SURVEY AND SUMMARY

Degradation of RNA in bacteria: comparison of mRNA and stable RNA

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ABSTRACT

Degradation of RNA plays a central role in RNA metabolism. In recent years, our knowledge of the mechanisms of RNA degradation has increased considerably with discovery of the participating RNases and analysis of mutants affected in the various degradative pathways. Among these processes, mRNA decay and stable RNA degradation generally have been considered distinct, and also separate from RNA maturation. In this review, each of these processes is described, as it is currently understood in bacteria. The picture that emerges is that decay of mRNA and degradation of stable RNA share many common features, and that their initial steps also overlap with those of RNA maturation. Thus, bacterial cells do not contain dedicated machinery for degradation of different classes of RNA or for different processes. Rather, only the specificity of the RNase and the accessibility of the substrate determine whether or not a particular RNA will be acted upon.

INTRODUCTION

RNA degradation is a major component of overall RNA metabolism, and plays an important role in determining intracellular levels of RNA species. For mRNAs, rapid decay serves to continuously adjust the message population to the needs of the cell for specific proteins (1–4). In contrast, stable RNAs, primarily rRNA and tRNA, are degraded only under certain stress conditions or when an RNA molecule is defective (i.e. quality control) (5). Traditionally, these two processes have been regarded as separate areas of investigation, and while considerable effort has gone into understanding mRNA decay, studies of stable RNA degradation generally have languished. In addition, RNA degradation has also been considered to be a distinct process compared with RNA maturation or

RNA processing during which RNA precursors, largely of the stable RNAs, are converted to their mature, functional forms. Consequently, new information obtained in one of these areas often has not transferred easily to studies in other areas.

Nevertheless, each of the aforementioned processes requires the action of ribonucleases (RNases). As more of these enzymes have been identified, and as we have learned more details about their functional roles, it has become increasingly clear that many of them participate in multiple RNA metabolic pathways, and that there is considerable overlap among the diverse processes mentioned above. Thus, while this article will focus on RNA degradation as it is currently understood in bacteria, particular emphasis will be placed on discussion of the many similarities between the turnover of mRNA and the removal of stable RNAs during stress or quality control, as well as on how the degradative machinery may overlap with that of RNA maturation.

mRNA DECAY

The rapid turnover of bacterial mRNAs has been known since the time of their discovery, and over the years much effort has been devoted to understanding the mechanisms responsible for this dramatic instability [recent reviews are in Refs (1–3)]. Such studies have identified multiple *cis*-acting structural features within the message itself as well as the participation of specific RNases that together contribute to the relative stabilities of different mRNAs. In addition, the translatability of a particular message, as determined by the strength of its Shine–Dalgarno sequence and other factors that influence the extent of ribosome loading also affect how rapidly an mRNA is degraded (6).

In considering mRNA decay, it is useful to distinguish between factors that influence initiation of the process from those that ultimately lead to complete breakdown of the message to mononucleotides, although since intermediates rarely accumulate, it is likely that the two phases of degradation are

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tightly coupled. In *Escherichia coli*, initiation of mRNA degradation is primarily due to endonucleolytic attack, generally mediated by the essential enzyme, RNase E (1,2). The action of RNase E is favored by an accessible 5′ terminus carrying a monophosphate residue, and cleavage usually occurs in AU-rich regions with little secondary structure. An important role for RNase E in mRNA decay was first suggested from studies of total mRNA turnover, and subsequently confirmed by many studies examining breakdown of individual messages (1,2). More recent genomic analyses using microarrays have established that RNase E is a major participant in the turnover process (7). However, a number of other endoribonucleases also participate in mRNA decay to a limited degree. These include RNase G, a homolog of RNase E (8), RNase III, an enzyme acting specifically on double-stranded structures (8), and RNase P, whose action is primarily on tRNA precursors (8). In addition, under certain circumstances, which are not well understood, a number of bacterial toxins such as RelE, MazF and Kid may also initiate mRNA degradation (8).

