Review Letter

PROCESSING OF BACTERIAL RNA

David APIRION and Peter GEGENHEIMER
Department of Microbiology and Immunology, Washington University School of Medicine, Box 8093, St Louis, MO 63110, USA

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1. Introduction

Processing of RNA is a feature of RNA metabolism which contributes to the determination of the final population of active RNA molecules in the cell. Processing is the sum of events which converts a primary RNA transcript into a functional molecule. RNA processing in bacteria consists mainly of two parts, modification of nucleotides and cutting and trimming of the molecule; here, we shall concentrate only on the latter. There is ample evidence that rRNA and tRNA go through extensive processing events in the bacterial cell (this area is covered by a number of articles in recent symposia [1,2]). During the processing events, which occur mainly during transcription [3], specific endoribonucleases introduce cuts into the growing transcripts. At present at least three such nucleases are known in *Escherichia coli*: RNase P [4]; RNase III [3,5]; and RNase E [6–8]. (This short review cannot be all-inclusive, and will reflect our own bias. Because of space limitations we shall emphasize only certain aspects of RNA processing and in each area we shall be able to mention only some of the references.)

Mutants which affect these three enzymes are available and strains have been constructed which are defective in one, two or three of these enzymes [9]. By studying tRNA and rRNA synthesis at temperatures permissive and non-permissive for these strains, it was deduced that these three enzymes are the major participants in the primary processing (processing which occurs during transcription) of tRNA and rRNA molecules in the cell and that there is probably one more enzyme which participates in endonucleolytic primary processing of rRNA and tRNA [10].

While primary processing of rRNA yields in *E. coli* the immediate precursors of the mature rRNA species, it is the secondary processing events which yield the final mature RNAs: 16 S, 23 S and 5 S. At present, while the gross features of primary rRNA processing are emerging, knowledge about secondary RNA processing is very minimal. However, there is one essential feature which seems to distinguish these two processes, i.e., namely the requirement for proteins. While all steps of RNA processing occur in the cell at the level of ribonucleoprotein particles, there does not seem to be a need for any protein beside the RNA processing enzymes in primary processing, whereas in secondary processing the substrate seems to be obligatorily an RNP particle [11–13]. The overall process of primary and secondary rRNA processing in *E. coli* is depicted in fig.1.

2. Enzymes involved in RNA processing

A large number of enzymes has been implicated in RNA processing. However, of these only five are sufficiently characterized to be dealt with here. These are RNase P [14,15], RNase III [5,16], RNase E [8], RNase D [17] and RNase M5 [18].

From analysis of tRNA precursors accumulated in RNase P^{ts} mutants, it is evident that RNase P is required for 5′-maturation of most *E. coli* tRNA species [15]. RNase P is an interesting processing enzyme since its enzymatic activity requires both a polypeptide and an RNA component [19,20].

Since no primary sequence similarities exist among all the numerous RNA molecules which are substrates for RNase P, it is apparent that secondary structure, and tertiary conformation play a major role in determining the specificity of RNase P. The correct conformation of the mature portion of a tRNA precursor is a part of the recognition signal [15,21].

RNase III was first purified as an endonucleolytic activity which converted double-stranded RNA to

