RNA Processing

Frederique Braun, Ciaran Condon

► **To cite this version:**

Frederique Braun, Ciaran Condon. RNA Processing. Encyclopedia of Microbiology, 2019, 10.1016/B978-0-12-801238-3.02455-7 . hal-02348500

HAL Id: hal-02348500
<https://hal.science/hal-02348500>

Submitted on 5 Nov 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1
2
3
4 **RNA Processing**

5
6 **Frédérique Braun and Ciarán Condon**
7

8
9
10
11 UMR8261 (CNRS, Université de Denis Diderot)
12 Institut de Biologie Physico-Chimique
13 13 rue Pierre et Marie Curie
14 75005 Paris
15
16
17

18 Correspondence:

19 Tel: (33)-1-58 41 51 23

20 Fax: (33)-1-58 41 50 20

21 E-mail: condon@ibpc.fr; braun@ibpc.fr
22
23
24
25
26

27 **Keywords:** *Bacillus subtilis*/*Escherichia coli*/messenger RNA/ribonuclease/ribosomal
28 RNA/stable RNA/transfer RNA/RNA maturation/RNA processing/RNA stability
29
30

Glossary

distributive Describing an enzyme that releases its substrate after each catalytic cycle.

endoribonuclease Enzyme that cuts RNA internally.

exoribonuclease Enzyme that chews RNA one nucleotide at a time from either the 5' or 3' end.

homolog(ous) Having the same origin

intergenic region Sequence between two open reading frames

paralog(ous) Having the same origin, but in a single organism.

processive Describing an enzyme capable of performing many catalytic cycles on the same molecule before releasing it.

primary transcript Transcript first synthesized by RNA polymerase from point of transcription initiation to point of transcription termination.

ribonuclease Enzyme that cuts or chews RNA

RNA processing/maturation Conversion of precursor RNA to shorter, functional and usually more stable species by ribonucleases.

Shine-Dalgarno Bacterial ribosome binding site, complementary to 3' end of 16S rRNA, named after its discoverers.

Abstract

RNAs can be either transcribed directly as functional species or as molecules that require post-transcriptional processing by ribonucleases. Most messenger RNAs fall into the first category, while most structural RNAs fall into the second. There are, however, some specific examples of mRNAs that undergo processing to shorter, more stable species with a higher translational yield. Most of the work in this field has been performed in *E. coli* and, more recently, in *B. subtilis*. In this chapter, I will first describe some examples of mRNA processing these two organisms and then compare their pathways of stable RNA maturation. While there is some degree of overlap between their RNA processing enzymes and pathways, for the most part they are remarkably different, far more divergent than was ever predicted before the sequencing of their respective genomes. None of the late steps in rRNA maturation are conserved between the two bacteria, for example. In addition, *B. subtilis* utilizes an enzyme with 5' exoribonuclease activity for mRNA and rRNA processing, whereas *E. coli* does not appear to possess a 5' exoribonuclease. These observations highlight the importance of studying more than one model bacterial system in fine detail.

Defining Statement

RNAs are synthesized as so-called primary transcripts extending from the point of transcription initiation by RNA polymerase to the point of transcription termination. In some cases, the primary transcript is the final functional molecule. In others, the primary transcript is ‘processed’ to a shorter, more stable functional species by ribonucleases (RNases).

Introduction

While primary messenger RNA (mRNA) transcripts can typically be bound by ribosomes and used directly for translation RNAs, stable RNAs such as ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs), are almost always processed from the primary transcript into shorter functional molecules. RNases remove sequences from the 5’ or 3’ end of stable RNA precursors, usually both. As we will see later, some mRNAs are also processed, i.e. the major translated species found in the cell *in vivo* is not the primary transcript. The classic case is where an mRNA encodes more than one protein and is processed between cistrons. This can have important consequences for the stoichiometry of the different proteins produced from a single operon. At the outset, therefore, it is useful to distinguish the term “RNA processing” (or maturation), which is used to describe RNase-catalyzed events leading to the generation of a functional RNA, from “RNA degradation”, used to describe its decay. The first generally leads to an increase in RNA half-life relative to the primary transcript and, the second, to a decrease. In this chapter, I will first describe some specific examples of mRNA processing and then focus for the remainder of the review on the maturation of stable RNAs. The vast majority of the work I will describe will be from studies in *Escherichia coli* and *Bacillus subtilis*, the best-studied examples of the Gram-negative and Gram-positive bacteria, respectively. I have chosen to emphasize some of the historical examples that served as paradigms for the different types of processing events. New examples are regularly discovered that give further weight to these pioneering studies.

The Players

Ribonucleases come in two flavors; endoribonucleases, which cut or cleave RNAs internally, and exoribonucleases, which remove nucleotides one at a time from either the 3' or 5' end. The former are typically symbolized as a pair of scissors, while exoribonucleases are usually represented by a 'Pacman' symbol. Exoribonucleases can be either processive, remaining attached to the same RNA molecule for many rounds of catalysis, or distributive, releasing the substrate with each nucleotide removed. They can also be either hydrolytic, consuming a molecule of water upon the breaking of each phosphodiester bond, or phosphorolytic, using inorganic phosphate instead. Most RNases are hydrolases. Not surprisingly, bacterial RNases have been studied most extensively in *E. coli*. In recent years, however, significant progress has been made in *B. subtilis*, where 22 RNases have now been identified (vs. 26 in *E. coli*) and it is clear that most of the enzymes are different from those of *E. coli* (only 9 are in common). While *E. coli* appears to only possess 3' exoribonucleases, for example, exonucleases of either orientation (5' or 3') can be found in *B. subtilis* (as in eukaryotes). A Venn diagram showing the full set of currently known RNases of *E. coli* and *B. subtilis* is shown in Figure 1. <Figure 1 near here> Many of these enzymes have specialized functions in RNA processing, which we will describe as we go along. The three-dimensional structures of many of these enzymes is known, permitting dissection of their activity mechanisms at the molecular level (indicated by an asterisk in Figure 1).

Messenger RNA Processing

Ribonuclease processing of mRNAs leading to an increase in stability over the primary transcript has been described in a number of cases in both Gram-negative and Gram-positive bacteria. In many of the examples described in the literature, an endonucleolytic cleavage event occurs in the intergenic region between two open-reading frames encoded by the same RNA, maintaining the translational integrity of each fragment. In many cases, however, the upstream and downstream products of the cleavage reaction have different chemical and functional half-lives. Such mechanisms are typically exploited to produce different stoichiometric amounts of different products of the same operon. A regulator of an operon, for example, is often required in much lower quantities than the enzymes or structural components of the cognate pathway and thus the RNA fragment encoding a regulatory protein is typically destabilized by this kind of processing event, while the remaining fragment remains relatively resistant to RNases.

