to digest through two adjacent C residues, limiting its action on mature tRNAs to removal of only the 3′ terminal A residue of the -CCA sequence. Catalytic specificity is mentioned here briefly because the few examples discussed below foreshadow the idea that a change in catalytic specificity might be used as a means to regulate RNase action.

Polynucleotide phosphorylase (PNPase), a phosphorolytic 3′ exoribonuclease present in most organisms, can be isolated from *E. coli* in multiple forms: (*a*) as a free protein, (*b*) in association with the RNA helicase (RhlB) (48), and (*c*) as a constituent of the RNA degradosome, a multienzyme complex that also contains the endoribonuclease RNase E, RhlB, and the glycolytic enzyme enolase (50). In vitro, PNPase processively digests RNA chains, but it is inhibited by secondary structure elements with as few as seven base pairs (16, 75). Yet, in vivo, PNPase participates in degradation of highly structured mRNAs and rRNAs (7), tRNAs (42), and even highly structured REP sequences (16). Undoubtedly, association with the RNA helicase RhlB enables PNPase to function on these RNAs, effectively changing its catalytic specificity. Thus, one might imagine that altering the distribution of PNPase among its various free and complexed forms in response to some external stimulus could serve to regulate the catalytic specificity of PNPase. However, little information is available on the distribution of PNPase under different environmental conditions.

RNase Y is an endoribonuclease that in many organisms serves to initiate mRNA degradation in a manner similar to that of RNase E. It is the primary endoribonuclease responsible for mRNA decay in *B. subtilis*, and in its absence the half-life of bulk mRNA increases twofold (70). RNase Y interacts with a complex of three proteins (YlbF, YmcA, and YaaT) termed the Y-complex, which affects its cleavage specificity (17). While many RNase Y cleavages can occur in the absence of the Y-complex, others such as the cleavage of the *cggR-gapA* transcript require association with the Y-complex (17). Hence, the Y-complex alters the cleavage specificity of RNase Y. Moreover, the Y-complex is required for developmental processes such as sporulation (18), implying it may alter the catalytic specificity of RNase Y as its role changes during development. This appears to be an ideal system to investigate whether alterations in RNase catalytic specificity can serve a regulatory role.

Cellular Localization

Bacteria were long considered to be bags of enzymes with little internal organization. This view has changed as more and more cellular components and processes have been shown to display spatial organization (30). This is true for certain RNases as well (10). The simplest way to avoid unwanted degradation of cellular RNAs by uncontrolled RNases is to keep the enzymes separate from their potential substrates. The classic example of such regulation by physical separation involves *E. coli* RNase I, a highly active, nonspecific endoribonuclease (74). Normally, most RNase I is localized in the periplasmic space, presumably serving some unknown function, and is unable to access cellular RNAs (60). However, agents that damage the inner membrane allow entry of RNase I, leading to major degradation of RNA and ultimately to cell death (19). Periplasmic RNase I also is fully oxidized and essentially inactive (11). So, even if it were to enter cells, some time would be required until intracellular reducing conditions generated the active enzyme. This delay might allow repair of the cell membrane to stem further influx, enabling the cell to survive a limited entry of RNase I. However, nothing is known about this aspect of the process.

Key RNases of the mRNA-degrading machinery are localized in some bacterial cells. For example, RNase E of *E. coli* (37) and RNase Y of *B. subtilis* (41) and *Staphylococcus aureus* (38) are

localized at the cell membrane and form short-lived foci within the membrane. For RNase E, attachment to the inner cytoplasmic membrane is dependent on a membrane-targeting sequence (MTS), a 15-residue motif in the C-terminal, noncatalytic portion of the protein that forms an amphipathic helix upon interacting with a phospholipid bilayer (77). Mutations of residues within the MTS (37) or complete removal of the MTS confirmed the essentiality of this sequence for membrane localization and leads to the presence of a cytoplasmic RNA degradosome (31). RhlB helicase, another degradosome component, also changes its localization as a consequence of removal of the MTS from RNase E, indicating that the complete degradosome becomes cytoplasmic in the mutant strain (77).