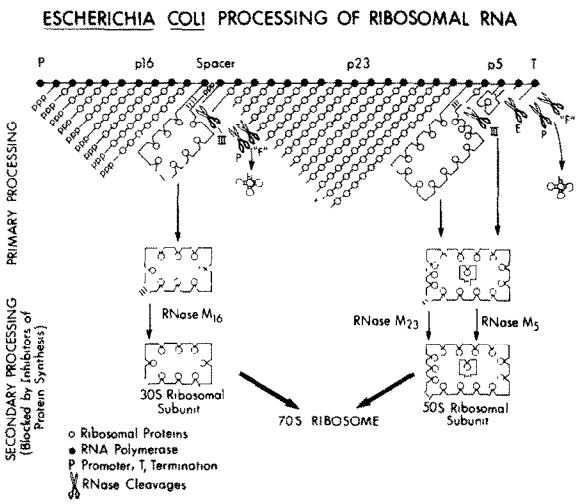

Fig.1. Maturation and assembly of ribosomes. Each nascent chain of ribosomal ribonucleoprotein represents a successive time point in the transcription of an rRNA operon by an RNA polymerase molecule. Many ribosomal proteins attach sequentially to the nascent rRNA, and endonuclease cleavages by RNases III and E can occur only after the 3′-terminal region of the substrate has been synthesized and hydrogen-bonded to the 5′-terminal region. The spacer and trailer tRNAs are not drawn in the nascent transcript, but they too must assume their mature configuration before being cleaved by RNases P and 'F'. Secondary maturation of p16, p23, and p5 rRNAs occurs only in ribonucleoprotein particles, and involves removal of some or all of the duplex formed between 5′- and 3′-termini in the precursor rRNA (see fig.2).

acid-soluble form [5]. Synthetic riboheteropolymeric duplexes are digested to products averaging 13–16 bases long containing 5′-phosphoryl and 3′-hydroxyl termini [22–24]. Cleavage is random with respect to sequence [23]. Under certain conditions, RNase III can also cleave single-stranded RNA [16].

In 1973, two cellular functions were found for RNase III. It converts a polycistronic transcript of the bacteriophage T7 'early' region into discrete monocistronic messenger RNAs [25], and it was shown to be involved in ribosomal RNA processing [25,26].

RNase E is involved in the production of p5 rRNA [6–8]. (p5 is the precursor to m5, mature 5 S rRNA, and it contains three extra nucleotides at the 5′-end [27].) RNase D is an exonuclease which can remove step-wise nucleotides from the 3′-end of tRNA precursor molecules [17]. Comparison of RNase D and RNase II, another known 3′-exoribonuclease, showed that RNase D is much more selective than RNase II in the removal of 3′-nucleotides from tRNA-like precursors [17]. The four enzymes discussed above, RNase P, RNase III, RNase E and RNase D, were isolated from *E. coli* cells.

The enzyme RNase M5 has been isolated from *Bacillus subtilis*. It processes an 179 base precursor to mature 5 S rRNA [18]. The partially purified enzyme is composed of two subunits, α and β [18]. The β component has substrate-binding capability, whereas the α component appears to be the catalytic subunit [28]. Substrate recognition and cleavage rate are affected partly by the precursor-specific, but largely by the mature portions, of the p5 molecule [28].

Other enzymatic activities have been implicated in RNA processing, but, there is not sufficient information about any of them to conclude whether or not they are identical with any of the enzymatic activities mentioned above. However, at least one further endonucleolytic activity is required to explain cleavage at or near the 3′-end of spacer and trailer ribosomal tRNAs. Such an activity was generally denoted RNase 'F' [10]. In a cell-free extract, Sekiya et al. [29] detected cleavage of an in vitro transcript of a synthetic $tRNA^{Tyr}_{su\,3}$ gene seven bases past the mature 3′-terminus. Other studies also suggested endonucleolytic processing activities [30]. Whether or not all of those endonucleolytic RNA processing activities are due to one or more new enzymes remains to be seen.

3. Genetics of RNA processing

Mutants are available which affect the three RNA processing ribonucleases, RNase E, III and P. The genes, mutations in which affect these enzymes are *rne, rnc, rnpA* and *rnpB*, respectively. They are located at min 24, 55, 82 and 70 of the *E. coli* chromosome, respectively. (For further details concerning isolation and mapping of these mutations consult [31].) At present only single mutations are available in the *rnc* and *rne* genes, whereas at least four mutations are known to affect RNase P. These four mutations fall into two genes [31,32]. While it is not known whether the mutation in the *rnc* gene affects the structure of RNase III, the mutations in the *rne* and the *rnp* genes do affect the structure of the corresponding enzymes [20,32,33].