Messenger RNA processing in *E. coli*

RNase E is the primary enzyme involved in intergenic RNA processing in *E. coli*. This is without doubt the key endoribonuclease of *E. coli*, and plays a role in mRNA processing and decay, and in stable RNA maturation. It is a large (1061 amino acids), essential protein that has an N-terminal catalytic domain and an intrinsically disordered C-terminal domain permitting both association with the inner membrane and interactions with other proteins, such as the RNA helicase RhlB, the 3' exoribonuclease polynucleotide phosphorylase (PNPase), and the glycolytic enzyme enolase. This complex is known as the degradosome and similar RNase E-based degradosomes have been found in many organisms, with some interesting variations in the associated proteins. Although the importance of the degradosome in *E. coli* RNA decay is generally accepted, its role in RNA processing is less clear. Only the catalytic domain is necessary for the maturation of 5S rRNA and RNase P RNA subunit, for example. The structure of the catalytic domain of RNase E has been solved in complex with RNA ([pdb 2C0B](#)). *E. coli* also possesses a non-essential paralog of the catalytic domain of RNase E, known as RNase G. Both enzymes have overlapping (although non-identical) cleavage site specificities, that is to say, AU-rich single stranded regions.

Other enzymes, such as RNase III and RNase P, have been implicated in intergenic cleavages of a small number of specific mRNAs in *E. coli* and *Salmonella*. These enzymes play a more important role in rRNA and tRNA maturation and will be described in more detail later.

The rpsU dnaG rpoD operon

One of the first described examples of intergenic processing of mRNA in *E. coli* was the *rpsU dnaG rpoD* operon, encoding ribosomal protein S21, the DNA primase DnaG and the sigma-70 subunit of RNA polymerase, respectively (Figure 2A). <Figure 2 near here> Primary transcripts of this operon are transcribed from tandem promoters and extend to either a weak transcription terminator located between *rpsU* and *dnaG* or a strong terminator at the 3' end of the operon. The long primary transcripts are cleaved between the *dnaG* and *rpoD* open reading frames by RNase E, leading to rapid degradation of the DnaG-encoding upstream portion and a stable downstream fragment encoding sigma-70. The terminator hairpin between *rpsU* and *dnaG* presumably protects the S21-encoding RNA from attack by 3' exoribonucleases degrading the *dnaG* RNA fragment. The particular processing and

degradation pattern of this operon leads to the production of an estimated 50,000 molecules of S21, 50 molecules of DNA primase and 3,000 copies of sigma-70 per cell.

The pap operon

The *pap* locus of uropathogenic *E. coli* strains encodes eleven proteins involved in pilin formation. Transcription of the rightward operon begins at the *papB* promoter and is attenuated at a terminator hairpin downstream of the second cistron *papA* (Figure 2B). The *papBA* primary transcript is processed between the two open reading frames by RNase E, and the upstream fragment, encoding the regulator of the operon PapB, is rapidly degraded, while the downstream portion, encoding the major pilin protein PapA, is stable. The primary transcript has a half-life of 2.5 mins while that of the PapA encoding fragment is 27 mins, accounting in large part for the relative proportions of the regulatory and structural proteins of this operon.

Many other examples of intergenic processing have been found in *E. coli*, too numerous to describe here. The principal is always the same; endonucleolytic cleavage followed by selective degradation of the upstream fragment by 3' exonucleases, while the downstream fragment remains relatively stable.

Bacteriophage mRNA processing

Bacteriophage mRNAs tend to be highly processed. In some cases, the processing event does not lead to an increase in mRNA stability but rather to an increase in translation. The *N* gene of phage lambda and the *0.3* gene of phage T7 are two such examples.

The lambda N gene

The *N* gene of lambda is the first gene transcribed from the P_L promoter of the leftward operon. Transcription of the *nutL* sequence between the P_L promoter and the *N* gene leads to the assembly of an antitermination complex on RNA polymerase, that includes the *nut* RNA itself, the N protein and at least four host factors NusA, NusB, NusG and ribosomal protein S10 (Figure 3A). <Figure 3 near here> This antitermination complex is critical for lambda development, as it allows RNA polymerase to read through multiple transcription terminators in this operon. The proximity of the N protein in the antitermination complex to its own Shine Dalgarno (SD) also causes steric inhibition of ribosome binding and hence auto-repression at the translational level. This translational auto-repression can be relieved (about 200-fold) by

1 cleavage of a hairpin structure between *nutL* and the ribosome binding site by the double-
2 strand nuclease RNase III, freeing up the SD element for ribosome binding. Lambda
3 development is therefore highly sensitive to the RNase III activity of its host. As we will see
4 below RNase III is occupied primarily in rRNA maturation in *E. coli*. Thus, changes in
5 growth conditions, which affect rRNA synthesis, would be predicted to temporarily alter the
6 amount of free RNase III in the cell and have a knock-on effect on lambda growth.

8 ***The phage T7 0.3 gene***

9 Bacteriophage T7 early mRNAs are quite stable, with half-lives in the 10 to 20 min range. A
10 7kb precursor transcript, made by the host RNA polymerase soon after infection, is processed
11 in five places by RNase III (Figure 3B). In most cases, RNase III processing occurs on the
12 distal side of hairpin structures, creating recessed 3' ends that are resistant to 3'
13 exoribonuclease action, likely accounting for the high degree of early transcript stability. The
14 *0.3* gene is the first gene of the early T7 operon and it plays a role in relieving host DNA
15 restriction. Like the lambda *N* gene, the *0.3* gene transcript possesses a stem-loop structure in
16 its 5' untranslated region. This hairpin is also cleaved on its distal side by RNase III. The
17 stability of the downstream *0.3* portion of the transcript is unaffected by processing, however.
18 Like lambda *N*, processing rather appears to influence the accessibility of the SD sequence to
19 ribosome binding.

21 ***Retroregulation of the lambda int gene***

22 The lambda *int* gene, encoding the integrase protein, provides an example where processing
23 leads to a decrease rather than an increase in mRNA stability. One can justify considering this
24 a processing pathway rather than a simple RNA decay mechanism on the grounds that
25 targeted degradation of the *int* mRNA is part of a regulatory switch that determines whether
26 the phage will enter the lysogenic or lytic development cycle. The *int* gene of lambda is
27 transcribed from either the P_I or P_L promoter, depending on the stage of the phage life-cycle,
28 but only the P_I transcript is translated. The P_I transcript traverses the *int* gene and ends at a
29 Rho-independent terminator immediately downstream. The P_L transcript, as we saw earlier, is
30 synthesized by terminator-resistant RNA polymerase, and extends further downstream. The
31 longer read-through transcript can fold into a hairpin structure called *sib*, which is a site for
32 endonucleolytic processing by RNase III (Figure 3C). Cleavage at this site provides access to
33 the mRNA by the 3' exoribonuclease PNPase ([pdb 1E3P](#)) and the *int* transcript is rapidly
34 degraded. In this mode, the phage does not synthesize the integrase protein and remains in the

lytic cycle. This phenomenon has been called retroregulation because the regulatory elements are located downstream of the target gene.

