JOURNAL OF BACTERIOLOGY, Aug. 1993, p. 4577–4583
0021-9193/93/154577-07$02.00/0

Vol. 175, No. 15

MINIREVIEW

Promiscuous Exoribonucleases of *Escherichia coli*

MURRAY P. DEUTSCHER

Department of Biochemistry, University of Connecticut Health Center, Farmington, Connecticut 06030-3305

INTRODUCTION

RNases play a central role in the metabolism of RNA. Among the many reactions catalyzed by these enzymes are those that separate individual entities of multicomponent transcripts, those that generate the mature 5′ and 3′ termini of RNAs, and those that remove introns. RNases also degrade RNA molecules that are no longer needed by the cell or that are damaged in some way, and under some conditions they also digest RNAs to supply nucleotides for continued RNA synthesis. As knowledge of the details of RNA metabolism has expanded, there has been a growing realization that many RNases with distinct specificities might be needed to catalyze the diverse reactions undergone by RNA molecules. Our understanding of this problem has progressed most rapidly in studies with *Escherichia coli*, so that for this organism close to 20 RNases are already known (17), and others undoubtedly exist.

Exoribonucleases are a class of RNases that digest RNA molecules from their ends. The exoribonucleases currently known generate mononucleotide residues which can be reutilized for RNA synthesis. Exoribonucleases have been implicated in a number of RNA metabolic events, including RNA maturation and RNA degradation. In the case of RNA maturation, exoribonucleases participate in the final processing steps, trimming terminal residues from certain RNA precursors. Exoribonucleases also play a role in degrading unwanted RNA molecules to the mononucleotide level. In *E. coli*, two exoribonucleases that act nonspecifically in vitro on all types of RNA molecules, PNPase and RNase II, have been known for many years (45, 66). The properties of these two enzymes, and those of nonspecific exoribonucleases identified from other organisms, incorrectly colored the early thinking about this class of enzymes. However, in contrast to the small number of nonspecific, degradative exoribonucleases initially identified, it is now clear that a single cell can contain a large number of distinct proteins that function to digest RNA exonucleolytically. This minireview will discuss the information that has come to light in recent years concerning this family of enzymes from *E. coli*.

MULTIPLE EXORIBONUCLEASES

Catalytic properties. Eight exoribonucleases have been isolated from *E. coli* (Table 1). At least seven of them, PNPase and RNases II, D, BN, T, PH, and R, are distinct enzymes on the basis of their structures, catalytic properties, and genetics (see below). It is not yet clear whether the eighth enzyme, oligoribonuclease, is a separate entity or an activity of one of the other RNases. When oligoribonuclease was identified it was shown not to be related to PNPase or RNase II, the only two exoribonucleases known at that time (15, 51). However, with the subsequent discovery of the other enzymes shown in Table 1, a possible relationship to one of them has not been eliminated, especially since the latter enzymes have not been tested against the oligoribonucleotide substrates favored by oligoribonuclease. Additional genetic and biochemical studies will be needed to resolve this question.

The other seven exoribonucleases differ from one another in their catalytic properties, but they also show a significant amount of overlap. PNPase and RNase II have similar specificities in that they act on all unstructured RNAs and homopolymers. Both enzymes degrade RNA processively in the 3′ to 5′ direction (33, 48). However, they differ dramatically in their mechanisms of action. RNase II hydrolyzes RNA, releasing nucleoside 5′ monophosphates (66), whereas PNPase degrades RNA phosphorolytically, generating nucleoside diphosphates as products (45). The latter mode of action recaptures the energy present in the phosphodiester bond and can serve to preserve energy under poor nutritional conditions (26). In fact, in *Bacillus* species, which generally live under energy-poor conditions, the phosphorolytic mode of degradation predominates (29) because these cells lack RNase II (26).

Another exoribonuclease that acts relatively nonspecifically with regard to substrates, RNase R, was originally identified and partially purified from an *E. coli* strain deficient in RNase II (39). RNase II accounts for more than 95% of the activity against poly(A) in cell extracts (41, 67), but the residual activity is due to RNase R (17, 19, 23). RNase R can degrade rRNA and mRNA in addition to homopolymer substrates (23, 39) and can be distinguished from RNase II by its higher relative activity on rRNA compared with homopolymers, by its chromatographic properties, and by mutations which inactivate one or the other enzyme (39). On the basis of these results it is very likely that RNase R represents a distinct protein, but additional work is necessary to solidify this conclusion.

The remaining four exoribonucleases (D, BN, T, and PH) were all identified by their action on tRNA substrates in the course of searching for the enzyme that matures the 3′ ends of tRNA precursors (18). RNase D was originally identified as an enzyme that would degrade the denatured tRNA molecules that arise upon removal of the -CCA sequence from certain species of tRNA or upon denaturation of intact tRNA (30). Subsequent studies with specially designed artificial tRNA precursors (20) revealed that RNase D also could remove the last few residues following the -CCA sequence of these molecules (31). Although it had been suggested earlier that RNase II was responsible for the 3′ processing of tRNA precursors (64), comparison of homogeneous preparations of RNase II and RNase D indicated that only the latter enzyme could generate functional tRNA molecules (11). RNase D removes precursor residues following the -CCA sequence