E. coli strain BN (a B strain) is a viable mutant [34] which after infection with bacteriophage T4 accumulates several phage-coded tRNAs with extra

sequences at their 3′-ends [35]. The mutant strain is apparently defective in the 'BN' nuclease [35], which removes nucleotides from the 3′-ends of a subset of T4-coded tRNAs. The relationship between this enzyme and RNase D is not clear.

4. Processing of ribosomal RNA

The understanding of rRNA processing in *E. coli* has been greatly facilitated by the analysis of rRNA metabolism in various mutants defective in one or more prcoessing enzymes. Therefore, we shall explain in some detail rRNA processing in a wild type strain and in various mutants.

4.1. *Structure of RNase III processing regions*

It has been proposed that RNase III cleavages occur in duplex structures formed by base-pairing

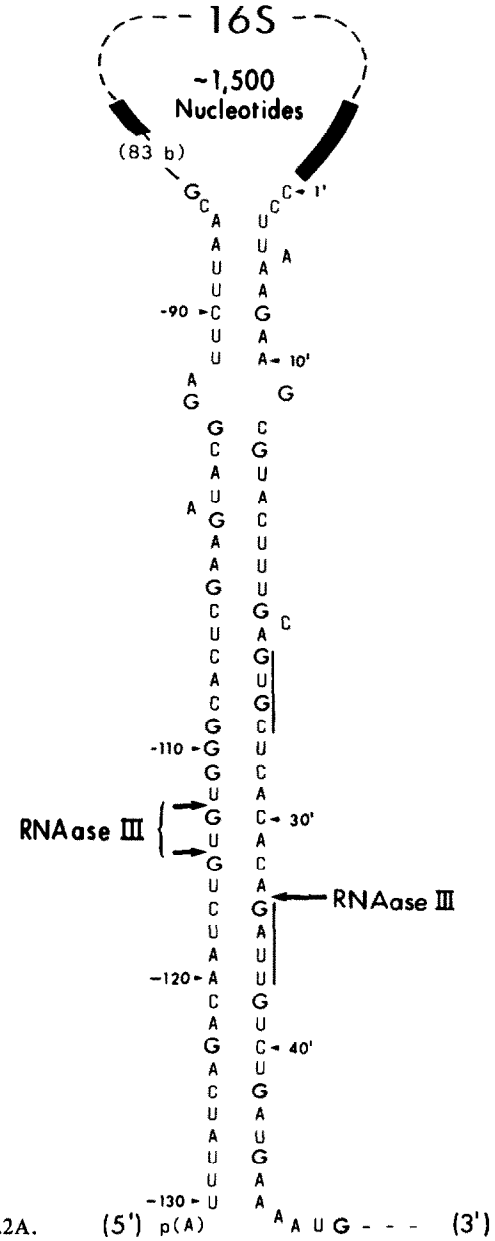

Fig.2A.