Messenger RNA processing in *B. subtilis*

Examples of mRNA processing have also been seen in *B. subtilis*. Interestingly, many Gram-positive bacteria do not possess RNase E or RNase G homologs, with the *Clostridia* and the *Actinobacteria* being notable exceptions. Although there has been evidence for some time of an RNase E-like activity in *B. subtilis*, the identity of this enzyme remained elusive until recently. Although RNases J1 and J2 were originally proposed to fulfill this role, there is now a general consensus that a different enzyme, RNase Y, is the functional analog of RNase E in *B. subtilis*, despite a complete lack of homology between the two enzymes. RNase Y has similar cleavage specificity to RNase E and is found associated with the membrane through its N-terminal trans-membrane domain like its protobacterial analog. RNase Y has been proposed to be a scaffold for a degradosome-like network (DLN) in *B. subtilis* involving PNPase, an RNA helicase, CshA, and glycolytic enzymes. It has also been shown to interact with three proteins, YlbF, YmcA and YaaT, to form a separate complex, known as the Y-complex. Although RNase Y has mostly been shown to be involved in mRNA turnover, a number of RNase Y-dependent cleavages have been discovered that can be considered as processing events. Recent data suggest that the Y-complex might be specifically involved in RNase Y processing events allowing differential abundance of mRNA fragments.

In addition to RNase Y, *B. subtilis* has another major RNA enzyme involved in RNA processing and decay, the 5'-exoribonuclease RNase J1. While neither RNase J1 nor RNase Y are essential on their own, a double mutant lacking RNase Y and RNase J1 is inviable, as is a double mutant lacking RNase J1 and the 3'-exoribonuclease PNPase. RNase J1 forms a complex with its paralog RNase J2, with RNase J1 providing most of the 5'-exonucleolytic activity. Although both enzymes have endonucleolytic activity at high concentrations *in vitro*, the *in vivo* relevance of this additional activity is not clear. *B. subtilis* RNase J1 is a member of the β -CASP family of metallo- β -lactamases and its structure has been solved ([pdb 3ZQ4](#)). Genome analysis has revealed that most bacterial phyla contain at least two of three major ribonucleases: RNases E/G, J, or Y.

The thrS mRNA

1 The threonyl-tRNA synthetase gene of *B. subtilis* *thrS* is one of about twenty so-called T-box
2 genes in this organism, regulated by a tRNA-dependent antitermination mechanism. Genes
3 regulated by this mechanism are involved in amino acid metabolism and are sensitive to the
4 ratio of uncharged to charged levels of the cognate tRNA. Hence, expression of the *B. subtilis*
5 *thrS* gene is induced in response to threonine starvation. Binding of uncharged tRNA^{Thr} to the
6 *thrS* leader sequence stabilizes an antiterminator structure in the mRNA at the expense of a
7 mutually exclusive transcription terminator upstream of the *thrS* coding sequence. Under
8 threonine starvation conditions, the major 5' end found *in vivo* corresponds to a processing
9 event that occurs nine nucleotides (nts) upstream of the leader terminator hairpin (Figure 4A).
10 <Figure 4 near here> The cleaved transcript is about five-fold more stable than the primary
11 transcript, presumably due to the presence of a hairpin structure protecting each extremity
12 from further degradation by exonucleases. At least five other T-box mRNAs are processed in
13 a similar manner just upstream of the leader terminator and thus this appears to be a general
14 mechanism to amplify the antitermination effect for this family of genes in *B. subtilis*.
15 Although cleavage at this site was originally attributed to the endonucleolytic activity of
16 RNase J1/J2 based on *in vitro* studies, it was subsequently shown that RNase Y is a more
17 likely candidate for this cleavage *in vivo*. Indeed, the leader regions of at least 5 of the 6 T-
18 box mRNAs known to be processed upstream of their terminators accumulate in strains
19 depleted for RNase Y, but not in strains depleted for RNase J1.

21 ***The hbs gene***

22 Evidence for mRNA processing by RNase J1, exploiting its 5' exoribonuclease activity, has
23 also been seen in *B. subtilis*. The *hbs* gene, is transcribed from two promoters, about 210 and
24 180 nts upstream of the open reading frame encoding the histone-like protein HU. A third
25 major transcript is also observed *in vivo*, whose 5' end maps about 20 nts upstream of the *hbs*
26 start codon. Although originally thought to represent transcription from yet another promoter,
27 the 5' end of this stable transcript is in fact generated by 5'-exoribonuclease digestion of the
28 *hbs* mRNA by the RNase J1/J2 complex until it bumps up against ribosomes initiating
29 translation at its very strong Shine-Dalgarno (SD) sequence. Increasing the strength of the *hbs*
30 SD increases the level of this mRNA species in the cell, while weakening only slightly it
31 causes this transcript to disappear completely (Figure 4B). The 5' ends of the two primary *hbs*
32 transcripts are highly protected by secondary structure. RNase Y cleaves in the 5'-UTR
33 sequence, allowing RNase J1/J2 access to the mRNA just upstream of the ribosome. Such a
34 pathway potentially provides a built-in destruction mechanism for the *hbs* RNAs once it has

surpassed its usefulness for translation, as access to the coding sequence is no longer protected by ribosomes from 5'-exonucleolytic attack. A similar example of processing and RNA protection by ribosomes has been identified for the *cryIIIA* mRNA, encoding the insecticidal crystal toxin of *B. thuringiensis*, expressed in *B. subtilis*. In this case a strong SD-like sequence with no associated start codon, called the stab-SD, binds a 30S ribosomal subunit and blocks RNase J1/J2 progression, stabilizing the mRNA for many kilobases downstream.

Intergenic mRNA processing

Several cases of intergenic processing have been described in *B. subtilis*, similar to that described for the *rpsU dnaG rpoD* and *papBA* operons of *E. coli* above. The best known examples are those of the *gap* operon, encoding six genes involved in the inter-conversion of triose phosphates of the glycolytic pathway (Figure 4C), and the *dnaK* heat shock operon (Figure 4D). In both of these cases, processing occurs between the first and second ORFs of the operon. The RNA fragment containing the first ORF, encoding the regulator of the operon is quickly degraded, while the downstream fragment is relatively stable. The differential stability of the upstream and downstream cleavage products can account for the differences in stoichiometry between the regulator and other proteins of the operon. RNase Y has been shown to be responsible for the cleavage in the *gapA* operon mRNA. This processing event was recently shown to require the Y-complex. Interestingly, the *cggR (gapR)-gapA* operon of *S. aureus*, is similarly matured by RNase Y and requires YlbF, one of the Y-complex protein, suggesting that the function of the Y-complex is conserved among the Firmicute. Tiling array data suggests that RNase Y is also the prime candidate for the cleavage downstream of *hrcA* in the *dnaK* operon transcript.

Stable RNA Processing

Ribosomal RNA processing

In *E. coli* and *B. subtilis*, ribosomal RNAs are transcribed from *rrn* operons containing the genes for 16S, 23S and 5S rRNA, in that order, sometimes interspersed with tRNAs (Figure 5A). <Figure 5 near here> Although this is the most commonly encountered scenario throughout the bacterial kingdom, there are scattered cases where the 16S and 23S genes are

not co-transcribed. The *rrn* operons of *E. coli* and *B. subtilis* are transcribed as long 30S precursor RNAs from tandem promoters upstream of the operon. The 5' and 3' flanking sequences of both 16S and 23S rRNA can base pair to form long 'processing stalks' which are substrates for RNase III. RNase III is the founder member of a large family of double-stranded RNases including Dicer, well known for its role in post-transcriptional gene silencing eukaryotes. RNase III consists of an N-terminal catalytic domain and a C-terminal double-stranded RNA binding domain (dsRBD) and forms dimers ([pdb 1RC7](#)). It generally cleaves on both strands of long double-stranded helices to produce RNAs with a recessed 5' end and a 2-nt 3'-overhang. Particular sequence and structure contexts can cause it to cleave on just one of the two strands, however. In *E. coli*, RNase III cleavage of the 30S rRNA precursor is known to occur as it is being transcribed and leads to separation of the 16S, 23S and 5S rRNA species. Processing by RNase III is not absolutely necessary for the production of functional rRNAs in either of these two organisms, however. The RNase III-generated processing intermediates are whittled down to their mature sizes by other enzymes, in many cases subsequent to subunit assembly or even translation initiation. With one notable exception (see below), the enzymes responsible for the late steps of rRNA maturation in *B. subtilis* have proven to be different to those in *E. coli*. In fact, in most cases, the enzymes involved are unique to each species.