Cells containing a cytoplasmic degradosome grow more slowly than wild type and display a slowdown of global mRNA degradation (31). The degradosome apparently assembles normally, but RNase E becomes more unstable. The level and activity of RNase E remain the same because its instability is compensated by an autoregulation mechanism (34) that is discussed in the section titled Feedback Control. Despite the slowdown of mRNA decay in the mutant strain, ribosome-free transcripts are more unstable, likely due to the lack of protection by ribosomes against the action of cytoplasmic RNase E (31). These findings indicate that membrane localization affects both RNase E stability and the stability of cellular transcripts that normally would not be in contact with RNase E. The importance of cellular localization is further emphasized during growth under anaerobic conditions, which leads to release of RNase E from the cell membrane and its diffusion throughout the cytoplasm, a decrease in RNase E stability and activity, and cell filamentation (59). These changes are dependent on the presence of another component of the RNA degradosome, enolase. For *E. coli*, at least, membrane localization of RNase E and the degradosome clearly has important physiological consequences. However, binding to the cell membrane is not a universal attribute of RNase E. For example, RNase E from *Caulobacter crescentus* lacks an MTS and is apparently cytoplasmic (4). In addition, it forms foci within the cytoplasm that are related to cell stress. These interesting differences in compartmentalization strategies among RNase E's of different organisms are not understood.

RNase Y, the major mRNA-decay-initiating endonuclease in many organisms (70), like RNase E, is membrane bound (41). Association with the membrane is through an N-terminal membrane-spanning domain of ~25 amino acids. The exact role of its membrane association is not understood, but removal of the transmembrane domain of RNase Y leads to slowed growth (41), implying an important function. RNase Y is dynamic within the cell membrane, forming short-lived foci, but these are currently thought not to be the most active form of the enzyme (32), in contrast to the foci formed by RNase E, which are dependent on the presence of RNA substrates (77). Thus, while presently available information suggests that membrane association of RNase Y has the potential to serve a regulatory purpose, no conclusive evidence yet exists.

Constituents of the RNA degradosome and other enzymes involved in RNA processing and/or degradation have been found as components of cytoskeleton-like structures that coil around the periphery of *E. coli* cells (80, 81). However, since the initial reports of this phenomenon (all from one lab) no additional information has been forthcoming. This lab also reported that RNase II, the major exoribonuclease in *E. coli*, is associated with the cytoplasmic membrane through an N-terminal amphipathic helix (49). Most importantly, removal of the helix affected cell viability in strains in which RNase II was essential for growth (49). These findings would be of considerable interest if confirmed. However, no evidence could be obtained that either RNase E or RhlB, known degradosome components, forms cytoskeleton-like structures (31), and removal of the amphipathic helix of RNase II had no effect on rRNA metabolism under conditions in which this enzyme played an important role (78). Thus, unless additional information becomes available, it is probably best to consider these initial reports with caution.

In addition to membrane localization, RNases can also be sequestered on ribosomes. However, one must be careful in drawing conclusions about association with ribosomes based solely on studies in cell extracts since positively charged regions of proteins often interact with ribosomes subsequent to cell rupture, especially under low salt conditions. The most telling example of such an artifact is the specific binding of *E. coli* RNase I to the 30S ribosome subunit in cell extracts (74) despite the fact that the enzyme resides in the periplasmic space in vivo (60), as discussed above.