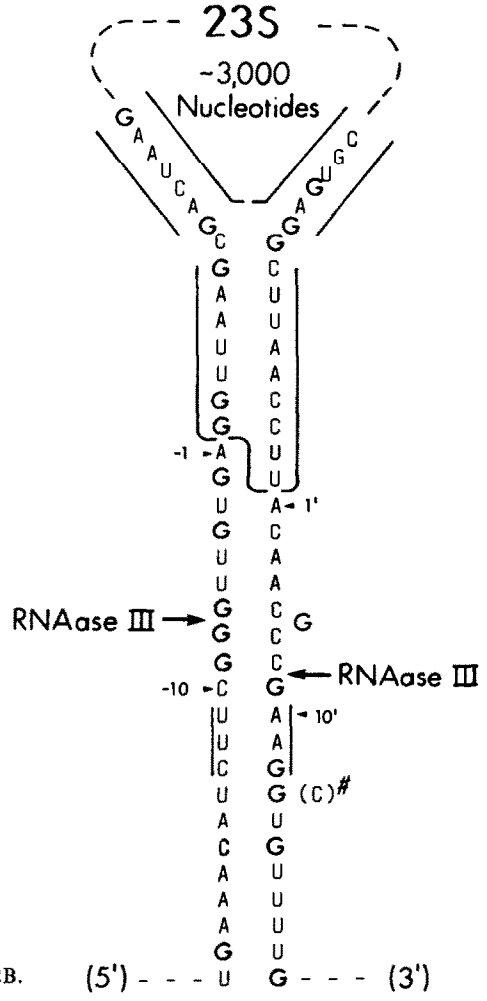

Fig.2B.

Fig.2. RNase III processing sites for ribosomal RNA. Duplex RNA 'stems' were isolated from the p16b and p23b rRNA precursors found in RNase III⁻ cells. (A) Terminal duplex and RNase III cleavage site for p16 rRNA. Mature 16 S sequences start at the solid bars (B) Terminal duplex and RNase III cleavage site for p23 rRNA. The terminal sequences of mature 23 S are enclosed within solid lines. Vertical lines denote regions of homology with other RNase III cleavage sites (for further details see [39,41,46]).

between regions of the ribosomal RNA transcript flanking the 5′- and 3′-ends of 16 S or of 23 S sequences [3,36,37]. These duplex structures were isolated from specific RNAs which accumulate in RNase III⁻ mutants [38,39]. The sequence and secondary structure of the p16 and p23 RNase III processing regions is shown in fig.2. Thus, it seems that ribosomal RNA transcripts in the cell take on the form of giant ribonucleoprotein loops held together by duplex stems (fig.1); p16 and p23 rRNA precursors arise by cleavage within these stems.

The cleavage sites indicated in fig.2 are compatible with the 'preferred' 5′-termini of many sequenced RNase III cleavage products [40,41]. In addition, some sequences (fig.2) are conserved in the vicinity of the RNase III cuts in many naturally occuring substrates [40–42]. Although the significance of 'preferred' termini and 'conserved' sequences remains to be determined, it seems plausible that some pattern of primary sequence information may guide RNase III towards cleavage of a specific phosphodiester bond.

4.2. *Processing in wild-type cells*

The detailed sequence of processing events discussed here is illustrated by the models presented in fig.1,3. An RNA polymerase molecule which initiates transcription of a ribosomal RNA gene cluster (fig.1) continues to synthesize the various components of the polycistronic rRNA primary transcript. Before transcription is terminated, however, processing endonucleases are already acting on the nascent transcript. As polymerase molecules complete synthesis of p16 rRNA, the inverted complementary sequences flanking the m16 transcript anneal to form a double-stranded 'stem' (fig.2) which is cleaved endonucleolytically by RNase III (fig.3, cuts 1A,1B) to release p16 S rRNA plus a 5′-leader fragment. As the spacer region is synthesized, tRNAs are removed by endonucleolytic cleavage (RNase 'F') at or near the 3′-side (cut 3), and at the 5′-end by RNase P (cut 2). Trimming of the 3′-end, perhaps by RNase D [17], might also be required to produce mature spacer tRNAs. As the RNA polymerase completes transcription of 23 S genes, RNase III excises p23 sequences, again by cleaving in the double-stranded stem formed by complementary sequences surrounding m23 (fig.3, cuts 4A,4B).