16S rRNA maturation

The mature 5' side of *E. coli* 16S rRNA is generated by the co-operative action of RNase E and RNase G (Figure 5B). RNase E first cleaves 66 nts from the mature 5' end and this facilitates the final endonucleolytic cleavage by RNase G, presumably by permitting some structural rearrangement of the RNA. These reactions occur after ribosome assembly.

In *B. subtilis*, 16S rRNA 5' maturation is catalyzed by the 5'-exoribonuclease activity of RNase J1, presumably in complex with RNase J2, since this is the primary form of both enzymes found *in vivo*. Some feature close to the 5' end of 16S rRNA in the assembled ribosome, e.g. a bound ribosomal protein, is required to tell RNase J1 where to stop. A processing intermediate, generated by an unknown RNase, accumulates 38 nts from the mature 5' end in *B. subtilis* cells lacking RNase J1.

Two different pathways have been proposed for the maturation of the 3' end of 16S rRNA in *E. coli*. One involves a new enzyme called YbeY ([pdb 1XM5](#)). Although a mutation in the *ybeY* gene was shown to affect processing of all three rRNAs, it was proposed that YbeY specifically catalyzes the endonucleolytic processing of the 3' end of 16S rRNA.

Another group has more recently proposed that 3'-maturation can also be catalyzed by a number of redundant 3'-exoribonucleases including PNPase, RNase PH, RNase R and RNase II. One of these enzymes, RNase R, has also been shown to perform exonucleolytic trimming of 16S precursors in the psychrophilic γ -Proteobacterium *Pseudomonas syringae*. Recently, it was shown that the 3' processing pathway of 16S rRNA in *B. subtilis* involves the YqfG enzyme, an ortholog of *E. coli* YbeY. Indeed, the *B. subtilis* *yqfG* gene can complement the *E. coli* *ybeY* deletion mutant for 16S rRNA processing. In contrast to *E. coli* YbeY, YqfG is essential in *B. subtilis*, but the lethality of the *yqfG* gene knockout can be suppressed by also deleting the gene encoding RNase R (*rnr*). This suggests that, unlike the proteobacterial RNase R, which can act in an alternative processing pathway for 16S rRNA 3'-maturation, RNase R in *B. subtilis* acts mainly in a quality control pathway to rapidly degrade 16S rRNA species that YqfG has failed to process. In the absence of RNase R, 16S rRNA species with 3'-extensions, which would be expected to be highly inefficient in translation, accumulate to sufficient levels to allow enough translation for cell viability.

23S rRNA maturation

Although accurate processing of *E. coli* 16S rRNA still occurs in the absence of RNase III, maturation of 23S rRNA fails completely. Thus, RNase III processing is an obligatory first step for 23S rRNA maturation in this organism. The 23S precursor RNA can still be assembled into functional ribosomes, however. RNase III cleavage of the 23S precursor produces an intermediate species with three to seven extra nucleotides on the 5' end and seven to nine extra nucleotides on the 3' end (Figure 5C). The enzyme responsible for the subsequent generation of the mature 5' end remains unknown, while the 3' end is matured by the distributive 3'-exoribonuclease RNase T. RNase T ([pdb 2F96](#)) is an important enzyme in *E. coli* that can degrade to within one or two nucleotides of double-stranded stems. It is a member of the DEDD family of exoribonucleases that includes oligoribonuclease, which degrades small oligonucleotides in the two to five nt range, and RNase D, which is involved in tRNA maturation (see below). The final maturation steps of both the 5' and 3' end of *E. coli* 23S rRNA are most efficient under conditions of protein synthesis, suggesting that some structural rearrangement occurs at the onset of translation that allows better access to the two extremities.

Maturation of 23S rRNA in *B. subtilis* follows a different pathway than *E. coli*. In this case, the base-paired 5' and 3' ends of 23S rRNA are matured by an enzyme homologous to

1 the catalytic domain of RNase III, and lacking the dsRBD, called Mini-RNase III ([pdb](#)
2 [4OUN](#)). Endonucleolytic cleavage by Mini-III on both sides of the processing stalk to directly
3 yield 5'- and 3'-mature 23S rRNA requires prior binding of the large subunit RNA by the
4 ribosomal protein L3. This provides a potential quality control check for correct ribosome
5 assembly before proceeding with the final maturation step. Interestingly, this L3 quality
6 control step is also used in the maturation of the large subunit RNA in *S. cerevisiae*, but with
7 a different processing enzyme. In *B. subtilis*, prior cleavage of the large 23S processing stalk
8 by RNase III is not necessary for Mini III action, and an alternative back-up pathway exists in
9 Mini-III deletion mutants ($\Delta mrnC$) that results in slightly different 5' and 3' ends. This
10 pathway is catalyzed by exoribonucleases: RNase J1 at the 5' end and RNases PH and YhaM
11 at the 3' end, but its physiological relevance is unknown. It may be the vestige of an older
12 pathway that can also be found in some proteobacteria such as *Sinorhizobium meliloti*.

13 Some variations on the pathways of 23S rRNA processing can be seen in other
14 organisms. In some species, including *Salmonella*, the 23S rRNA contains short intervening
15 sequences that require removal by RNase III processing. In these cases, the "23S" rRNA is
16 assembled into 50S subunits as fragments. In *Deinococcus radiodurans*, maturation of the 3'
17 end of 23S rRNA is catalyzed principally by RNase PH and RNase II.

18 19 **5S rRNA maturation**

20 RNase III processing of 23S rRNA releases the 5S rRNA precursor in both *E. coli* and *B.*
21 *subtilis* and defines the 5' end of the naturally occurring precursor species. The 3' end is quite
22 variable, however, and depends on the distance to the terminator hairpin or downstream tRNA
23 for each of the individual *rrn* operons (there are seven of these in *E. coli* and ten in *B.*
24 *subtilis*). Once again, these two organisms have found very different strategies for the final
25 maturation steps of an rRNA. In *E. coli*, RNase E cleaves in a single-stranded region on either
26 side of the 5S rRNA, producing an intermediate species with 3 extra nucleotides at each end
27 (Figure 5D). The 3' exonuclease RNase T catalyzes the final maturation of the 3' end, while
28 the enzyme that removes the 3 nts from the 5' end is still unidentified. In *P. syringae*, the 3'
29 maturation reaction is carried out by RNase R at cold temperatures.