TABLE 1. Summary of properties of *E. coli* exoribonucleases and their genes

RNase	Size (kDa), protein structure	Gene[a]	Map position (min)	Gene sequence known	Preferred substrates in vitro	Suggested function(s) in vivo
PNPase	~260, α_3 ~360, $\alpha_3\beta_2$	*pnp*	69 (α)	+	Unstructured RNAs	mRNA degradation
II	~70	*rnb*	28	+	Unstructured RNAs	mRNA degradation, tRNA maturation
D	42.7	*rnd*	40	+	Denatured tRNAs, tRNA-CCA-CN	Denatured tRNA degradation, tRNA maturation
BN	~60	(*rbn*)		−	tRNA-CU, tRNA-CA	tRNA maturation
T	47, α_2	*rnt*	36	+	tRNA-CCA	tRNA end turnover, tRNA maturation
PH	Subunit = 25.5 (aggregates)	*rph*	81.7	+	tRNA-CCA-Cn	tRNA maturation
R	~80	(*rnr*)		−	rRNA, homopolymers	Unknown
oligo	~38			−	Oligoribonucleotides	Unknown

[a] Mutations affecting RNase BN (46) and RNase R (39) are known; however, since the genes have not been mapped or named, the designations given here in parenthesis should be considered tentative. The map position shown for PNPase is for the α subunit. The native size of RNase PH is not known, because this enzyme aggregates (37, 40).

about 20- to 40-fold more rapidly than it acts on mature tRNA (14), and it also hydrolyzes the RNA in a random (nonprocessive) manner (11, 14), providing ample time for an aminoacyl-tRNA synthetase to charge the mature tRNA generated by RNase D action. However, upon overexpression of RNase D in strains that lack tRNA nucleotidyltransferase and are thus unable to repair the 3′ end of tRNA (72), even the low rate of action of RNase D on intact tRNA is sufficient to lead to tRNA degradation in vivo (69).

RNase D is highly specific for tRNA substrates in vitro, with no detectable activity against homopolymers (14, 31), which is in marked contrast to PNPase, RNase II, and RNase R. The discovery of RNase D demonstrated for the first time that exoribonucleases can be highly specific enzymes that recognize more than just the terminus of an RNA molecule. This was shown most dramatically by the addition of a second -CCA sequence following the mature -CCA terminus in tRNA. While the mature terminus is relatively resistant to RNase D action, as noted above, the second -CCA sequence is removed rapidly (14). These data indicate that the relative resistance of tRNA to RNase D is not due to the -CCA sequence per se but to its location within the three-dimensional structure of the tRNA molecule. Thus, as already suggested by its more rapid hydrolysis of denatured tRNA, RNase D is markedly influenced by the subtleties of tRNA structure.

The existence of another exoribonuclease, RNase BN, was initially inferred from studies of the 3′ processing of certain tRNAs encoded by bacteriophage T4 (65). These tRNA precursors lack the -CCA sequence present in those of the host and are acted on poorly by RNase D (31), suggesting that another enzyme might be involved. Moreover, two *E. coli* strains, BN and CAN, were known that could not support growth of a phage T4 mutant that required the suppressor function of a T4 $tRNA^{Ser}$ (46). The defect in strain BN was associated with decreased maturation of the 3′ terminus of phage tRNAs (64), and with decreased hydrolysis of tRNA-CU, a phage tRNA precursor analog (63). The mutation in strain BN did not affect RNase II or RNase D, supporting the idea that another RNase was involved (63). RNase BN was subsequently identified in strains lacking RNase II and RNase D and was shown to be absent in strains BN and CAN (3, 4). Use of the RNase II^- RNase D^- background was necessary because the latter two active enzymes also hydrolyze tRNA-CU to some degree and mask RNase BN activity.

RNase BN has not been purified completely, but it is sufficiently pure to ensure that its catalytic properties differ from those of all the other known RNases (50). In contrast to the other exoribonucleases, RNase BN is most active at pH 6 in the presence of Co^{2+}. The preferred substrates are tRNA-CU, tRNA-CA, and tRNA-U, in which the -CCA sequence has been replaced by other residues. tRNA-CC, which contains the first two correct residues, results in much less activity (3). Likewise, the enzyme works relatively poorly on tRNA-CCA-C_{2-3}, an analog of the host-type precursors which contains two or three C residues following the -CCA sequence, and it is also inactive against poly(A). Comparison of the activities of RNase D and RNase BN using the substrates tRNA-CCA-C_{2-3} and tRNA-CU revealed a difference of over 60-fold between the two enzymes in their abilities to remove terminal residues from the two types of precursor analogs, with each enzyme favoring one or the other substrate (3, 50). These findings demonstrate the high degree of substrate specificity that can be exhibited by exoribonucleases.

RNase T was discovered in a search for the enzyme responsible for the end turnover of tRNA. Removal and resynthesis of the 3′ terminal AMP and penultimate CMP residues of tRNA has been known since the earliest studies of tRNA metabolism (18). Although the synthetic phase of the process was known to require tRNA nucleotidyltransferase (21), the nuclease responsible for removal of the terminal residues was not known. Since multiple mutant cells lacking RNases II, D, and BN still underwent the turnover process (23), it was clear that another enzyme had to be involved. RNase T was identified in extracts of this multiply RNase-deficient strain using as a substrate intact tRNA-CCA labeled in its terminal residue (23), and it was purified to homogeneity on the basis of removal of AMP from this molecule (22). The in vitro catalytic properties of RNase T are completely consistent with a role for this enzyme in tRNA end turnover. Thus, RNase T action on intact tRNA is 5- to 10-fold more rapid than on either type of tRNA precursor, only uncharged tRNA molecules are substrates for the enzyme, and removal of the penultimate CMP residue is only 10% as rapid as removal of the terminal AMP (22). In its mode of action, RNase T shows yet another type of specificity that differs from those of all the enzymes discussed previously.