Transcription of the distal portion of the gene cluster now procedes into the 5 S gene (fig.1). As

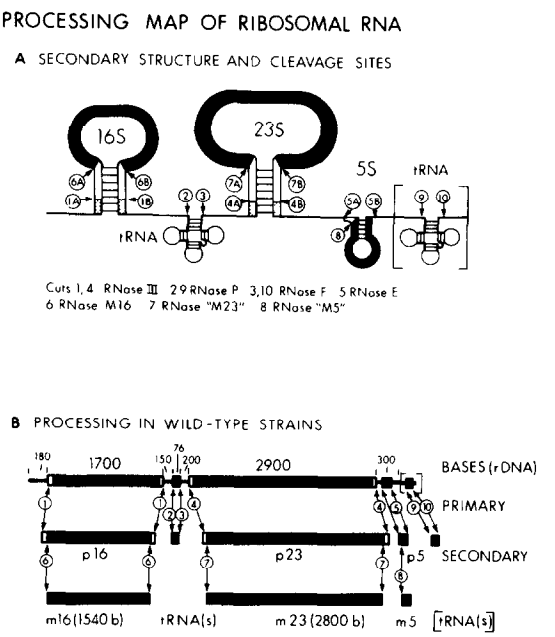

Fig.3. Structure and processing map of ribosomal RNA transcripts. (A) Structure and cleavage sites of the rRNA primary transcript (not to scale). Derived from data from a number of laboratories, summarized in [3,10]. Distal (trailer) tRNAs are bracketed, since not all rDNAs contain them. Transcripts may contain one or two spacer tRNAs, and zero, one, or two trailer tRNAs. Arrows indicate endonucleolytic cleavage sites. Each cutting event is given a separate number, referring to the enzyme involved: 'A' and 'B', where used, indicate that two separate cuts might be required. Thick solid segments represent mature rRNA sequences, thick open segments represent precursor-specific sequences removed during secondary processing steps, stippled segments are sequences found only in p16b and p23b of RNase III⁻ cells, and thin lines (except for tRNAs) represent non-conserved sequences discarded during primary processing. Enzymes are discussed in the text. (B) Processing in wild-type strains. The first line shows the transcriptional map of a representative rDNA unit, approximately to scale. Distances in bases are between vertical bars above the map. The primary and secondary cuts, numbered as in 3A, are shown above the products they generate. Open and solid segments are as in 3A.

soon as p5 rRNA sequences are formed they are excised by RNase E (fig.1; fig.3, cuts 5A,5B), and distal or 'trailer' tRNAs are removed by RNase P (cut 9) and another activity (cut 10) perhaps identical with RNase 'F'.

That p23 and p5 rRNA are excised from rRNA transcripts prior to transcription termination is evidenced by the failure to detect 25 S rRNA (p23 plus p5) [3] or 9 S [43] in wild-type strains. Thus, it seems that the primary processing cleavages by RNase

III and RNase E are rapid events and virtually all sites are cleaved within seconds after synthesis of the sequences which comprise them.

4.3. *Processing in mutant strains*

The origin of rRNA species seen in mutants defective in RNA processing enzymes (fig.4) can readily be described by reference to the model shown in fig.3. In all mutants which contain RNase III (*rnp, rne, rne rnp*) synthesis of the large rRNAs is not affected (as to why 16 S does not appear in the *rne* and *rne rnp* mutants at 43°C, fig.4). In strains lacking the processing endonuclease RNase III, scission of nascent rRNA transcripts is initiated by RNase P and another enzyme(s) (RNase 'F') (cuts 2,3). The single-stranded region between cut 2 and the p16 stem is removed, possibly by enzyme(s) such as RNase II or polynucleotide phosphorylase, giving rise to 18 S RNA (fig.4). Subsequently, the 5′-leader sequence is removed from 18 S RNA in a slower process by single-strand specific (endo)nuclease(s) which leaves the duplex stem intact. The final product, a p16b molecule which contains the entire duplex stem and is thus slightly larger than normal p16 [39], is converted to normal m16 rRNA by the maturation enzyme(s) RNase(s) M16 (cuts 6A,B). RNase E cleavage in the distal portion of the nascent rRNA transcript would generate p5 rRNA and a p23-like molecule, the latter containing extra single-stranded spacer sequences extending from cleavages 3–5A (fig.3) which are probably non-specifically removed at the 3′- and 5′-sides, giving rise to the p23b rRNA of the *rnc* strain [39]. This p23b is further processed to mature m23 rRNA via the maturation enzyme(s) RNase(s) 'M23' (cuts 7A,B).