30 *B. subtilis*, on the other hand, uses the double-stranded maturase RNase M5, cleaving
31 once on each strand and producing the mature 5S rRNA in a single endonucleolytic step.
32 RNase M5 is a small protein (20.5 kDa) that belongs to the Toprim domain family of
33 enzymes, including the topoisomerases and the primase DnaG. It is essentially confined to the
34 low G+C Gram-positive bacteria. Cleavage requires the binding of ribosomal protein L18 to

1 5S rRNA, presumably to fold it correctly for processing. Thus, like 23S rRNA, final
2 processing of 5S rRNA is subjected to a quality control step. Strains lacking RNase M5 can
3 no longer mature 5S rRNA, but yet are viable despite the presence of long (up to 165 nts) 5S
4 rRNA precursors in their translating ribosomes.

6 **Transfer RNA processing**

8 Transfer RNA genes are found in many different contexts in bacteria, as single transcription
9 units, co-transcribed with other tRNAs, or co-transcribed with other components of the
10 translational apparatus, such as ribosomal RNAs or elongation factors. In *E. coli*, all tRNAs
11 have the so-called CCA motif encoded by their genes. This is the single-stranded motif found
12 at the 3' end of all tRNAs to which the cognate amino acid is attached. In *B. subtilis*, about
13 one third of tRNA genes lack the CCA motif and in these cases the CCA is added post-
14 transcriptionally by an enzyme called nucleotidyl transferase. The proportion of tRNA genes
15 with a CCA motif in any particular organism is extremely variable throughout the bacterial
16 kingdom, ranging from all to none and everything in between. As we shall see this plays an
17 important role in determining which of two possible tRNA maturation pathways is used for
18 the 3' processing of a particular tRNA.

19 All tRNAs, even those that occur as single transcription units, are synthesized as
20 primary transcripts bearing extraneous sequences at both their 5' and 3' ends. The 5'
21 extensions are universally removed by the endoribonuclease RNase P (Figure 6). <Figure 6
22 near here> RNase P is a fascinating enzyme consisting of a catalytic RNA subunit and a
23 protein subunit, both of which are essential. The 13 kDa protein subunit has been proposed to
24 play multiple roles, the most important of which appears to be to increase the affinity of the
25 350-400 nt RNA subunit for the precursor tRNA, by changing its conformation near the
26 tRNA binding site. The crystal structure of RNase P holoenzyme bound to tRNA has been
27 solved ([pdb 3Q1Q](#)). Two different protein-only forms of RNase P have also been identified,
28 one in some bacteria, notably the *Aquificaceae* (HARP), and the archaea, and one found only
29 in eukaryotes (PRORP), but the ribozyme form is by far the most dominant form found in
30 nature.

31 Maturation of the 3' end of tRNAs can occur via a primarily exonucleolytic or
32 endonucleolytic pathway, depending mainly on the presence or absence of a CCA motif in the
33 precursor species. In *B. subtilis*, an enzyme called RNase Z endonucleolytically cleaves those
34 tRNA precursors lacking a CCA motif. RNase Z is a member of the Zn-dependent metallo-β-

1 lactamase family of enzymes, like RNase J. The structure of RNase Z has been solved in
2 complex with tRNA ([pdb 2FK6](#)). RNase Z generally cleaves tRNA precursors just
3 downstream of the discriminator nucleotide, the unpaired nucleotide (labeled N in Figure 6) at
4 the 3' end of the acceptor stem. Most CCA-less *B. subtilis* tRNAs have a naturally occurring
5 uracil (U)-residue immediately downstream of the cleavage site and RNase Z activity is
6 stimulated >200-fold by the presence this residue, explaining why this class of tRNAs is a
7 preferred substrate for RNase Z. In fact, the U-residue also determines the site of initial
8 cleavage. For the few CCA-less tRNA precursors where the U-residue is one or two
9 nucleotides further downstream of the discriminator base, endonucleolytic cleavage occurs
10 just 5' to the U-residue and then the enzyme chews back to the discriminator base in 3'-
11 exonucleolytic mode. Like many of the ribonucleases of the β -lactamase family, RNase Z has
12 both endo- and exonuclease activity.

13 The 3'-exonucleolytic tRNA maturation pathway was first discovered in *E. coli*. Here,
14 any of at least four redundant 3' exonucleases, RNase PH, RNase T, RNase D and RNase II
15 can perform the task, with the first two distributive enzymes being the most important. Should
16 any of these RNases degrade into the CCA motif present in *E. coli* tRNA precursors, the
17 damage can be repaired by nucleotidyl transferase. RNase PH ([pdb 1OYP](#)) is primarily
18 involved in maturation of small stable RNAs and is a member of the PDX family of
19 phosphorolytic 3'-exonucleases that includes PNPase. Homologs of RNase PH form the
20 hexameric core of the eukaryotic exosome that plays an important role in both mRNA
21 degradation and stable RNA maturation. RNase D ([pdb 1YT3](#)) is a distributive enzyme, also
22 dedicated to small stable RNA 3'-maturation in *E. coli* and a member of the same family of
23 RNases as RNase T. A homolog of RNase D is also associated with the yeast nuclear
24 exosome. Finally, RNase II ([pdb 2IX1](#)) is processive 3'-exoribonuclease that plays a more
25 important role in mRNA degradation. In the absence of these four redundant 3' exonucleases,
26 sufficient tRNA 3' maturation can be performed by RNase Z (also known as RNase BN) in
27 3'-exonuclease mode to maintain viability in *E. coli*. RNase Z progression is halted by the CC
28 residues of the CCA motif.

29 Of the four enzymes implicated in the *E. coli* 3'-exonucleolytic tRNA maturation
30 pathway, only RNase PH is found in *B. subtilis*. *B. subtilis* strains lacking RNase PH
31 accumulate precursors of those tRNAs with an encoded CCA motif. These precursors are one
32 to four nts longer than the mature tRNA species, indicating that RNase PH plays a key role in
33 the removal of the last few precursor nucleotides from the 3' end. RNase PH null mutants still

produce large quantities of accurately matured CCA-containing tRNAs, indicating that other enzymes can perform the same function. Indeed, *B. subtilis* strains lacking from one to four of its known 3'-exoribonucleases, RNase PH, PNPase, RNase R and YhaM accumulate progressively longer tRNA precursors, suggesting that, as in *E. coli*, multiple redundant RNases can perform the maturation reaction. PNPase is a processive phosphorolytic enzyme, related to RNase PH, whose principal role is mRNA degradation. RNase R is a processive hydrolytic 3'-exonuclease, with a much better ability to degrade through RNA secondary structures than either PNPase, or its cousin RNase II. It is known to play a major role in rRNA degradation and, more recently, in the decay of structured portions of mRNAs. The primary substrates of YhaM are unknown and this enzyme may actually play a more important role as a DNase than an RNase in *B. subtilis*. In the absence of other 3'-exoribonucleases, YhaM can clearly provide some back-up, however, as we saw for 23S rRNA. At least one other enzyme, likely to have an RNase T-like function, remains to be identified in the 3'-exonucleolytic tRNA maturation pathway in *B. subtilis*, since a significant portion of all CCA-containing tRNAs are still correctly processed at their 3' ends in strains lacking these four enzymes.

Processing of other stable RNAs

A number of small stable RNAs with specific properties have been identified in *E. coli* and *B. subtilis*. These too are synthesized as precursors requiring maturation by RNases. Identification of the RNases involved underscores the idea that the maturation pathways of stable RNAs are generally very different in these two organisms. .