An interesting feature of RNase E action is that it appears to function as part of a multiprotein complex, the RNA degradosome, that contains, in addition, the exoribonuclease, polynucleotide phosphorylase (PNPase), an RNA helicase, RhlB, and the glycolytic pathway enzyme, enolase (1,2). Other components in sub-stoichiometric amounts may also be present (9). The association of an endoribonuclease, an exoribonuclease and an RNA helicase would seem to make the degradosome ideally suited for the breakdown of RNA molecules. Nevertheless, it has been difficult to prove this point or even to demonstrate that the degradosome actually exists *in vivo*. In fact, cells containing truncated forms of RNase E, which precludes degradosome assembly, grow relatively normally and display normal half-lives for several mRNAs (10). On the other hand, a more extensive analysis of degradosome function using DNA microarrays revealed that the assembled multiprotein complex was necessary for decay of some mRNAs *in vivo* (7). In addition, the degradosome was shown to be important for removal of mRNA fragments containing highly structured repeated extragenic palindrome (REP) elements (11). Thus, the most recent evidence suggests that the degradosome does function *in vivo*, but that it may play only a limited role.

Following an initial endonucleolytic cleavage which likely inactivates the message for translation, additional cleavages result in breakdown of the mRNA into fragments. Details of these secondary cleavages regarding the enzymes involved, the number of cuts and the sites of cutting remain somewhat murky as intermediates in the process rarely accumulate due to subsequent degradation to the mononucleotide level by exoribonucleases. In *E.coli*, three large, processive 3′–5′ exoribonucleases are primarily responsible for degradation of the mRNA fragments [in contrast to eukaryotes, eubacteria and archaea lack 5′–3′ exoribonucleases, Ref. (12)]. These three nucleases are PNPase, RNase II and RNase R (8) (Table 1). PNPase is a phosphorolytic nuclease that generates nucleoside 5′-diphosphates, whereas the latter two enzymes are hydrolytic and release nucleoside monophosphates. Interestingly, although each of these three exoribonucleases has distinct catalytic properties *in vitro*, they display significant functional overlap *in vivo*. Thus, mutant cells lacking just one of the

Table 1. Processive exoribonucleases of *E.coli*

Nuclease	Subunit size (kDa)	Protein structure	Gene name	Gene location (min)	Mode of action
RNase II	73	α	*rnb*	29.0	Hydrolytic
RNase R	92	α	*rnr*	94.9	Hydrolytic
PNPase	77(α)	α₃ or	*pnp*(α)	71.3	Phosphorolytic
	47(β)[a]	α₃β₂	*rhlB*(β)	85.4	

[a]Ref. (57).

Table 2. Properties of cells lacking two processive exoribonucleases

Nucleases absent	Viability	Phenotype
PNPase, RNase II	–	Accumulates mRNA fragments[a]
PNPase, RNase R	–	Accumulates rRNA fragments and mRNA fragments containing REP elements[a]
RNase II, RNase R	+	None observed

[a]Based on ts strains at a non-permissive temperature.

three nucleases grow essentially normally, indicating that the remaining two enzymes can rescue the missing function. In marked contrast, the absence of both PNPase and RNase II (13) or of PNPase and RNase R (14) leads to inviability. These findings imply the existence of one or more essential functions that involve the action of either PNPase or RNase II, which cannot be carried out by RNase R, and of a second essential function requiring either PNPase or RNase R for which RNase II cannot substitute. On the other hand, since cells lacking RNase II and RNase R are relatively unaffected, the remaining enzyme in this situation, PNPase, appears to have sufficiently broad specificity to carry out all essential functions at rates that do not have a major impact on cell growth (Table 2).

What might these essential functions be? For cells lacking both PNPase and RNase R, it is likely to be their inability to degrade RNA molecules containing extensive secondary structure. RNase R, by itself (15), and PNPase, as part of the degradosome (16) or in association with RhlB (17), can degrade structured RNA fragments *in vitro*, and are required for such degradation *in vivo*. Thus, cells lacking these two enzymes accumulate large amounts of rRNA fragments (18) and of mRNA decay fragments containing highly structured REP elements (15), consistent with the fact that the remaining RNase II cannot digest highly structured RNA molecules (15,19). The reason that cells lacking PNPase and RNase II are inviable is less evident. One possibility is that there is simply insufficient RNase R to carry out all the essential functions normally carried out by the two missing nucleases. If this explanation is correct, overexpression of RNase R should rescue the double mutant strain. One additional prediction regarding double mutant strains is that in cells lacking the hydrolytic nucleases, RNase II and RNase R, the final products of RNA degradation should primarily be nucleoside 5′- diphosphates since under these conditions PNPase would be expected to be the major contributor.