The presence in *E. coli* of at least one more exoribonuclease was postulated on the basis of almost normal growth

of a cell lacking RNases II, D, and BN and 70% of RNase T (24). Moreover, extracts prepared from such cells were still able to process tRNA precursors in an exonucleolytic fashion (12). The surprising feature of this processing was that it required P_i; the residues removed were released as nucleoside diphosphates, indicating a phosphorolytic mode of attack. Further examination revealed that PNPase, the only phosphorolytic nuclease known at that time, was not involved, and that the activity was due to a new enzyme, termed RNase PH (25). Interestingly, purification and N-terminal sequencing of RNase PH revealed that it was the product of an unidentified open reading frame, *orfE* (53), located upstream of and cotranscribed with *pyrE*, the gene encoding orotate phosphoribosyltransferase (58). The *orfE* gene product was purified to homogeneity (37) and shown to be identical to RNase PH (40). A similar enzyme was also identified in *Bacillus subtilis* (10).

The specificity of RNase PH is similar to that of RNase D in that it favors tRNA precursors with extra residues following the -CCA sequence over other tRNA-type substrates and can regenerate amino acid acceptor activity. Mature tRNA has an apparent K_m value about 10 times higher than that of the tRNA precursor (40). However, in contrast to RNase D, poly(A) is an active substrate. RNase PH is completely dependent on P_i for activity, and in keeping with a phosphorolytic mode of degradation, the equilibrium constant of the reaction is close to unity. Consequently, RNase PH can also synthesize RNA chains using nucleoside diphosphates as substrates (52). Chains as long as 13 for A incorporation and 40 for C incorporation have been observed. In many respects, RNase PH is similar to PNPase; however, the activity of RNase PH is about 60 times higher on tRNA-type substrates than that of PNPase when the activities are normalized to their actions on poly(A) (40).

As can be gathered from the above descriptions, each of the *E. coli* exoribonucleases is a catalytically distinct enzyme with unique substrate specificities. However, there is also a great deal of similarity among them. All of the enzymes act on RNA molecules in the 3′ to 5′ direction, releasing mononucleotides (mono or diphosphates) in divalent cation-dependent reactions. In this regard, all of the enzymes probably utilize a similar mechanism of phosphodiester bond cleavage, although this point needs to be proven conclusively. Even more importantly, the enzymes show a high degree of overlap in substrate specificity. Although each of the enzymes may prefer certain substrates over others, most of the RNases can act on most of the RNAs to some degree. This functional overlap in vitro extends to their actions in vivo and explains why many RNase-deficient mutants remain viable. This will be discussed in detail below.

Genes and protein structure. Probably the main reason that our understanding and knowledge of *E. coli* exoribonucleases is so much more advanced than it is for other organisms is the availability in this system of mutants affecting individual enzymes. Of the eight RNases identified, mutations affecting seven of them have been isolated, and five have been mapped (Table 1). The strains harboring these mutations, alone or in combination, have been invaluable for purifying the RNases, for distinguishing among the various activities, and for providing insights into their functions. The genetic studies have reinforced the conclusions based on catalytic properties that the identified exoribonucleases are distinct proteins. First, the five mapped genes are located at different positions on the chromosome. Although the mutations affecting RNase BN and RNase R have not yet been located, it is clear that *rbn* does not affect RNases II or D (63) and that *rnr* does not influence RNase II (39). Second, each of the mutations dramatically decreases the activity of only one of the enzymes, leaving the others unaffected (19, 23, 67). Moreover, removal of multiple RNases does not alter the activities that remain (41). On the basis of these initial observations, it appears that each of the exoribonuclease genes functions independently.

Five exoribonuclease genes (*pnp*, *rnb*, *rnd*, *rnt*, and *rph*) have now been cloned and sequenced (Table 1), and a number of interesting points have emerged from these analyses. (i) There is little or no similarity among the protein sequences as determined by computer analysis. In multiple alignments, no regions common to all the enzymes are evident (36, 61), suggesting no common exoribonuclease motif. In pairwise alignments of the various enzymes, small regions of similarity can be identified for some pairs (61); however, their functional significance is not known. (ii) There is no common theme among the enzymes with regard to their subunit size or quaternary structure. On the basis of examination of the proteins and their genes, it is clear that the various exoribonucleases vary greatly in structural properties (Table 1). Thus, RNases II and D are single-chain proteins with masses of about 70 and 40 kDa, respectively (1, 11, 68); RNase T is an α_2 dimer of 47 kDa (22, 36); PNPase is found in two forms, an α_3 trimer of approximately 260 kDa and a pentameric form of ~360 kDa that contains in addition β subunits of 48 kDa each (56, 59); and RNase PH consists of a 25.5-kDa subunit which displays various native molecular weights from the dimer on up, depending on the protein concentration (37, 40, 58). The significance of such a diverse group of structures for enzymes that are catalytically quite similar is not understood. (iii) Exoribonuclease genes are present in operons. For the three exoribonuclease genes in which this point has been examined, *pnp*, *rph*, and *rnt*, the nuclease gene is cotranscribed with that of another protein. *pnp* is downstream of *rpsO*, the gene which encodes ribosomal protein S15 (59); *rph* is upstream of and required for the transcription of *pyrE* (7); and *rnt* is upstream of a recently discovered gene that apparently encodes an RNA helicase (36). It is not clear whether these gene arrangements serve any regulatory function. (iv) Two of the RNase coding regions initiate with UUG codons. Although UUG is used very infrequently as an initiation codon in *E. coli*, it is present in both the *pnp* (59) and *rnd* (68) genes. In the latter case, the presence of UUG serves to down-regulate translation because its conversion to AUG increases RNase D expression by over 10-fold (70).