4.5S/scRNA maturation

The 4.5S RNA of *E. coli* and its equivalent in *B. subtilis*, called small cytoplasmic RNA (scRNA), are part of the signal recognition particle (SRP) whose function is to direct proteins bearing an N-terminal export sequence to the plasma membrane as they emerge from the ribosome. This RNA is synthesized as a precursor with 5' and 3' extensions in both *E. coli* and *B. subtilis*. In *E. coli*, 4.5S RNA is matured on the 5' side by RNase P and on the 3' side by exonucleolytic trimming. As for tRNA 3'-maturation, several of the 3'-exonucleases appear capable of contributing to this process, with RNase T playing the most important role (Figure 7A). <Figure 7 near here>

B. subtilis has two possible pathways of scRNA maturation. The first step of both pathways is catalyzed by RNase III on the upstream side of a long double stranded region, to

1 produce the mature 5' end directly. The 3' end is then generated either by a second, less
2 efficient cleavage by RNase III on the 3' side of the stem, four nts downstream of the mature
3 3' end, or by endonucleolytic cleavage by RNase Y twelve nts downstream of the mature 3'
4 end. In both cases, the final maturation step is catalyzed by 3' exonucleolytic trimming by
5 redundant exonucleases, with RNase PH playing the predominant role. Although the cleavage
6 by RNase Y is shown in a double-stranded region in Figure 7A, it is thought that the
7 maturation of the 5' side by RNase III permits rapid degradation of the fragment of RNA
8 base-pairing with the RNase Y recognition site. Thus, these steps are likely to precede RNase
9 Y cleavage.

11 ***Maturation of the RNA subunit of RNase P***

12 The RNA subunit of RNase P (also known as P-RNA or M1 RNA) is transcribed from the
13 *rnpB* gene as a precursor molecule. The main promoter of the *rnpB* gene in *E. coli* produces a
14 P-RNA that does not require further maturation at the 5' end. Longer RNAs resulting from
15 read-through transcription from upstream genes accumulate in RNase E mutants, however,
16 and there is some debate about whether these RNAs can produce functional P-RNA.
17 Maturation of the 3' side of *E. coli* P-RNA requires cleavage by RNase E a few nts
18 downstream, followed by exonucleolytic trimming. RNase T plays the most important role
19 among the 3'-exonucleases of overlapping function (Figure 7B).

20 *B. subtilis* P-RNA maturation, on the other hand, has been proposed to be
21 autocatalytic. Indeed, accurate maturation of the 5' end of *B. subtilis* P-RNA can be
22 efficiently catalyzed *in vitro* by the RNase P holoenzyme. It has also been proposed that
23 RNase P processes its own 3', in this case producing a P-RNA four nts shorter than the
24 mature species identified *in vivo*. While autocatalytic maturation of the 3' end is still possible,
25 about 80% of RNase P RNA is in its precursor form in cells lacking RNase Y, strongly
26 suggesting this is the major 3' processing enzyme *in vivo*.

28 ***Maturation of tmRNA***

29 The transfer-messenger RNA (tm RNA or 10Sa RNA) plays an important role in the rescue
30 and recycling of ribosomes stalled on truncated mRNAs. Its tRNA-like structure can be
31 aminoacylated and inserted into the ribosome A-site. A conformational change then occurs on
32 the ribosome that causes it to switch templates from the truncated mRNA to the tmRNA. The
33 tmRNA encodes a small peptide tag that is added to the truncated protein, targeting it for
34 proteolysis. Maturation of tmRNA has been characterized in *E. coli* (Figure 7C). Not

surprisingly, for a molecule whose 5' and 3' extremities come together to form a tRNA-like structure, maturation of tmRNA employs the same pathway as *E. coli* tRNAs. RNase P removes the 5' leader sequence, while the 3' exoribonucleases perform the 3' reaction. RNase R, which co-purifies with tmRNA, has also been shown to play a role in 3' processing under conditions of cold growth. In *B. subtilis*, the tmRNA processing pathway is also very similar to that of its tRNAs. RNase P is responsible for processing at the 5' end, and since the tmRNA gene lacks the appropriate CCA motif for aminoacylation, RNase Z processes the 3' end. The CCA-motif is added subsequently to create a functional tmRNA, presumably by nucleotidyl transferase. Like the vast majority of tRNAs lacking an encoded CCA motif in *B. subtilis*, the tmRNA precursor also has a U-residue immediately downstream of the discriminator base to stimulate RNase Z cleavage.

Maturation of 6S RNA

The 6S RNA binds RNA polymerase and modulates sigma-70 holoenzyme activity in response to growth phase. It is thought to function as a mimic of the transcription bubble, thus inhibiting RNA polymerase activity. In *E. coli* 6S RNA is synthesized as a precursor molecule from two different promoters and has both 5' and 3' extensions. Transcription from the distal promoter P₂ is dependent on both sigma-70 and the stationary phase factor sigma-S, whereas the proximal promoter P₁ is sigma-70 dependent only. The long precursor is cleaved exclusively by RNase E to generate the mature 5' end of 6S RNA, while the short precursor can be processed by either RNase E or RNase G. Thus the biogenesis pathway of 6S is an interesting interplay between sigma factors and endoribonucleases. Maturation of the 3' side occurs via 3' exonucleolytic trimming, with RNases T and PH playing predominant roles. The 6S RNA processing pathway has not yet been published for *B. subtilis*.

Role of precursors in quality control?

Why synthesize stable RNAs as precursors, rather than directly as mature species? Although it would be feasible for cells to synthesize RNAs directly with mature 5' ends, simply by adjusting the point of transcription initiation (and this indeed is the case for *E. coli* RNase P RNA), direct production of the mature 3' end is more difficult. This is because the transcription termination signals are transcribed into RNA, making at least one processing step, i.e. removal of the terminator, almost unavoidable. It is also possible, however, to imagine that synthesis of stable RNAs as precursor molecules provides cells with a quality

control option, by leaving “access-ramps” in place for exonucleases to degrade these RNAs should they be prove to be non-functional. This idea is supported by the fact that the final steps of rRNA maturation generally only occur once subunit assembly is completed and, for some reactions, not until after the ribosome has initiated translation. Quality control mechanisms involving RNase R have been shown to exist in *E. coli* for rRNAs and tRNAs and these mechanisms rely on a re-synthesis of an exonuclease access-ramp by poly(A) polymerase. A similar phenomenon is seen with the role of RNase R in degrading unprocessed 16S rRNAs in *B. subtilis*. Moreover, in *B. subtilis*, RNase J1 can degrade 16S rRNA completely in 5'-exonuclease mode unless is stopped by some feature of the assembled 30S subunit. This phenomenon could be, and likely is, exploited by the cell as a quality control mechanism for 30S subunit assembly, afforded by a 5'-extension. The intervention of ribosomal proteins L3 and L18 in 23S and 5S rRNA maturation is thought to play similar role in the quality control of these rRNAs.