The clearest example of RNase binding to ribosomes comes from studies of *E. coli* RNase R (46). RNase R has the potential to be a very destructive enzyme, as it can processively digest all RNAs, even those with extensive secondary structure (16). Consequently, cells need to regulate its activity, and this is accomplished by a variety of regulatory mechanisms, including posttranslational modification, binding of factors, proteolysis, and sequestration on ribosomes (5). In exponential-phase cells, ~80% of RNase R is bound to ribosomes, which stabilizes the RNase and enables it to participate in *trans*-translation (46), a process that removes ribosomes from mRNAs on which they are stalled. Binding of RNase R is dependent on two factors required for *trans*-translation: transfer-messenger RNA (tmRNA) and its associated protein factor SmpB. Binding is also dependent on a truncated mRNA lacking a termination codon (nonstop mRNA), which leads to ribosome stalling, and on ribosomal protein S12. Inhibiting the ability of RNase R to bind ribosomes leads to slower growth and a large increase in RNA degradation, indicating that its sequestration regulates the deleterious RNase R, thereby protecting cells from unwanted RNase action (46). In stationary-phase cells, RNase R exists solely as the free form where it is needed for the degradation of rRNA that occurs when growth ceases (46). Additional strategies for regulating RNase R are described in other sections, and they emphasize the importance of maintaining the enzyme under strict control.

Several reports have suggested that RNA degradosomes also associate with ribosomes. In *E. coli*, degradosomes form a stable complex with 70S ribosomes and with polysomes (83). Interaction occurs through two domains on RNase E and through RhlB. Ribosome binding decreases the activity of RNase E against 9S RNA, a precursor of 5S RNA, whereas activity against unstructured substrates is unaffected. In *Helicobacter pylori*, a minimal RNA degradosome containing only RNase J and an RNA helicase associates with translating ribosomes, but not with ribosome subunits (67). Both reports propose that association of the degradosome with ribosomes serves to degrade mRNAs as part of the mRNA decay process. It is not known whether this mechanism is used for regulation, such as by altering the amount of bound degradosome under certain cellular conditions.

As information accumulates, it is apparent that cellular localization has the potential to be an important strategy for regulating RNases. Studies of this type of mechanism are limited by the difficulties of examining cell organization in bacteria and also by the likelihood that many of the interactions that serve to compartmentalize RNases are transient and weak, making isolation of localized enzymes that much more challenging.

Feedback Control

Cells growing at a constant rate maintain each RNase activity at a fixed set point that is appropriate for the growth rate at hand and is dependent on both RNase synthesis and processes that regulate the RNase. One regulatory strategy that maintains the set point of several RNases is feedback control. This limits the degree of variation for an RNase that can be tolerated under a specific set of conditions. However, if those conditions were to change such that more or less of a particular RNase is required, feedback regulation would be a relatively efficient mechanism for altering the set point, since the RNase controls its own level. When RNase activity becomes too high for the needs of the cell, the excess RNase then shuts off its own expression. Alternatively, if insufficient

RNase activity is present, no feedback occurs and expression proceeds at a maximal level. This mechanism can be quite sensitive, as was shown in quantification studies of RNase E (35, 73). Other RNases known to be regulated by feedback control include *E. coli* RNase III and PNPase, and perhaps *S. aureus* RNase Y is as well.

RNase E is a major participant in both RNA maturation and RNA degradative reactions in *E. coli* (5), making it imperative that its level be carefully regulated. Too much RNase E might result in unwanted removal of important RNA molecules, whereas insufficient enzyme could reduce maturation of essential RNAs. The *rne* message is itself a substrate for RNase E cleavage that is dependent on its 5′ untranslated region (UTR) (22, 69), thereby providing a mechanism by which RNase E regulates its own expression. Construction of a fusion between the 5′ UTR segment of *rne* and *lacZ* brought β-galactosidase synthesis under the control of RNase E expression, and this system was used to quantify the feedback effect. The half-life of the hybrid message could be modulated over a wide range, between 40 s and 8 min (35). Moreover, overexpression of RNase E is severely limited, such that a 20-fold increase in *rne* gene dosage leads to only an ~3-fold increase in RNase E protein, suggesting that cells cannot tolerate too much RNase E.