EXORIBONUCLEASE FUNCTION

Elucidation of the physiological roles of the individual exoribonucleases has proven to be extremely difficult, apparently because of their high degree of redundancy. Mutations affecting single enzymes generally have little or no effect, and many combinations of multiple RNase mutations likewise show minimal perturbations of function. Nevertheless, some clarification of the problem has emerged, particularly with regard to the functional overlap of the RNases in mRNA and tRNA metabolism.

Growth. The effect on growth of a particular mutation can serve as a crude measure of the degree that a gene's product is required for overall cell function. In order to affect growth, an enzyme's deficiency must slow some essential reaction sufficiently so that it becomes rate limiting for metabolism. In the extreme case, when no other enzyme can compensate,

even partially, for the one that is missing, growth will eventually cease and the mutant strain will be inviable. Alternatively, it is possible for the absence of an enzyme to slow down a specific pathway, yet not enough to become rate limiting for growth. In this situation, no effect on growth will be observed. In intermediate situations, alternate enzymes take over the missing enzyme's functions to allow some level of growth.

On the basis of the growth of single exoribonuclease mutant strains (containing either null or very defective mutations), none of these RNases are essential for cell viability (42, 60); consequently, none of them carries out a unique function that cannot be compensated for by another enzyme still present. For two of them, PNPase (42, 49) and RNase T (42, 54), the absence of either enzyme does lead to slightly slowed growth (~35- versus 24-min doubling time for the wild type), indicating that the compensatory RNases do not function as effectively as the one that is absent. Additional information about the interrelationships among the various exoribonucleases was obtained by combining mutations. Certain combinations of mutations have essentially no effect on growth, whereas others have dramatic consequences. For example, cells deficient in RNases II, D, BN, and most of T grow almost normally (24), whereas PNPase$^-$ RNase II$^-$ cells are inviable (28) and RNase T$^-$ PH$^-$ and PNPase$^-$ PH$^-$ cells grow slowly (42). From these types of experiments it is clear that for certain metabolic processes any one of a number of the exoribonucleases can function, whereas for other processes the range of effective enzymes is much more limited.

Considerable clarification about the degree of functional overlap in vivo among the exoribonucleases was obtained when it was found that a cell lacking RNases I, II, D, BN, T, and PH is inviable (42). This observation made it possible to analyze the effect of reintroducing a wild-type chromosomal copy of the gene for each of the five deficient exoribonucleases (41). Surprisingly, any one of the five enzymes is able to support cell growth despite the absence of four of the other RNases. The order of effectiveness for the enzymes in supporting growth is RNase T > RNase PH > RNase D > RNase II > RNase BN, with doubling times varying from 35 to 105 min in rich medium, compared to 24 min for the wild-type parent. Even a cell that contains only ~30% of RNase T also allows growth, with a 57-min doubling time. The data indicate that at least these five exoribonucleases are extremely promiscuous, with any one of them able to take over the functional roles of all the others, although with different degrees of effectiveness. Exactly what these functions are has not been solved completely, but some are known (see below). It should also be noted that PNPase, RNase R, and oligoribonuclease are still present in the mutant cells and undoubtedly contribute to the cells' ability to grow; nevertheless, the extent of functional overlap among the five enzymes is remarkable.

mRNA metabolism. One process for which exoribonuclease involvement has been delineated is mRNA breakdown. Mutant strains lacking both PNPase and RNase II accumulate fragments of mRNA that are 100 to 1,500 nucleotides long (28), and discrete fragments from specific mRNAs can also be detected (2). Cells lacking only one of the two RNases are unaffected, indicating that either enzyme is sufficient for the final breakdown of mRNA degradation products. RNase II and PNPase also participate in the 3′ processing or shortening of certain mRNAs, and in these instances the two enzymes are not necessarily functionally equivalent (33). PNPase also has a much greater effect on the instability of mRNAs from certain cloned genes expressed in *E. coli* (35). The apparently greater role of PNPase in some of these processes may account for the growth defect associated with its absence (42, 49). Another possible way of assessing an RNase's involvement in a metabolic process is by overexpression of a cloned gene. This has been done for RNase II, but no effect on mRNA degradation was observed (27). It is not clear whether these data contradict earlier studies describing a mutation leading to elevated RNase activity and increased mRNA degradation, since the involvement of RNase II, although suggested at the time, was not proven conclusively (32). With the discovery of many other RNases since that time, it is possible that some other activity was responsible.

tRNA metabolism. The involvement of exoribonucleases in various aspects of tRNA metabolism has been known or suspected for many years. The end turnover of tRNA requires an exoribonuclease to remove the last few residues within the -CCA sequence (18), and, as noted, RNase T is now known to catalyze this reaction in vivo. Mutant strains deficient in or devoid of RNase T are impaired in or unable to carry out the end turnover process (24, 54). Moreover, it is not possible to transform strains lacking tRNA nucleotidyltransferase with clones overexpressing RNase T, undoubtedly because of uncontrolled removal of the -CCA sequence of tRNA (54). On the basis of these observations, it has been concluded that the integrity of the 3′ terminus of tRNA is determined by the relative levels of RNase T and tRNA nucleotidyltransferase (24).