This latter point raises the question of what are the relative contributions of hydrolytic and phosphorolytic degradation to overall mRNA decay when all RNases are present. Early work, using ^{18}O analysis to determine the mode of phosphodiester

bond breakage, indicated that in *E.coli* mRNA decay is primarily hydrolytic, whereas in *Bacillus subtilis* it is predominantly non-hydrolytic (20,21). The enzymatic basis for this difference was demonstrated by the finding that crude extracts of *E.coli* degrade RNA using primarily RNase II, whereas *B.subtilis* lacks RNase II and RNA degradation in extracts is due largely to the phosphorolytic nuclease, PNPase (22). Indeed, a major role for PNPase in mRNA decay in *B.subtilis* has been confirmed (23). In PNPase mutant strains, fragments resulting from mRNA decay accumulate. These data indicate that while the initial endonucleolytic cleavages can continue in the absence of PNPase, the rate of removal of the resulting fragments is greatly slowed. Nevertheless, PNPase is not an essential enzyme (24) presumably because in its absence, other RNases assume a more important role.

The situation in *E.coli* is somewhat more confusing. Inasmuch as mRNA decay is primarily hydrolytic (20), the role of PNPase, and by inference the degradosome, must be limited, at least under usual laboratory growth conditions. Perhaps, there are certain conditions in which phosphorolytic decay assumes a greater role. For example, it is already known that PNPase levels increase during cold shock (25). Moreover, in the wild, where famine conditions may be more prevalent, phosphorolytic degradation could help to conserve energy during the constant synthesis and decay of mRNAs. However, under conditions in which hydrolytic degradation is the norm, then the relative contributions of RNase II and RNase R need to be considered. Until recently, RNase II was thought to be a major contributor to mRNA decay (13). However, this idea was thrown into question based on a genome-wide analysis of message levels in cells lacking RNase II (26). In fact, many mRNAs became less stable in the absence of this nuclease, suggesting that for these molecules, RNase II might serve to protect their 3′ ends, rather than degrade them. In addition, an important role for RNase R in mRNA decay was subsequently discovered, especially for those molecules with considerable secondary structure (15). RNase R was also found to increase dramatically in response to a variety of stress conditions (27,28). As a consequence of this new information, the relative contributions of RNase II and RNase R to overall mRNA decay need to be re-evaluated.

The processive exoribonucleases, PNPase, RNase II and RNase R, are unable to complete the degradation of mRNA fragments because they are relatively inactive against short oligonucleotides (8). These residual products are digested to mononucleotides by oligoribonuclease, an exoribonuclease specific for very short chains (8). In the absence of oligoribonuclease small fragments derived from mRNA, 2–5 nt in length, accumulate to high levels (29). Since oligoribonuclease is essential for cell viability, it is presumed that the presence of these RNA fragments is deleterious to the cell, but this remains to be proven.

STABLE RNA DEGRADATION

Stable RNA, which consists primarily of rRNA and tRNA, is generally not degraded during exponential growth. However, under certain physiological conditions or treatment of bacteria with certain agents, extensive degradation of these molecules may occur. In addition, quality control of stable RNA metabolism also is accompanied by RNA degradation. Since a detailed review of this area was published relatively recently (5), only a few relevant highlights will be discussed here.