Details of the feedback mechanism are not yet fully understood, but it is thought that when RNase E levels exceed needs, the excess enzyme binds to a highly conserved stem-loop structure, hp2, present in the 5′ UTR of the *rne* message (69). This binding leads to cleavage by RNase E at an unknown site elsewhere in the mRNA, preventing further translation. When RNase E levels are too low, growth is impacted, likely due to insufficient maturation of stable RNAs, and under these conditions, no RNase E binds and the *rne* message is stabilized (35). Autoregulation is also decreased by polyadenylation of the *rne* transcript (58), or by removal of the 5′-phosphate sensor region of RNase E that normally recognizes the 5′-terminal phosphate of RNA substrates (27); however, the mechanisms are not understood. While autoregulation of RNase E is no doubt very important, questions about the details of the process remain. Nevertheless, it is clear that autoregulation would enable cells to respond to varied situations that require an altered level of RNase E activity.

RNase III is a double-strand-specific endoribonuclease that is present in essentially all bacteria and is critical for many aspects of RNA metabolism (5). In *E. coli*, RNase III expression is controlled by what appears to be a straightforward autoregulatory mechanism that acts on the message encoding RNase III, Era, and RecO (2, 54). This operon message contains three stabilizing stem-loop structures within the first 215 nucleotides (nt) of its 5′ UTR. When RNase III is present in excess, it cleaves the double-stranded stem of the largest central stem-loop, generating a single-stranded 5′ end that is bound by RNase E and leading to transcript degradation and reduced RNase III synthesis (55). Similar autoregulatory processes have been observed in *Salmonella enterica* Typhimurium (1) and *Streptomyces coelicolor* (88). In *B. subtilis*, two RNase III cleavage sites were identified in the coding sequence of the *rnc* gene, suggesting the likelihood of autoregulation of RNase III in this organism as well (21).

As noted, PNPase is a phosphorolytic, 3′ exoribonuclease that exists in multiple forms within cells and is primarily involved in RNA degradation (5). In *E. coli*, it is subject to complex autoregulatory mechanisms that can affect both the stability of its mRNA and its translation (7). PNPase is expressed from either of two promoters, leading to production of two mRNAs. The longer transcript encodes both upstream RpsO (ribosomal protein S15) and PNPase, whereas the shorter one encodes only PNPase (24). In the currently favored model for autoregulation (12), RNase III cleaves a long stem-loop structure in the 5′ UTR of *pnp* to remove the upper half of the structure. This generates a new 5′ end on the *pnp* mRNA about 80 nt upstream of the initiation codon and a complementary 37-nt fragment. This fragment, with its newly generated 3′-OH end, is removed by PNPase, thereby rendering the 5′ UTR of the *pnp* message single-stranded and a substrate

for subsequent degradation by RNase E, which binds to the newly generated 5′ end and cleaves within the *pnp* coding region. In the absence of PNPase action due to mutations that eliminate its activity or binding ability, the RNase III–cleaved mRNA is stable (87). Thus, PNPase is responsible for regulating the stability of its own message. In the absence of RNase III, PNPase can act as a translational repressor of the native message (64), although it is difficult to imagine that this mechanism operates in cells in which RNase III would normally be present. Autoregulation of *pnp* expression has also been observed in several other bacterial species (7).

PNPase is a critical enzyme during cold shock and is elevated under these conditions (36). Autoregulation is suppressed during the acclimation phase at low temperature, possibly because of an inability to remove the 37-nt fragment. As a result, *pnp* mRNA is stabilized, leading to higher levels of expression (6, 58). Once cells have acclimated, autoregulation is restored (52), suggesting that additional regulation is necessary to maintain the elevated levels of PNPase observed during cold shock.

Global analysis of RNase Y cleavage specificity in *S. aureus* using RNA-seq enabled mapping of 99 cleavage sites. Among those identified were sites in several RNase mRNAs, including RNase Y itself (38). While this suggests the possibility of an autoregulatory mechanism, much more work will be needed to determine whether this occurs. RNase activities associated with certain bacterial toxins are not discussed here in detail, but it should be mentioned that *E. coli* MazF cleaves its own transcript and that this fine-tunes MazF expression during stress (61).