Exoribonucleases also play an important role in processing tRNA precursors (16). Early studies implicated exoribonucleases in the 3′ maturation of both *E. coli* (5, 64) and phage-specified (47) tRNAs. In the latter case, a mutation in strain BN (46) defined the exoribonuclease responsible for the 3′ trimming reaction of the phage tRNAs (65), and this enzyme was subsequently identified on the basis of its absence from the mutant strain and was termed RNase BN (3). It is also possible that RNase II has some role in phage tRNA metabolism (6). The primary role of RNase BN in phage tRNA processing contrasts with that in uninfected cells. The large number of exoribonucleases present and the lack of a phenotype upon their individual removal by mutation initially made it extremely difficult to sort out which enzymes might participate in tRNA maturation in *E. coli* and what their role in the process might be. This problem has recently been at least partially answered, and the surprising conclusion is that most of the exoribonucleases participate in tRNA processing in vivo.

On the basis of the findings noted above, i.e., that any one of the five exoribonucleases (RNases II, D, BN, T, and PH) could support growth, it was likely that each of the enzymes has the capacity for 3′ maturation of tRNA. The evidence that they each actually contribute to the overall level of tRNA maturation came from examination of mutant strains lacking all combinations of RNase mutations and a quantitative suppression assay that measures the amount of functional $tRNA^{Tyr}su_3^+$ (60). The data from this study revealed that removal of any single RNase does not affect suppressor activity but that suppressor levels decrease concomitantly with removal of additional enzymes. Elimination of certain combinations of enzymes has greater effects than others, but all five of the RNases and PNPase play a role. In vitro analysis of $tRNA^{Tyr}su_3^+$ and $tRNA_2^{Arg}$ maturation using RNase-deficient extracts and purified enzymes indicate that RNase II and PNPase are most effective in shortening the 3′ trailer sequences to +2 to +4 residues, RNase PH is most

effective in trimming further to +1 residue, RNase T works best at removing the final residue following the -CCA sequence, and RNases D and BN are less active but function best with short sequences (44). Moreover, the relative contributions of the various RNases differ depending on the precursor substrate (44, 61). Thus, it appears that the total tRNA-processing capacity of the cell depends on a combination of RNases but that particular specificities among substrates and enzymes also come into play. Understanding how this all comes together and coordinates into the totality of tRNA metabolism will require much more analysis.

Other roles. Exoribonucleases undoubtedly participate in other aspects of RNA metabolism in addition to those already mentioned. For example, various reactions of rRNA maturation, particularly at the termini of 5S RNA, would appear to require exoribonuclease action (43). Likewise, conversion of 17S to 16S rRNA has been suggested to involve RNase II (9). It was noted earlier that the absence of either PNPase or RNase T leads to slowed growth. In neither case is there any effect on tRNA maturation as determined by the quantitative suppression assay, although the absence of PNPase does affect *lacZ* expression in this system (42, 60). These findings suggest that some unknown processes involving either PNPase or RNase T can become rate limiting for growth in their absence.

OUTSTANDING QUESTIONS

Are RNases regulated? Metabolic pathways in which RNases participate could be modulated if the activities or expression of the RNases themselves were subject to regulation. Although no definitive evidence for alterations in the levels of exoribonuclease synthesis in response to physiological needs has yet been found, several pieces of information suggest that this may occur. For example, the degradation of *pnp* mRNA encoding PNPase is initiated by an RNase III endonucleolytic cleavage in its 5′ untranslated region, and in the absence of RNase III, *pnp* mRNA is elevated and PNPase levels increase by close to 10-fold (57). Moreover, PNPase itself is negatively autoregulated at the translational level, but this is dependent on prior cleavage by RNase III (62). RNase D expression can also be affected at the translational level. As noted above, the *rnd* message contains a UUG initiation codon which down-regulates RNase D synthesis (70). The *rnd* mRNA also has a stem-loop structure followed by eight U residues that is essential for efficient binding by 30S ribosomal subunits and for translation (71). Thus, translation of the *rnd* mRNA can be affected by three separate sequences within the initiation region: the UUG initiation codon, the Shine-Dalgarno sequence, and the U-rich region. RNase D is also known to be altered upon infection of *E. coli* with bacteriophage T4 (13). In this situation, the mass of RNase D increases from ~40 to ~65 kDa as a result of its association with a phage-specified protein; the modification is completed early in infection. No change in the catalytic properties of RNase D is observed as a result of this association. Finally, as noted earlier, several exoribonuclease genes are parts of operons, and consequently are cotranscribed with other genes which may influence their expression.

Each of the aforementioned examples has the potential to function as a regulatory mechanism to influence the level of RNase activity. What is missing is clear evidence that RNase activity in cells is actually modulated by any of these processes.

What is the significance of phosphorolytic versus hydrolytic RNase action? Two pairs of exoribonucleases in which the enzymes have similar specificities are now known in *E. coli*, but one enzyme in each pair acts hydrolytically and the other acts phosphorolytically. One pair is RNase II and PNPase, and the second pair is RNase D and RNase PH. PNPase and RNase PH are the only phosphorolytic nucleases identified at this point, and the absence of both leads to slow growth (42). As noted above, phosphorolytic degradation would recapture energy present in the phosphodiester bond and would provide a means to help cells survive under energy-poor conditions. For example, it is known that PNPase levels increase in response to cold shock of *E. coli* (38). However, at present there is no conclusive evidence that the physiological function of phosphorolytic degradation is to conserve energy.