Stable RNAs encompass as much as 98% of total cellular RNA, thus representing a large potential storehouse of nutrients that could be used under conditions of starvation. In fact, it has been known for many years that stable RNA, particularly rRNA, can be extensively degraded under starvation conditions (5). Other slow growth conditions such as stationary phase or following a nutritional downshift also lead to rRNA degradation (5). The mechanisms that protect ribosomes from degradation during exponential phase, but allow extensive degradation under various slow- or no-growth conditions, are not understood. Likewise, the RNases contributing to the degradative processes under these conditions remain to be identified conclusively. Early work suggested that stable RNA degradation is initiated by endonucleolytic cleavages followed by exoribonuclease action to generate mononucleotides (30). This sequence of events clearly is reminiscent of what occurs during mRNA decay. Moreover, rRNA fragments have been found associated with the degradosome, and this structure can degrade rRNA *in vitro* (31). Whether, the degradosome or RNase E also participate in rRNA degradation *in vivo* is not yet known. There is evidence that PNPase, another degradosome constituent, participates in RNA degradation during carbon starvation (30); however, whether it does so as a free enzyme or as part of the degradosome remains to be determined.

Stable RNA is also degraded in response to a variety of agents that alter membrane permeability (5). The extensive degradation that accompanies such treatment is probably due to loss of ions, such as Mg^{2+}, which help to stabilize ribosome structure, and to the entry of RNase I into the cell cytoplasm. RNase I is a nonspecific endoribonuclease that is most active in the absence of divalent cations (8). It resides primarily in the periplasmic space in *E.coli*, and breakdown of membrane integrity would be expected to lead to its entry into the cell resulting in extensive destruction of RNA. In confirmation of this scenario, extensive RNA degradation due to these agents does not occur in cells lacking RNase I (5).

Finally, stable RNAs are degraded as a consequence of quality control processes that operate during the course of their metabolism (5). Thus, a mutant form of tRNA^{Trp} that does not fold properly is present in cells at only ~15% of wild-type levels due to extensive degradation (32). Degradation occurs primarily at the level of the tRNA precursor and is greatly stimulated by its prior polyadenylation. In the absence of poly(A) polymerase, large amounts of the defective precursor accumulate because degradation is slowed. Degradation is carried out by PNPase (32), and also by RNase R (S. Chebolu and M.P. Deutscher, unpublished data). These data establish the existence of a quality control mechanism for defective tRNAs, and they provide a possible explanation for why stable RNAs are synthesized as precursors.

A second example of quality control, in this case for rRNA, comes from studies of a temperature sensitive mutant strain that lacks PNPase and RNase R at the non-permissive temperature (18). As noted earlier, strains lacking these two enzymes are inviable. In the absence of these two exoribonucleases, cells accumulate large amounts of fragments of 16S

and 23S rRNA, implying that they normally would be degraded by the missing RNases. The fragments are thought to arise by endonucleolytic cleavage of rRNAs that are not properly assembled into ribosomes due to insufficient ribosomal proteins or errors during the assembly process. These data indicate that PNPase and RNase R play an important role in the degradation of stable RNAs.

RNA MATURATION

Most RNA molecules, particularly stable RNAs, are synthesized as precursors that must be processed to their mature forms in order to function. These processing reactions, which can be quite extensive, also require the action of multiple RNases [reviewed in Ref. (33)]. Over the years, there has been considerable progress in identifying the RNases responsible for many of the processing steps such that in *E.coli* a number of maturation pathways are now fairly well defined. The general picture that emerges from all of this information is that individual RNAs are first separated from other RNAs with which they may be co-transcribed by endonucleolytic cleavages. These include individual rRNAs and tRNAs within the ribosomal transcript, individual tRNAs present in multimeric tRNA precursors, or tRNAs associated with mRNAs in multifunctional transcripts. Following the cleavages that separate individual RNAs, additional processing reactions remove extraneous terminal sequences to expose the mature 5′ and 3′ termini. In what follows, a brief description of the various known maturation reactions will be presented.

The 16S, 23S and 5S rRNAs are co-transcribed as a single RNA molecule. Depending on the particular rRNA locus (there are seven in *E.coli*), one or several tRNAs are also part of the transcript, located between the 16S and 23S sequences and downstream of the 5S RNA. Owing to the existence of complementarity between sequences flanking 16S RNA and sequences flanking 23S RNA, the double strand-specific endoribonuclease, RNase III, cleaves in these regions to release precursors to 16S RNA, 23S RNA and the tRNA between them [reviewed in Ref. (34)]. The resulting downstream fragment containing 5S RNA and tRNA is subsequently cleaved by RNase E to generate precursors to each of these molecules. In the absence of RNase III, a 30S transcript accumulates; yet, cells remain viable because secondary cleavages by other endoribonucleases allow some functional rRNA to be made. These secondary cleavages are thought to be part of the normal processing pathways for the individual RNAs, but apparently can also occur on the original, unprocessed transcript at a rate sufficient to maintain cell viability.