When RNase E is inactivated in the *rne* mutant, p5 rRNA is not removed from the distal portion of the transcript, but instead accumulates as 9 S RNA (cuts 4B–9; fig.5) [7]. If RNase P is also inactivated

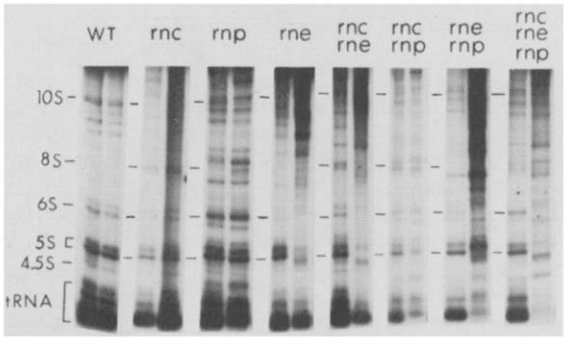

Fig.4. Ribosomal RNAs produced in different RNA processing mutants. For each of the genotypes indicated, cultures of the corresponding strain were labeled with $^{32}P_i$ at 30°C or at 43°C and nucleic acids were fractionated on a 3% polyacrylamide gel (details as in [3]). After electrophoresis the gel was dried, autoradiographed, and photographed. For each strain the left lane represents RNA from cells grown at 30°C, while the right lane represents RNA from cells labeled at 43°C. While the *rne* mutant behaves like a classical *ts* mutant, the *rnc* mutant does not. Strains carrying the *rnc-105* allele are completely missing RNase III [39] and therefore the patterns observed at 30°C and 43°C are similar. The *rnp-A49* mutation used here is apparently defective in the synthesis but not in the function of RNase P [20] and certain features of this mutation are already expressed at temperatures where the mutant can grow [55] (see also fig.5). The *rne* and *rne rnp* strains used here contain an additional *ts* mutation(s) which blocks protein synthesis [6], and therefore 16 S rRNA, the product of secondary rRNA processing, is not observed in these strains. It can be seen that in the *rnc rne* double mutant and in the *rnc rne rnp* triple mutant 16 S rRNA appears. While the difference between p16 and m16 is substantial (~150 nucleotides) and can be easily dected on gels, the difference between p23 and m23 is only 15 nucleotides [39,41] and cannot be observed in the gel. The differences between p16a and p16b, and p23a and p23b (see text) are also not readily observed in gels.

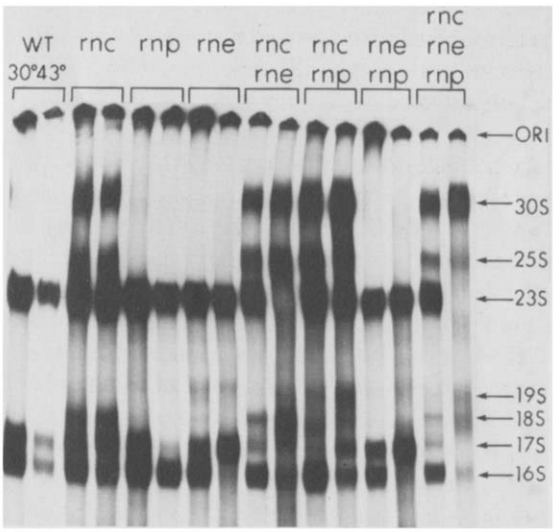

Fig.5. Display of small RNAs in various RNA processing mutants of *E. coli*. Cells were labeled with $^{32}P_i$ at 30°C, or at 43°C. The cells were processed and their contents analysed on a 5%/10% tandem thin-slab polyacrylamide gel [3]. The 5% portion of the gel was removed from the photograph. The strains used here and in fig.5 were the same or similar to those described in [9].