Is there a 5′ exoribonuclease in *E. coli*? Although 5′ exoribonucleases have been found in eukaryotic cells, such an activity has not been identified conclusively in *E. coli* (17). Nevertheless, there are a number of processes for which such an enzyme might be expected. Many mRNAs are degraded in an overall 5′ to 3′ direction, and certain 5′ leader sequences can function as stabilizing elements (55), suggesting that mRNA decay can be initiated at or near the 5′ end. Early work described an enzyme, RNase V, that degraded mRNA 5′ to 3′ in a ribosome-dependent reaction, but subsequent studies attributed this reaction to RNase II (8, 34, and references therein). In spite of this, 5′ to 3′ degradation apparently was observed, and it is not entirely clear how this could be explained by RNase II. Whether some type of 5′-to-3′-degrading enzyme for mRNA is ultimately found awaits further work, but its existence would not be surprising. Furthermore, such an enzyme also may be required for removing the last three 5′ nucleotides from 5S RNA precursors (43).

Why are there so many exoribonucleases? Many possibilities, such as multiple functions, specificities, backup systems, etc., can be suggested to explain the large number of overlapping enzymes, but, in fact, we don't have a clue!

ACKNOWLEDGMENTS

I thank Peter Setlow for helpful discussions.

Work from the laboratory of M.P.D. was supported by NIH grant GM16317.

REFERENCES

1. **Arraiano, C. M.** Personal communication.
2. **Arraiano, C. M., S. D. Yancey, and S. R. Kushner.** 1988. Stabilization of discrete mRNA breakdown products in *ams pnp rnb* multiple mutants of *Escherichia coli* K-12. J. Bacteriol. **170:**4625–4633.
3. **Asha, P. K., R. T. Blouin, R. Zaniewski, and M. P. Deutscher.** 1983. Ribonuclease BN: identification and partial characterization a new tRNA processing enzyme. Proc. Natl. Acad. Sci. USA **80:**3301–3304.
4. **Asha, P. K., and Deutscher.** 1983. *Escherichia coli* strain CAN lacks a tRNA processing nuclease. J. Bacteriol. **156:**419–420.
5. **Bikoff, E. K., B. F. LaRue, and M. L. Gefter.** 1975. *In vitro* synthesis of transfer RNA. II. Identification of required enzymatic activities. J. Biol. Chem. **250:**6248–6255.
6. **Birenbaum, M., D. Schlessinger, and Y. Ohnishi.** 1980. Altered bacteriophage T4 ribonucleic acid metabolism in a ribonuclease II-deficient mutant of *Escherichia coli*. J. Bacteriol. **142:**327–330.
7. **Bonekamp, F., K. Clemmesen, O. Karlstrom, and K. F. Jensen.** 1984. Mechanism of UTP-modulated attenuation at the *pyrE* gene of *Escherichia coli*: an example of operon polarity control through the coupling of translation to transcription. EMBO J. **3:**2857–2861.