Maturation of the 5′ terminus of 16S rRNA requires the combined action of two endoribonucleases, RNase E and RNase G (35,36). The long 115 nt leader sequence is first cleaved 66 nt upstream of the mature terminus by RNase E, followed by a second cleavage at the mature 5′ terminus by RNase G. Maturation still can proceed when only one of the two enzymes is present, but it is much less efficient and some incorrect product may be generated. No processing occurs when both RNases are absent. Maturation of the 3′ terminus of 16S rRNA has not yet been elucidated, but it most likely is due to a single endonucleolytic cut to release the 33 nt 3′

trailer. The RNase(s) responsible for removing the short 5′ leaders of 23S RNA and 5S RNA also have not yet been identified. However, their 3′ termini are generated by action of the exoribonuclease, RNase T (37,38). In its absence, precursors with extra 3′ residues accumulate that, nevertheless, can still be assembled into functional ribosomes.

Maturation of tRNA molecules also requires a series of processing steps (39,40). As mentioned, precursors to individual tRNAs that are part of an rRNA transcript are released during the course of rRNA maturation either by the action of RNase III or of RNase E. Separation of individual tRNA precursors from multifunctional transcripts containing mRNAs or other tRNAs is accomplished primarily by RNase E action (40,41). Once released from the rest of the transcript, or directly in the case of monomeric tRNA precursors, additional processing reactions generate the mature 5′ and 3′ termini. Cleavage by the ribonucleoprotein (RNP), RNase P, serves to mature the 5′ terminus of all tRNA molecules (8). However, 3′ maturation is more complex, and may differ depending on the organism and the particular tRNA precursor (Figure 1).

In *E.coli*, an organism in which all tRNAs have their 3′-CCA sequence encoded in the gene, 3′ maturation is carried out by exoribonucleases, primarily RNase T and RNase PH (42). However, in the absence of these two enzymes, other exoribonucleases, such as RNase II and RNase D or RNase BN, which has both exo- and endoribonuclease activity (43), may also contribute (42). Any one of the five mentioned nucleases is sufficient to maintain *E.coli* viability, although with markedly different growth rates (44), most likely because the latter three enzymes process tRNA less efficiently (45). When the 3′ trailer sequence on the tRNA precursor is relatively long (>15 nt), PNPase and RNase II may participate in shortening it prior to final trimming by the other enzymes (40). Which of the exoribonucleases plays the major role in processing the 3′ end of a particular tRNA precursor is stochastic, and is influenced by the secondary structure and sequence of the 3′ trailer (42). For example, RNase T slows down dramatically at two consecutive pyrimidine residues and is stopped almost completely by two consecutive C residues (46). Thus, in molecules containing such precursor sequences, RNase PH would act preferentially.
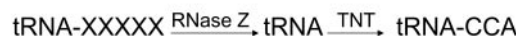