Future Orientations

Although the majority of the processing reactions of stable RNAs have now been characterized in *E. coli* and *B. subtilis* there are still a few loose ends. The 6S processing pathway and the enzyme that gives access to RNase J1 for the maturation of the 5' end of 16S rRNA remain to be identified in *B. subtilis*, as do the enzymes that catalyze the maturation of the 5' side of 23S and 5S rRNA in *E. coli*. The future of RNA processing probably lies outside of these two organisms, however. Many were taken by surprise by the extent of the differences between *E. coli* and *B. subtilis* maturation enzymes and pathways, despite their evolutionary distance (estimates vary between 1 and 3 billion years, greater than that of plants and animals). Clearly, other highly divergent model organisms, arepresentative of the archaea, for example, and perhaps a representative of the photosynthetic bacteria, require intense study before we can claim to have a global understanding of RNA processing throughout the bacterial kingdom.

Change History: September 2018. F. Braun and C. Condon updated the main text and Figures 1, 4 and 7. Additional articles were added for further reading,

Futher Reading

- Bechhofer, D.H. and Zen, K.H. (1989) Mechanism of erythromycin-induced *ermC* mRNA stability in *Bacillus subtilis*. *J. Bacteriol.* **171**, 5803-5811.
doi:10.1128/jb.171.11.5803-5811.1989
- Baumgardt, K., Gilet, L., Figaro, S. and Condon, C. (2018) The essential nature of RNase M16 (YqfG) required for 3' maturation of *B. subtilis* 16S ribosomal RNA is suppressed by deletion of RNase R. *Nucleic Acids Res.* doi: 10.1093/nar/gky488
- Condon, C. (2003) RNA processing and degradation in *Bacillus subtilis*. *Microbiol. Mol. Biol. Rev.* **67**, 157-174. doi: 10.1128/MMBR.67.2.157-174.2003
- Condon, C. and Putzer, H. (2002) The phylogenetic distribution of bacterial ribonucleases. *Nucleic Acids Res.* **30**, 5339-5346. doi: 10.1093/nar/gkf691
- Braun, F., Durand S. and C. Condon (2017) Initiating ribosomes and a 5'/3'-UTR interaction control ribonuclease action to tightly couple *B. subtilis* *hbs* mRNA stability with translation *Nucl. Acids Res.* **45**, 11386-11400. doi:10.1093/nar/gkx793
- Dunn, J.J. and Studier, F.W. (1973) T7 early RNAs and *E. coli* ribosomal RNAs are cut from large precursor RNAs *in vivo* by ribonuclease III. *Proc. Natl. Acad. Sci. USA* **70**, 3296-3300. doi: 10.1073/pnas.70.12.3296
- Even, S., Pellegrini, O., Zig, L., Labas, V., Vinh, J., Brechemmier-Baey, D. and Putzer, H. (2005) Ribonucleases J1 and J2: two novel endoribonucleases in *B. subtilis* with functional homology to *E. coli* RNase E. *Nucleic Acids Res.* **33**, 2141-2152.
doi:10.1093/nar/gki505
- Gilet, L, DiChiara, J.M., Figaro, S., Bechhofer, D.H. and C. Condon. (2015) Small stable RNA maturation and turnover in *Bacillus subtilis*. *Mol. Microbiol.* **95**, 270-282.
doi: 10.1111/mmi.12863
- Li, Z., Pandit, S. and Deutscher, M.P. (1999a) Maturation of 23S ribosomal RNA requires the exoribonuclease RNase T. *RNA* **5**, 139-146.
- Li, Z., Pandit, S. and Deutscher, M.P. (1999b) RNase G (CafA protein) and RNase E are both required for the 5' maturation of 16S ribosomal RNA. *EMBO J.* **18**, 2878-2885. doi: 10.1093/emboj/18.10.2878

- 1 Mathy, N., Benard, L., Pellegrini, O., Daou, R., Wen, T. and Condon, C. (2007) 5'-to-3'
2 exoribonuclease activity in bacteria: role of RNase J1 in rRNA maturation and 5'
3 stability of mRNA. *Cell* **129**, 681-692. doi: 10.1016/j.cell.2007.02.051
4
- 5 Pellegrini, O., Li de la Sierra-Gallay, I., Piton, J., Gilet, L. and Condon, C. (2012) Activation
6 of tRNA maturation by downstream uracil residues in *B. subtilis*. *Structure* **10**, 1769-
7 1777. doi: 10.1016/j.str.2012.08.002
- 8 Redko, Y., Li de la Sierra-Gallay, I. and Condon, C. (2007) When all's zed and done: the
9 structure and function of RNase Z in prokaryotes. *Nat. Rev. Microbiol.* **5**, 278-286.
10 doi: 10.1038/nrmicro1622
- 11 Sirdeshmukh, R. and Schlessinger, D. (1985) Ordered processing of *Escherichia coli* 23S
12 rRNA *in vitro*. *Nucleic Acids Res.* **13**, 5041-5054. doi: 10.1093/nar/13.14.5041
- 13 Yao, S., Blaustein, J.B. and Bechhofer, D.H. (2007) Processing of *Bacillus subtilis* small
14 cytoplasmic RNA: evidence for an additional endonuclease cleavage site. *Nucleic*
15 *Acids Res.* **35**, 4464-4473. doi: 10.1093/nar/gkm460
16
17

Figure Legends

Figure 1. Venn diagram of ribonucleases identified in bacteria. Exoribonucleases (Exos) are above the horizontal dividing line, mostly in blue type and represented by a ‘Pacman’ symbol. Endoribonucleases (Endos) are below the dividing line, mostly in black type and represented by a scissors symbol. Enzymes unique to *E. coli* are in the blue square on the left, those unique to *B. subtilis* are in the pink square to the right, and those in common are in the grey square in the center. Essential enzymes are in red type. Shared enzymes that are essential to *B. subtilis*, but not *E. coli* are in black type on the *E. coli* side of the Venn diagram, and in red type on the *B. subtilis* side. RNase J1 and J2 are in gray type as endonucleases as the relevance of this activity *in vivo* is under question. Only two bacterial enzymes have so far been identified that are not present in either *E. coli* or *B. subtilis*, Barnase, found in some Gram-positive species, and Cas6, part of the CRISPR defense mechanism.

Figure 2. Processing of mRNA in *E. coli*. (A) Organization of the *rpsU dnaG rpoD* operon and processing of its primary transcripts. Operon promoters are indicated as P_i and P_r. Open reading frames and their orientation are indicated by light blue arrows. Rho-independent transcription terminators (ter) are also shown. Transcripts are indicated by wavy lines and amounts detected *in vivo* are roughly proportional to the thickness of the lines. The site of RNase E cleavage in the intergenic region between *dnaG* and *rpoD* is represented by a blue scissors. (B) Organization of the *pap* locus of uropathogenic *E. coli* and processing of its primary transcripts. Promoters are indicated as P_i and P_r. Other symbols and conventions are as in (A). The 3' end of the long rightward *pap* transcript is unknown and indicated by a question mark.