Posttranslational Modification

Posttranslational modification is a common mechanism of enzyme regulation, and several examples have been identified that control bacterial RNases. Posttranslational modifications involve either phosphorylation or acetylation of the RNase leading to alterations in its catalytic activity or stability.

When bacteriophage T7 infects *E. coli*, the early T7 protein kinase phosphorylates two host endoribonucleases, RNase III (56) and RNase E (51). The activity of RNase III increases three- to fourfold due to phosphorylation of Ser33 and Ser34 residues in its N-terminal catalytic domain (28). Phosphorylation increases the rate-limiting product release step, leading to an approximately seven-fold increase in catalytic efficiency. Since host RNase III is required for cleavage of the phage early region polycistronic mRNA, its increased activity leads to elevation in functional T7 messages. Also, since RNase III initiates autoregulation of PNPase, its elevation may also affect expression of PNPase, further reducing another potential inhibitor of T7 mRNAs. On the other hand, the phosphorylation of RNase E leads to inhibition of its activity due to extensive phosphorylation in its C-terminal region (51). T7 transcripts normally are quite unstable because T7 RNA polymerase moves so rapidly that ribosome translation cannot keep up, leaving naked RNA gaps behind itself. The inhibition of host RNase E helps to stabilize these mRNAs, keeping them available for productive T7 infection. From these limited observations, it is clear that additional studies of host RNase regulation after phage T7 infection would be extremely informative.

A more detailed examination of RNase regulation by posttranslational modification comes from studies of the *E. coli* exoribonuclease, RNase R. The amount and activity of RNase R increase three- to tenfold under certain stress conditions such as stationary phase and cold shock (9, 13) due to stabilization of RNase R protein (13). RNase R has a very short average half-life of ~10 min in exponentially growing cells, reflecting the combination of a stable portion (80%) bound to ribosomes and free enzyme (20%) with a half-life of 2 min (46). RNase R in stationary-phase cells is stable. Instability of exponential-phase enzyme is a consequence of its association with two components of the *trans*-translation machinery: tmRNA and its cofactor SmpB, which bind near

the C-terminal basic region/S1 domain of RNase R (43). This binding directly affects RNase R stability but has no effect on RNase R activity.

tmRNA/SmpB binds approximately ten times more weakly to stationary phase RNase R than to the exponential-phase protein, indicating that the two forms of the enzyme must differ in some manner. Immunological and mass spectrometry analyses revealed that exponential-phase RNase R is posttranslationally modified by an acetyl group on Lys544 (47), whereas the stationary-phase protein is unmodified. No other modification was found on the exponential-phase protein. Site-directed mutation of Lys544 indicated that a positive charge at this position due to the original Lys residue or to a substituted Arg stabilized RNase R, whereas an uncharged acetyl-Lys or an Ala substitution at the same position destabilized the protein. Additional structural and mutagenic analyses revealed that the positively charged Lys544 interacts with acidic residues in the C-terminal region of the protein, but if the positive charge is eliminated, this interaction is prevented and tmRNA/SmpB is now able to bind to the newly exposed C-terminal region (47).

Acetylation of RNase R is limited to the exponential-phase protein because the acetylating enzyme, termed Pka, is absent from late-exponential-phase and stationary-phase cells (44). As cells approach stationary phase, the preexisting acetylated, unstable RNase R continues to be removed, while newly synthesized protein is no longer acetylated, leaving only nonacetylated, stable RNase R to persist in stationary phase. Likewise, Pka is not present in cold-shocked cells, explaining why RNase R also is stable under these conditions (44). Thus, these observations explain how the presence of Pka leads to acetylation of a single Lys residue in RNase R, to binding of tmRNA/SmpB to the C-terminal region, and ultimately to removal of the RNase. The mechanism by which binding of tmRNA/SmpB leads to proteolysis is discussed below (see section titled Proteolysis).