Figure 1. Maturation of the 3′ ends of tRNA precursors. In *E.coli*, where the –CCA sequence is encoded, primarily the exoribonucleases, RNase T and RNase PH, remove the extra 3′ residues. In *B.subtilis*, precursors containing the –CCA sequence are processed as in *E.coli*, using RNase PH. Precursors lacking the –CCA sequence are first cleaved by the endoribonuclease, RNase Z, followed by addition of the –CCA sequence by tRNA nucleotidyltransferase (TNT). However, a few CCA-less precursors also use RNase PH (47). X represents precursor-specific residues.
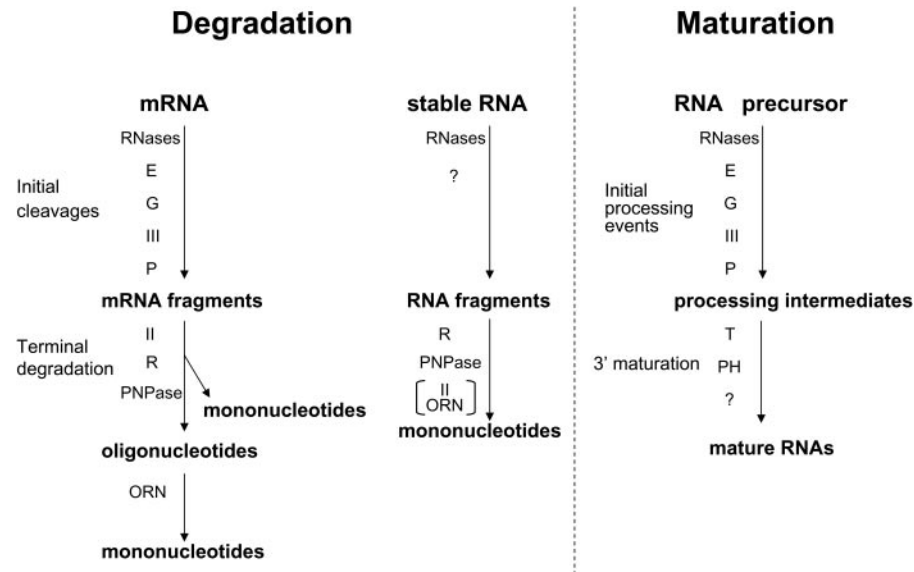
Degradation

mRNA

RNases

Initial
cleavages

E

G

III

P

mRNA fragments

Terminal
degradation

II

R

PNPase

mononucleotides

oligonucleotides

ORN

mononucleotides

stable RNA

RNases

?

RNA fragments

R

PNPase

[II
ORN]

mononucleotides

Maturation

RNA precursor

RNases

Initial
processing
events

E

G

III

P

processing intermediates

3' maturation

T

PH

?

mature RNAs

Figure 2. Comparison of mRNA and stable RNA degradation and RNA maturation in *E.coli*. Initial cleavages during mRNA decay or maturation of rRNA and tRNA are carried out by the endoribonucleases, RNases E, G, III and P, although depending on the process, a different enzyme may serve the primary role. The enzyme(s) responsible for generating fragments of stable RNA is (are) not known. Terminal degradation of mRNA decay intermediates utilizes the processive exoribonucleases, RNases II, R or PNPase, followed by oligoribonuclease (ORN) to remove 5′ terminal oligonucleotides. Stable RNA degradation also utilizes RNase R or PNPase, but roles for RNase II and ORN have not yet been shown. Where known, maturation of the 3′ termini of tRNAs and some rRNAs uses RNases T or PH, but for 16S rRNA, the 3′ maturase has not been identified.

In *B.subtilis*, 3′ processing of tRNA precursors is somewhat different. In this organism, only about two-thirds of the tRNAs have their –CCA sequence encoded. Precursors to these tRNA are matured exonucleolytically using RNase PH to remove the last few nucleotides adjacent to the –CCA (*B.subtilis* does not contain an RNase T ortholog) (47). Other redundant exoribonucleases contribute to shortening of the 3′ trailer, but apparently do not participate in the final trimming steps. For the population of tRNA precursors lacking the –CCA sequence, a different pathway for 3′ processing prevails. In this case, an endonuclease, termed RNase Z, cleaves the precursor after the discriminator base, and tRNA nucleotidyltransferase subsequently adds the 3′ terminal –CCA residues (48). A few tRNA precursors lacking the –CCA sequence can use either maturation pathway.

Interestingly, *E.coli* also possesses an RNase Z ortholog, RNase BN (43). This enzyme is required for maturation of the 3′ termini of four bacteriophage T4 tRNA precursors that lack an encoded –CCA motif (49), but as all *E.coli* tRNAs encode the –CCA, its role in uninfected cells is unclear. As noted earlier, RNase BN can process *E.coli* tRNA precursors *in vivo* when RNases T, PH, D and II are absent, but it does so extremely poorly (44,45). *E.coli* RNase BN is unusual in that it can function as either an exo- or endoribonuclease *in vitro* (43). However, it's mode of action on tRNA precursors *in vivo* has not yet been ascertained.