8. **Bothwell, A. L. M., and D. Apirion.** 1971. Is RNase V a manifestation of RNase II? Biochem. Biophys. Res. Commun. **44**:844–851.
9. **Corte, G., D. Schlessinger, D. Longo, and P. Venkov.** 1971. Transformation of 17S to 16S ribosomal RNA using ribonuclease II of *Escherichia coli*. J. Mol. Biol. **60**:325–338.
10. **Craven, M. G., D. J. Henner, D. Alessi, A. T. Schauer, K. A. Ost, M. P. Deutscher, and D. I. Friedman.** 1992. Identification of the *rph* (RNase PH) gene of *Bacillus subtilis*: evidence for suppression of Cs mutations in *Escherichia coli*. J. Bacteriol. **174**:4727–4735.
11. **Cudny, H., and M. P. Deutscher.** 1980. Apparent involvement of RNase D in the 3′ processing of tRNA precursors. Proc. Natl. Acad. Sci. USA **77**:837–841.
12. **Cudny, H., and M. P. Deutscher.** 1988. 3′ processing of tRNA precursors in ribonuclease-deficient *Escherichia coli*. J. Biol. Chem. **263**:1518–1523.
13. **Cudny, H., P. Roy, and M. P. Deutscher.** 1981. Alteration of *Escherichia coli* RNase D by infection with bacteriophage T4. Biochem. Biophys. Res. Commun. **98**:337–345.
14. **Cudny, H., R. Zaniewski, and M. P. Deutscher.** 1981. *E. coli* RNase D. Catalytic properties and substrate specificity. J. Biol. Chem. **256**:5633–5637.
15. **Datta, A. K., and S. K. Niyogi.** 1975. A novel oligoribonuclease of *Escherichia coli*. II. Mechanism of action. J. Biol. Chem. **250**:7313–7319.
16. **Deutscher, M. P.** 1984. Processing of tRNA in prokaryotes and eukaryotes. Crit. Rev. Biochem. **17**:45–71.
17. **Deutscher, M. P.** 1985. *E. coli* RNases: making sense of alphabet soup. Cell **40**:731–732.
18. **Deutscher, M. P.** 1990. Ribonucleases, tRNA nucleotidyltransferase and the 3′ processing of tRNA. Prog. Nucleic Acid Res. Mol. Biol. **39**:209–240.
19. **Deutscher, M. P.** Unpublished results.
20. **Deutscher, M. P., and R. K. Ghosh.** 1978. Preparation of synthetic tRNA precursors with tRNA nucleotidyltransferase. Nucleic Acids Res. **5**:3821–3829.
21. **Deutscher, M. P., J. C. Lin, and J. A. Evans.** 1977. Transfer RNA metabolism in *Escherichia coli* cells deficient in tRNA nucleotidyltransferase. J. Mol. Biol. **117**:1081–1094.
22. **Deutscher, M. P., and C. W. Marlor.** 1985. Purification and characterization of *E. coli* RNase T. J. Biol. Chem. **260**:7067–7071.
23. **Deutscher, M. P., C. W. Marlor, and R. Zaniewski.** 1984. Ribonuclease T: a new exoribonuclease possibly involved in end-turnover of tRNA. Proc. Natl. Acad. Sci. USA **81**:4290–4293.
24. **Deutscher, M. P., C. W. Marlor, and R. Zaniewski.** 1985. RNase T is responsible for the end-turnover of tRNA in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82**:6427–6430.
25. **Deutscher, M. P., G. T. Marshall, and H. Cudny.** 1988. RNase PH: a new phosphate-dependent nuclease distinct from polynucleotide phosphorylase. Proc. Natl. Acad. Sci. USA **85**:4710–4714.
26. **Deutscher, M. P., and N. B. Reuven.** 1991. Enzymatic basis for hydrolytic versus phosphorolytic mRNA degradation in *Escherichia coli* and *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **88**:3277–3280.
27. **Donovan, W. P., and S. R. Kushner.** 1983. Amplification of ribonuclease II (*rnb*) activity in *Escherichia coli* K-12. Nucleic Acids Res. **55**:265–275.
28. **Donovan, W. P., and S. R. Kushner.** 1986. Polynucleotide phosphorylase and ribonuclease II are required for cell viability and mRNA turnover in *Escherichia coli* K12. Proc. Natl. Acad. Sci. USA **83**:120–124.
29. **Duffy, J. J., S. G. Chaney, and P. D. Boyer.** 1972. Incorporation of water oxygen into intracellular nucleotides and RNA. I. Predominantly non-hydrolytic RNA turnover in *Bacillus subtilis*. J. Mol. Biol. **64**:565–579.
30. **Ghosh, R. K., and M. P. Deutscher.** 1978. Identification of an *E. coli* nuclease acting on structurally altered transfer RNA molecules. J. Biol. Chem. **253**:997–1000.
31. **Ghosh, R. K., and M. P. Deutscher.** 1978. Purification of potential 3′ processing nucleases using synthetic tRNA precursors. Nucleic Acids Res. **5**:3831–3842.
32. **Gorelic, L., and D. Apirion.** 1973. Increased ribonuclease II activity in a temperature sensitive mutant of *Escherichia coli*. FEBS Lett. **30**:133–136.
33. **Guarneros, G., and C. Portier.** 1990. Different specificities of ribonuclease II and polynucleotide phosphorylase in 3′ mRNA decay. Biochimie **72**:771–777.
34. **Holmes, R. K., and M. F. Singer.** 1971. Inability to detect RNase V in *Escherichia coli* and comparison of other ribonucleases before and after infection with coliphage T7. Biochem. Biophys. Res. Commun. **44**:837–843.
35. **Hautala, J. A., C. L. Bassett, N. H. Giles, and S. R. Kushner.** 1979. Increased expression of a eukaryotic gene in *Escherichia coli* through stabilization of its messenger RNA. Proc. Natl. Acad. Sci. USA **76**:5774–5778.
36. **Huang, S., and M. P. Deutscher.** 1992. Sequence and transcriptional analysis of the *Escherichia coli rnt* gene encoding RNase T. J. Biol. Chem. **267**:25609–25613.
37. **Jensen, K. F., J. T. Andersen, and P. Poulsen.** 1992. Overexpression and rapid purification of the *orfE/rph* gene product, RNase PH of *Escherichia coli*. J. Biol. Chem. **267**:17147–17152.
38. **Jones, P. G., R. A. Van Bogelen, and F. C. Neidhardt.** 1987. Induction of proteins in response to low temperatures in *Escherichia coli*. J. Bacteriol. **169**:2092–2095.
39. **Kasai, T., R. S. Gupta, and D. Schlessinger.** 1977. Exoribonucleases in wild type *Escherichia coli* and RNase II-deficient mutants. J. Biol. Chem. **252**:8950–8956.
40. **Kelly, K. O., and M. P. Deutscher.** 1992. Characterization of *Escherichia coli* RNase PH. J. Biol. Chem. **267**:17153–17158.
41. **Kelly, K. O., and M. P. Deutscher.** 1992. The presence of only one of five exoribonucleases is sufficient to support the growth of *Escherichia coli*. J. Bacteriol. **174**:6682–6684.
42. **Kelly, K. O., N. B. Reuven, Z. Li, and M. P. Deutscher.** 1992. RNase PH is essential for tRNA processing and viability in RNase-deficient *Escherichia coli* cells. J. Biol. Chem. **267**:16015–16018.
43. **King, T. C., R. Sirdeskmukh, and D. Schlessinger.** 1986. Nucleolytic processing of ribonucleic acid transcripts in procaryotes. Microbiol. Rev. **50**:428–451.
44. **Li, Z., and M. P. Deutscher.** Unpublished observations.
45. **Littauer, U. Z., and H. Soreq.** 1982. Polynucleotide phosphorylase, p. 518–553. *In* P. D. Boyer (ed.), *The enzymes*, vol. XV, part B. Academic Press, Inc., New York.
46. **Maisurian, A. N., and E. A. Buyanovskaya.** 1973. Isolation of an *Escherichia coli* strain restricting bacteriophage suppressor. Mol. Gen. Genet. **120**:227–229.
47. **McClain, W. H.** 1977. Seven terminal steps in a biosynthetic pathway leading from DNA to transfer RNA. Accounts Chem. Res. **10**:418–425.
48. **McLaren, R. S., S. F. Newbury, G. S. C. Dance, H. C. Causton, and C. F. Higgins.** 1991. mRNA degradation by processive 3′-5′ exoribonucleases *in vitro* and the implications for prokaryotic mRNA decay *in vivo*. J. Mol. Biol. **221**:81–95.
49. **McMurry, L. M., and S. B. Levy.** 1987. Tn*5* insertion in polynucleotide phosphorylase (*pnp*) gene in *Escherichia coli* increases susceptibility to antibiotics. J. Bacteriol. **169**:1321–1324.
50. **Neri-Cortes, D., and M. P. Deutscher.** Unpublished results.
51. **Niyogi, S. K., and A. K. Datta.** 1975. A novel oligoribonuclease of *Escherichia coli*. I. Isolation and properties. J. Biol. Chem. **250**:7307–7312.
52. **Ost, K. A., and M. P. Deutscher.** 1990. RNase PH catalyzes a synthetic reaction, the addition of nucleotides to the 3′ end of tRNA. Biochimie **72**:813–818.
53. **Ost, K. A., and M. P. Deutscher.** 1991. *Escherichia coli orfE* (upstream of *pyrE*) encodes RNase PH. J. Bacteriol. **173**:5589–5591.
54. **Padmanabha, K. P., and M. P. Deutscher.** 1991. RNase T affects growth and recovery of *Escherichia coli* from metabolic stress. J. Bacteriol. **173**:1376–1381.
55. **Petersen, C.** 1992. Control of functional mRNA stability in bacteria: multiple mechanisms of nucleolytic and non-nucle-