Table 1
Ribosomal RNA molecules which appear in various RNA processing mutants

Genotype			Containing 5 S			Containing 16 S					Containing 23 S				Intact transcript
rnc	*rne*	*rnp*	5 S	9 S	11 S	16 S	17 S[a] (p16a)	17 S[a] (p16b)	18 S	19 S	23 S	p23a[b]	p23b[b]	25 S	30 S
+	+	+	v			v	v				v	v			
–	+	+	v			v		v	v		v		v	v	v
+	+	–	v			v	v				v	v			
+	–	+		v		v	v				v	v			
–	–	+				v		v	v					v	v
–	+	–	v			v		v	(v)[c]	v	v		v	v	v
+	–	–			v	v	v				v	v			
–	–	–				v		(v)[c]	v	v				v	v

[a] p16a is slightly smaller than p16b, the difference being in the double-stranded stem of the molecule [39] (fig.2,3)
[b] p23a is slightly smaller than p23b, (fig.2,3)
[c] Low levels were observed

This table shows the rRNA molecules which can be observed (v) in the various strains at elevated temperatures. For further details see text and fig.2–5. Some of the molecules cannot be clearly observed in fig.2–5 and the reader is advised to consult specific references which are mentioned in the text: 11 S contains 5 S and trailer tRNAs; 18 S contains 16 S and leader sequences; 19 S contains 16 S, leader sequences and spacer tRNAs; 25 S contains 23 S and 5 S; 30 S is the whole RNA from the rRNA transcription units, containing leader sequences, 16 S, spacer tRNA, 23 S, 5 S and trailer tRNAs

(*rne rnp* strains), trailer tRNAs are found linked to 5 S rRNA (cuts 4B–10) [44]. Failure to perform RNase E and RNase III cleavages, as in the *rnc rne* strain at the non-permissive temperature, yields p5 sequences linked to p23 rRNA forming a 25 S RNA which accumulates at the expense of p23 and p5 [6,45].

Spacer tRNAs are linked to 18 S RNA, giving 19 S RNA, if RNase P is inactivated in an *rnc* strain (*rnc rnp* mutants). Each 19 S molecule is initiated with a nucleoside 5′-triphosphate and, at its distal end, contains a spacer tRNA which terminates with the mature 3′ CCA_{OH} sequence [10,46]. Production of 19 S RNAs therefore requires endonucleolytic cleavage near or at the 3′-ends of tRNAs (cut 3, fig.3). Inactivation of RNase III, RNase P, and RNase E allows the primary production only of 25 S and of 19 S RNA (fig.4, triple mutant). Uncleaved 30 S RNA is also synthesized. It appears, then, that in the *rnc rne rnp* strain at least one distinct endonuclease does exist which can cleave nascent rRNA chains into proximal 19 S and distal 25 S portions. The simplest hypothesis is that an endonuclease which recognizes and cleaves at the 3′-end of spacer tRNA molecules (fig.3, cut 3) is responsible for release of 19 S rRNA from growing rRNA chains. We refer to this activity as RNase 'F'.

The various rRNA molecules observed in the different strains are listed in table 1.

4.4. *Efficiency and order of processing steps*

Since RNase III$^-$ cells are viable (the mutation is not conditional), and form normal, functional mature m16, m23, and m5 ribosomal RNAs, the physiological necessity for RNase III might well be questioned. However, in *rnc*$^+$ cells no uncleaved primary transcripts are detected, whereas in *rnc-105* cells a large proportion of the newly synthesized rRNA transcripts are uncleaved 30 S and partially cleaved 25 S molecules, neither of which can be productively converted in vivo to mature rRNA species [3]. The uncleaved 25 S and 30 S RNAs appear in an *rnc* strain even though the cells still contain the enzymes RNase P, E and 'F' which could cleave such substrates. Apparently while these enzymes are very efficient when cleaving after RNase III and hence confronting a relatively small RNA molecule, their efficiency is decreased when, in the absence of RNase III, they have to face a very large substrate (consult fig.3) [3,10].