E. coli RNase II, a close relative of RNase R, also is acetylated (72). RNase II is acetylated primarily on Lys501, but several other residues may also be modified at a low level. Acetylation decreases the activity of RNase II by weakening its binding to RNA substrates, but it does not affect the stability of RNase II. Interestingly, acetylation of RNase II can be reversed by the action of the deacetylase CobB; this does not occur with RNase R. Slow growth increases the acetylation level of RNase II and reduces RNase II activity. Thus, this system has the ability to reversibly regulate the activity of RNase II in response to changes in growth rate.

Recently, a protein was identified in *S. aureus*, YabJ, in which a ribonuclease activity is activated upon chlorination (39). Reversal of chlorination by reduction with dithiothreitol diminishes the RNase activity. Interestingly, YabJ is homologous to the *E. coli* protein RidA, but its chlorination induces RNA chaperone activity.

So far, only a few examples of RNase regulation by posttranslational modification have been identified, but proteomic analysis indicates that other RNases may be modified as well. Given the ubiquitous role of posttranslational modification in cell regulatory processes, it would not be surprising if other RNases also were regulated by this mechanism.

Proteolysis

One strategy for efficiently eliminating an unwanted RNase is to specifically target it for removal by proteolysis. Only one example of this mechanism has been identified definitively, namely, the aforementioned *E. coli* RNase R, although there is a second possibility involving *E. coli* RNase PH. In both cases, proteolysis appears to be the final step in a complex regulatory process that singles out the RNase for degradation.

As already discussed, acetylation of Lys544 on RNase R leads to turnover of enzyme not bound and protected by ribosomes (47). Degradation is so rapid that unbound RNase R has a half-life of only two minutes, extremely unusual for bacteria (46). Turnover is due to the action of either of two

proteases, Lon or HslUV (45). The binding of tmRNA/SmpB in the C-terminal region of RNase R facilitates binding of a protease to the N terminus of the protein due to direct interactions among RNase R, the protease, and SmpB, ultimately leading to initiation of proteolysis. Interference with acetylation of Lys544 or SmpB binding, or removal of the N- or C-terminal regions of RNase R, or removal of the proteases leads to stabilization of the RNase. It is not understood why regulation of RNase R is such a complicated process; nevertheless, it serves to effectively remove the free form of the RNase and avoids unchecked degradation of RNA.

Initial results suggest a similar mechanism may operate for removal of the exoribonuclease RNase PH. The amount of this RNase normally declines as much as 90% during starvation and stationary phase (79; A. Hussain and M. P. Deutscher, unpublished results) because the protein is unstable under conditions of nutrient deprivation. RNase PH plays a role in the decay of rRNA under these conditions (3), but if too much RNase PH is present, rRNA removal is uncontrolled. In fact, reduction in RNase PH depends on the presence of another RNase, RNase II. In its absence, RNase PH levels remain high, ribosome degradation is excessive, and cells ultimately become inviable (79). However, details of the regulatory process, particularly the role of RNase II, are not yet known.

Modulatory Factors

A wide variety of factors have been identified that can affect the activity, stability, or expression of RNases. These include proteins, small RNAs, and even small molecules. In many cases, the effects of these factors have not yet been studied in sufficient detail to completely understand their physiological significance, although their widespread occurrence strongly suggests they may be important to overall RNase regulation. Several such factors have already been discussed, namely, Y-complex regulation of RNase Y specificity and tmRNA/SmpB regulation of RNase R stability. Also, the association of RNases within degradosome complexes clearly influences their action. Other examples are discussed here.