olytic inactivation. Mol. Microbiol. **6**:277–282.
56. **Portier, C.** 1975. Quaternary structure of polynucleotide phosphorylase from *Escherichia coli*: evidence of a complex between two types of polypeptide chains. Eur. J. Biochem. **55**:573–582.
57. **Portier, C., L. Dondon, M. Grunberg-Manago, and P. Regnier.** 1987. The first step in the functional inactivation of the *Escherichia coli* polynucleotide phosphorylase messenger is a ribonuclease III processing at the 5′ end. EMBO J. **6**:2165–2170.
58. **Poulsen, P., F. Bonekamp, and K. F. Jensen.** 1984. Structure of the *Escherichia coli pyrE* operon and control of *pyrE* expression by a UTP modulated intercistronic attenuation. EMBO J. **3**:1783–1790.
59. **Regnier, P., M. Grunberg-Manago, and C. Portier.** 1987. Nucleotide sequence of the *pnp* gene of *Escherichia coli* encoding polynucleotide phosphorylase. J. Biol. Chem. **262**:63–68.
60. **Reuven, N. B., and M. P. Deutscher.** 1993. Multiple exoribonucleases are required for the 3′ processing of *Escherichia coli* tRNA precursors *in vivo*. FASEB J. **7**:143–148.
61. **Reuven, N. B., and M. P. Deutscher.** Unpublished observations.
62. **Robert-LeMeur, M., and C. Portier.** 1992. *E. coli* polynucleotide phosphorylase expression is autoregulated through an RNase III-dependent mechanism. EMBO J. **11**:2633–2641.
63. **Roy, P., H. Cudny, and M. P. Deutscher.** 1982. The tRNA processing defect in *E. coli* strains BN and CAN is not due to a mutation in RNase D or RNase II. J. Mol. Biol. **159**:179–187.
64. **Schedl, P., J. Roberts, and P. Primakoff.** *In vitro* processing of *E. coli* tRNA precursors. Cell **8**:581–594.
65. **Seidman, J. G., F. J. Schmidt, K. Foss, and W. H. McClain.** 1975. A mutant of *Escherichia coli* defective in removing 3′ terminal nucleotides from some transfer RNA precursor molecules. Cell **5**:389–400.
66. **Shen, V., and D. Schlessinger.** 1982. RNases I, II, and IV of *Escherichia coli*, p. 501–515. *In* P. D. Boyer (ed.), *The enzymes*, vol. XV, part B. Academic Press, Inc., New York.
67. **Zaniewski, R., E. Petkaitis, and M. P. Deutscher.** 1984. A multiple mutant of *Escherichia coli* lacking the exoribonucleases RNase II, RNase D and RNase BN. J. Biol. Chem. **259**:11651–11653.
68. **Zhang, J., and M. P. Deutscher.** 1988. *Escherichia coli* RNase D: sequencing of the *rnd* structural gene and purification of the overexpressed protein. Nucleic Acids Res. **16**:6265–6278.
69. **Zhang, J., and M. P. Deutscher.** 1988. Transfer RNA is a substrate for RNase D *in vivo*. J. Biol. Chem. **263**:17909–17912.
70. **Zhang, J., and M. P. Deutscher.** 1989. Analysis of the upstream region of the *Escherichia coli rnd* gene encoding RNase D: evidence for translational regulation of a putative tRNA processing enzyme. J. Biol. Chem. **264**:18228–18233.
71. **Zhang, J., and M. P. Deutscher.** 1992. A uridine-rich sequence required for translation of prokaryotic mRNA. Proc. Natl. Acad. Sci. USA **89**:2605–2609.
72. **Zhu, L., and M. P. Deutscher.** 1987. tRNA nucleotidyltransferase is not essential for *Escherichia coli* viability. EMBO J. **6**:9927–9930.