COMPARISON OF mRNA DECAY AND STABLE RNA DEGRADATION

Based on the foregoing discussion, it is evident that mRNA decay and stable RNA degradation share many common features. Thus, the overall processes, involving initial endonucleolytic cleavages followed by exonucleolytic digestion of the resulting fragments are remarkably similar for mRNA and rRNA (it is not yet known whether endonucleolytic cleavages play any role in quality control of defective tRNA) (Figure 2). What differs in the two processes is that mRNA decay is an ongoing aspect of cell metabolism, whereas rRNA degradation occurs only under special conditions. What stabilizes rRNA during normal growth, in contrast to mRNA, is not clear, but is likely to be due to its association with ribosomal proteins in a stable RNP particle that precludes accessibility to RNases. However, if a ribosome is misassembled such that the rRNA is not fully protected, degradation would ensue, resulting in what we call quality control (18). This would provide a simple mechanism for removing defective ribosomes. The process during starvation that leads to rRNA degradation is not understood, but it is likely due to some alteration in ribosome structure that results in increased accessibility to an RNase. In those situations in which rRNA degradation results from RNase I entry into the cell, its small size (27 kDa) presumably allows it to act on rRNA within the ribosome structure, as ribosomes are sensitive to RNase I *in vitro* (50).

Perhaps, the most obvious similarity between mRNA decay and stable RNA degradation is that the same RNases participate in both processes (Figure 2). This is most apparent for the exoribonucleases, and it is not surprising that the three major participants, PNPase, RNase II and RNase R, all act processively, as this would be the most efficient way to degrade RNA fragments. Also, what has emerged from various studies is that it is the RNA structure, not the type of RNA (mRNA or stable RNA) that determines which RNases will be used for degradation. Thus, PNPase and RNase R seem to be the all-purpose degraders of RNAs with secondary structure, whether it be mRNA REP elements (15), defective tRNAs (32) or fragments

of rRNA (18), because of their ability to digest such molecules. When long stretches of unstructured RNA need to be degraded, RNase II will probably be the major contributor in *E.coli* because of its rapid action on such molecules and its presence in cells at high levels (22). Likewise, oligoribonuclease is the major enzyme removing residual oligonucleotides generated during RNA degradation because it is the only RNase that can do so efficiently (29).

Another similarity between mRNA decay and stable RNA degradation that was initially quite surprising is the significance of polyadenylation for both processes. The presence of poly(A) tails on mRNAs, and also on mRNA fragments, was clearly established, and its importance for mRNA decay *in vivo* demonstrated (51). What was unexpected, however, is that stable RNA precursors also accumulate poly(A) tails when they cannot be matured (52), and that the poly(A) tail is needed for efficient removal of defective tRNA *in vivo* (32). It is also known that incomplete rRNAs can accumulate poly(A) tails (53), but their role in rRNA degradation has not yet been demonstrated. However, such a role would be expected because it is now understood that poly(A) tails serve as a required single-stranded binding site for each of the three degradative exoribonucleases, PNPase, RNase II and RNase R (15). None of these enzymes is able to act on a completely double-stranded RNA molecule or even one with a short 3′ tail, presumably due to their inability to bind the substrate (15). The importance of a single-stranded tail for enzyme binding has now been confirmed directly for RNase R by RNA binding experiments (H. Vincent and M.P. Deutscher, unpublished data).

One feature of RNA degradation that needs to be clarified to be able to fully compare mRNA decay and stable RNA degradation is to identify the endoribonucleases involved in stable RNA degradation and to assess the role of the degradosome in each process. In the absence of PNPase and RNase R, cells accumulate large amounts of fragments derived from 16S and 23S rRNA (18). However, the endoribonuclease responsible for generating these fragments has not been identified. In a similar fashion, fragments derived from mRNAs containing REP elements also accumulate in cells lacking PNPase and RNase R (15). However, these fragments are known to be generated by RNase E, as part of the degradosome (11). Continuing the idea of common mechanisms for all RNA degradation, it would not be surprising if RNase E or RNase G served a similar role in initiating degradation of rRNA. In fact, these enzymes have been shown to participate in ribosome degradation in an *in vitro* system (M. Zundel and M.P. Deutscher, unpublished data).