Not surprisingly, given the importance of RNase E in so many aspects of RNA metabolism, several factors are known that modulate it. Two proteins, termed RraA and RraB, bind at separate positions in the C-terminal half of *E. coli* RNase E and inhibit its activity and alter the degradosome (26). Additional studies of RraA binding revealed that it occludes RNA-binding domains and also interacts with the C-terminal extension of RhlB helicase (29). A major concern about the significance of these factors is that they must be overexpressed to exert their effects in vivo (29). Ribosomal protein L4 also binds to the C-terminal half of RNase E, inhibiting its activity (71). Ectopic expression of L4 affects levels of many transcripts, particularly those involved in stress responses, supporting a possible regulatory function since L4 increases under such conditions.

Certain phage-encoded proteins also modulate RNase E activity. Bacteriophage T4 protein Srd stimulates RNase E activity, leading to enhanced host mRNA degradation, and expression of Srd in uninfected cells increases mRNA decay, dependent on RNase E (66). Inactivation of *srd* decreases phage growth, emphasizing the importance of this system for efficient phage infection. In contrast to the stimulatory effect of Srd, the gp37/Dip protein of phage ϕKZ inhibits the RNase E activity of *Pseudomonas aeruginosa* (85).

Other examples of known protein modulators of RNases include YmdB, an ~19-kDa protein that inhibits the activity of *E. coli* RNase III (40). This protein interferes with the dimerization of RNase III subunits by interacting with residue Arg40 at the subunit interface (65). YmdB increases during stationary phase and cold shock, but only in the latter condition does it correlate with reduced RNase III activity (40). Additionally, *E. coli* RNase II, encoded by the *rnb* gene, is affected by removal of a gene downstream of *rnb*, termed *gmr* (8), that encodes a cyclic di-GMP

phosphodiesterase (86). Upon deletion of *gmr*, RNase II protein and activity increase approximately threefold due to stabilization of the RNase II protein (8). How the stability of RNase II might be affected by cyclic di-GMP is not known. It should be noted in this context that cyclic di-GMP also binds to PNPase with a K_D of 2.9 μM and that this binding enhances some activities of PNPase (84).

The preceding modulatory factors all act directly on the RNase to alter its activity or stability; however, some factors are known that alter RNase expression. For example, the global regulator CsrA strongly represses translation of PNPase by binding to the leader sequence of *pnp* transcripts that have already undergone the cleavages associated with the autoregulation process discussed above (64). A second modulator of PNPase expression is SraG, a small RNA encoded in the reverse direction by the region between *rpsO* and *pnp*. SraG destabilizes the mRNA and inhibits translation initiation of PNPase (25). In a novel mechanism of RNase regulation, the amount of RNase BN/Z is maintained at high levels in exponential-phase cells because its mRNA is stabilized against breakdown by RNaseE due to the binding of the protein Hfq and the small RNA GcvB (14). The message is not protected in stationary-phase cells because GcvB levels are reduced, and as a consequence, RNase BN is present at much lower levels during this growth phase.

RNases may also be modulated by low-molecular-weight metabolites. The Krebs cycle intermediate citrate directly binds and inhibits PNPase activity (63). In an *E. coli* strain dependent on PNPase for viability, elevated levels of citrate inhibit growth, demonstrating that this mechanism operates in vivo. Citrate also inhibits PNPase from other bacteria, archaea, and eukaryotes (76). Additionally, glucosamine-6-phosphate, a precursor of peptidoglycans and lipopolysaccharides, inhibits RNase E (53). These findings suggest links between metabolic intermediates and RNA metabolism, although details of this communication are not yet understood.

CONCLUDING REMARKS

Study of RNase regulation is still in its infancy. Yet, it is already clear that many RNases are subject to strict control by a wide variety of mechanisms. These regulatory processes serve both to protect RNA against unwanted degradation and to adjust RNases to levels appropriate for particular cellular conditions. Given the central role of these enzymes in RNA metabolism, and their large number, there is no doubt that many more regulatory strategies will be uncovered and that RNase regulation will be an essential component to our understanding of RNA metabolism.

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