Figure 3. Processing of phage mRNA. (A) Transcriptional and translational control of the lambda *N* gene. The top panel shows the assembly of the antitermination complex on the leftward operon of lambda, transcribed from promoter P_L. A similar complex is formed during transcription of the rightward operon from promoter P_R. The *N* open reading frame is indicated by a leftward oriented arrow. The positions of *nutL*, RNase III hairpin (hp) and Shine-Dalgarno (SD) elements are indicated. Transcription of *nutL* by RNA polymerase (RNA Pol) leads to the formation of an antitermination complex, containing the *nutL* RNA itself, N protein, and host factors NusA, NusB, NusG and NusE (=ribosomal protein S10), represented

by the letters A, B, G and E, respectively. The 5' end of the *N* transcript (represented by a curved black line) remains attached to RNA polymerase through the interaction with *nutL*. In this conformation, ribosomes do not have access to the SD element of the *N* coding sequence. The lower panel shows cleavage of the hairpin (hp) by RNase III, freeing up the SD element for ribosome binding and translation of *N*. (B) Organization of the phage T7 early operon and processing of its primary transcript. The operon's three promoters (P) are indicated by the triple headed arrow. Open reading frames and their orientation are indicated by brown arrows. The operon's Rho-independent transcription terminator (ter) is also shown. Transcripts are indicated by wavy lines and sites of RNase III cleavage in five intergenic regions are indicated with blue arrows. (C) Organization of the phage lambda leftward operon and retroregulation of *int* gene expression. For description, see text. Promoters are indicated as P_i and P_r. Open reading frames and their orientation are indicated by light blue arrows. The transcription terminator (ter) and site of RNase III cleavage (sib) are indicated. PNPase is represented by a red 'Pacman' symbol.

Figure 4. Processing of mRNA in *B. subtilis*. (A) Antitermination and processing of the *thrS* transcript. The left panel shows binding of tRNA^{Thr} (red cloverleaf structure) to the *thrS* leader mRNA, stabilizing the antiterminator hairpin. The position of suspected RNase Y cleavage is indicated by a blue scissors. The right panel shows the transcriptional organization of the *thrS* gene, with promoter (P) and terminators (ter). Transcripts are indicated by wavy lines, with the thickness of the line roughly proportional transcript abundance *in vivo*. (B) Organization of the *B. subtilis hbs* gene and processing of its transcript. The 5' exoribonuclease activity of the RNase J1/J2 complex is represented by a 'Pacman' symbol. The RNase Y cleavage is indicated by a scissors symbol. Ribosomal subunits are represented by purple ovals. Nucleotide co-ordinates are given above the *hbs* transcript, indicated by a wavy line. (C) Organization of the *gap* operon and processing of its primary transcripts. Symbols and conventions are the same as in previous figures. RNase J1 is the prime candidate for cleavage at the site indicated by the scissors symbol. (D) Organization of the *dnaK* heat-shock operon and processing of its primary transcripts. Symbols and conventions are the same as in previous figures. RNase J1 is also the prime candidate for cleavage at the site indicated by the scissors symbol.

Figure 5. Processing of rRNA in *E. coli* and *B. subtilis*. (A) General organization of rRNA operons. Transcription begins at tandem promoters (P₁ and P₂) and ends at the transcription

terminator (ter). Transfer RNA genes [tRNAs] sometimes occur in the spacer regions between the 16S and 23S rRNA genes or downstream of the 5S gene. (B) Processing of 16S rRNA in *E. coli* (left) and *B. subtilis* (right). The mature 16S sequence is in light blue, precursor sequences in black. The number of extra nts remaining following processing at each site is indicated below the enzyme in question. Unknown enzymes are indicated in red type. Endonucleolytic cleavages are represented by black arrows perpendicular to the transcript; exonucleolytic digestion is represented by black arrows parallel to the transcript. Although the different cleavage steps are numbered, the order of cleavages is not obligatory for 16S rRNA maturation in either organism. Adapted from Britton *et al.* Mol. Microbiol. (2007) 63:127-138. (C) Processing of 23S rRNA in *E. coli* (left) and *B. subtilis* (right). The mature 23S sequence is in green, precursor sequences in black. Mini-III processing of *B. subtilis* 23S rRNA requires r-protein L3. Symbols and conventions are as in (A). In *E. coli*, RNase III cleavage is an obligatory first step for generation of mature 5' and 3' ends. The order of steps 2 and 3 is not known. In *B. subtilis*, accurate maturation can occur in the absence of RNase III. (D) Processing of 5S rRNA in *E. coli* (left) and *B. subtilis* (right). The mature 5S sequence is in mauve, precursor sequences in black. RNase M5 processing of *B. subtilis* 23S rRNA requires r-protein L18. Symbols and conventions are as in (A). As for 16S rRNA, prior cleavage by RNase III is not obligatory in either organism.

Figure 6. Processing pathways of tRNAs in *E. coli* and *B. subtilis*. (A) Exonucleolytic pathway. The mature tRNA is represented by a red cloverleaf structure, precursor sequences in black. Endonucleolytic cleavages are represented by black arrows perpendicular to the transcript; exonucleolytic digestion is represented by black arrows parallel to the transcript. The key 3' exonucleases are in bold type. In *E. coli*, extremities accessible to the 3' exonucleases are often generated by prior cleavage by RNase E. An RNase T-like enzyme remains to be identified in *B. subtilis*. (B) Endonucleolytic pathway. The 'mature' tRNA is represented by a red cloverleaf structure, although this requires further addition of the CCA motif by nucleotidyl transferase, with the discriminator base (N) and the downstream uracil (U) residue indicated. Symbols and conventions are as in (A). Adapted from Redko *et al.* Nature Rev. Microbiol. (2007) 5:278-286.

Figure 7. Processing of various small stable RNAs in *E. coli* and *B. subtilis*. (A) Processing of 4.5S/scRNA. The mature RNA is in light blue, precursor sequences in black. Endonucleolytic cleavages are represented by black arrows at an angle to the transcript;

1 exonucleolytic digestion is represented by black arrows parallel to the transcript. The key 3'
2 exonucleases are in bold type. (B) Processing of RNase P RNA. The mature RNA is in
3 mauve, precursor sequences in black. Symbols and conventions are as in (A). Although most
4 RNase P RNA is synthesized with a mature 5' end in *E. coli*, some precursor can be produced
5 by read-through transcription from upstream genes (represented by a dotted line). The details
6 of the processing pathway of these transcripts are unknown, but involves RNase E in *E. coli*
7 and RNase Y in *B. subtilis*. (C) Processing of tmRNA. The mature RNA is in red, precursor
8 sequences in black. Symbols and conventions are as in (A). (D) Processing of 6S RNA in *E.*
9 *coli*. The mature RNA is in green, precursor sequences in black. Symbols and conventions are
10 as in (A).

Exos

Endos

E. coli

B. subtilis

RNase II* RNase T* Oligo-RNase* RNase D*		PNPase* RNase PH* RNase R*	RNase J1* YhaM KapD	RNase J2 NrnA* NrnB
RelE* ChpB YoeB* YhaV HigB* YafO	RNase E* RNase G RNase I* RNase LS RNase HI* YafQ* MqsR*	RNase III* RNase P* RNase Z* MazF/EndoA* RNase HII* YbeY/YqfG*	RNase Y* Mini-III* RNase M5 RNase Bsn	Rae 1* YhcR RNase HIII*

Cas6

Barnase

Figure 2

A

B

C

Figure 3

Figure 4

Figure 5

Figure 5

A

Exonucleolytic pathway

B

Endonucleolytic pathway

Figure 6

A

B

C

D

Figure 7