If RNase E is involved in stable RNA degradation, the question then arises as to whether it does so as part of the degradosome, as in mRNA breakdown. It is known that RNase E can function almost normally for 5S rRNA maturation even in the absence of degradosome formation (10), indicating that the degradosome is not required for all actions of RNase E. On the other hand, even a small loss in processing efficiency may have profound effects on growth under natural conditions. Considering a role for the degradosome in stable RNA degradation, it is interesting to note that the protein composition of the *E.coli* degradosome changes under at least one stress condition, cold shock, leading to replacement of the RNA helicase, RhlB, with another helicase, CsdA (54).

Inasmuch as two other enzymes involved in stable RNA removal, PNPase and RNase R, are greatly elevated during cold shock (25,27,28), these data suggest that this stress condition may affect RNA stability. The concomitant alteration of the degradosome lends support to its participation, as does the ability of isolated degradosomes to degrade rRNA *in vitro* (31). Along these lines, it is intriguing that the degradosome present in *Pseudomonas syringae* Lz4W contains RNase R rather than PNPase (55). It is also of considerable interest that other recent work indicates that the degradosome may vary in composition in response to the environment (9), and that RNase E may be programmed for decay of specific RNAs by association with small, noncoding RNAs (56). All of this information suggests that RNase E may be part of multiple RNP complexes, and that the RNA degradosome is just one of them.

RELATION OF RNA DEGRADATION AND RNA MATURATION

It is also evident that there is significant overlap between the RNases involved in RNA degradation and those participating in RNA maturation, especially with respect to the endoribonucleases (Figure 2). Thus, RNases E, G, III and P contribute to both mRNA decay and to maturation of stable RNAs. What seems to differ between the degradative and maturation processes are the exoribonucleases that carry out the various reactions. For degradative reactions, the processive enzymes, PNPase, RNase II and RNase R, are used exclusively. In contrast, while these latter enzymes may also contribute to RNA maturation, generation of mature 3′ termini generally utilizes the distributive enzymes, RNase T, RNase PH and RNase D. Presumably, the action of these RNases avoids the possibility of unwanted degradation that might occur if their processive counterparts were used. How coordination between the two classes of exoribonucleases is achieved when both participate in maturation of the same RNA precursor remains to be explored.

CONCLUSIONS

From the information presented, it is evident that mRNA decay and stable RNA degradation are largely the same process, and that they also overlap to some degree with RNA maturation. The conclusion that emerges is that bacterial cells do not contain dedicated degradative machinery for different classes of RNA, and for different processes, as might have been expected given the large number of RNases present in an organism such as *E.coli*. Rather, RNases will initiate action on an RNA based on their intrinsic specificity, the amount of RNase present and the accessibility of the RNA substrate. Unless protected in some fashion, any RNA is a potential target at any time. Based on this argument, what differentiates the stability of one mRNA from another, or mRNA from stable RNA, is simply whether an RNase can gain access to a site on the RNA at which it can act. Likewise, RNA precursors are rapidly converted to mature forms because the precursor sequences are open to RNase action. However, once converted to a mature, stable RNA, the RNA is somehow protected against further RNase action. This

protection may be due to tertiary structure, assembly into an RNP particle, or even blocking or burying the RNA's 3′ terminus.

Considered in this light, mRNAs are unstable because they are relatively unprotected, and stable RNAs can become substrates if they do not fold properly, are not properly assembled into an RNP, or if their 3′ terminus is not blocked. Moreover, under certain physiological conditions, a normally stable RNA can become a substrate for degradation if it loses its protection or if RNase activity changes. Thus, future work in this area will focus on how a stable RNA might be altered such that it becomes a substrate in response to changed environmental conditions, or what regulatory processes might come into play that would elevate pre-existing RNases or lead to production of new ones. Another area of interest is to understand why prokaryotes vary so much in the number and types of RNases that they contain even though their RNA metabolism may be quite similar. It is clear that despite the tremendous progress in our knowledge of RNA metabolism in recent years, there is still much to be learned and more surprises to come.

ACKNOWLEDGEMENTS

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