4.5. *Secondary processing of rRNA*

The primary processing events described here give rise to precursor forms of 16 S, 23 S and 5 S rRNAs,

whose maturation seem to occur obligatorily in ribonucleoprotein particles. The processing of p16 to m16 rRNA by RNase(s) M16 [11–13] is probably endonucleolytic (fig.3, cuts 6A,6B). Similarly, cuts 7A,7B (fig.3) are proposed to mature p23 to m23 rRNA, and p5 rRNA might be trimmed to m5 exonucleolytically [27] by RNase 'M5' (cut 8, fig.3).

Since these secondary maturation enzymes are unaffected by the processing mutations described here, the viability of an RNase III$^-$ cell can be ascribed to the ability of the maturation enzymes (RNases 'M16' and 'M23') to recognize and process the abnormal p16b and p23b substrates produced in the absence of RNase III. The short extension of the duplex stem observed in these species (fig.2,3), does not seem to alter the accuracy of cleavages which give rise to m16 and m23 rRNAs.

5. Processing of transfer RNA

The immediate transcriptional products of *E. coli* tRNA genes are molecules containing extra nucleotides at both their 5′- and 3′-termini. Reflecting the nature of tRNA gene organization, many transcripts are polycistronic, containing as many as four or five different or identical tRNA species [1,2,4,15,21]. These intact transcripts are not detected in wild-type cells in appreciable quantities, suggesting that the endonucleolytic processing cleavages begin to occur before transcription is terminated.

That RNase P is producing the 5′-ends of many and perhaps all tRNA molecules of *E. coli* is well established [4,15,21]. The other endonucleolytic RNA processing enzymes mentioned here, RNase III and RNase E also play a role in tRNA processing [10]. When cultures of strains lacking RNase III (*rnc*), or containing thermolabile RNase P (*rnp*) or RNase E (*rne*), either singly or in all possible combinations, were labeled at 30°C and 43°C, and their RNA species fractionated on 5%/10% polyacrylamide slab gels [10], as can be seen in fig.5, the level of RNA appearing in the tRNA region of the gel diminishes in the conditional mutants at the non-permissive temperature and is very much reduced in the triple *rnc rne rnp* mutant. The lack of tRNA processing is genuine and is not due to an effect of lack of processing on transcription, since in the triple mutant strain a large variety of larger RNA molecules accumulate (fig.5).

The RNAs in the 4–5 S range were further analysed by two-dimensional gel electrophoresis [10]. Analysis of RNA synthesized at 43°C showed that, beside RNase P, RNase E plays a major role in tRNA processing and RNase III also has some function. Since *rnc* strains grow even at 43°C [3,10] they must be able to mature all the necessary tRNA molecules in the absence of RNase III. (RNase III is completely missing in *rnc-105* strains [39].) We suggest that RNase III is not required for the final maturation steps of tRNA, but rather in earlier steps and that cutting of tRNA precursors by RNase III is not an obligatory prerequisite for subsequent maturation events. This is precisely the situation which was clearly demonstrated for the role of RNase III in the maturation of rRNA, where it was shown that the cell can produce mature functional rRNA, albeit at a much reduced rate, in the absence of RNase III (see above).

In the absence of RNase E (in an *rne* mutant grown at 43°C) molecules accumulate which contain tRNA sequences. These molecules can be processed to tRNAs in a reaction which requires RNase E, RNase P as well as some other cellular component(s) [47].

Apart from RNases III, E, P, and one more endonuclease (RNase F), at least one additional activity, an exonuclease, is required for trimming extra sequences from the 3′-termini. Since in all *E. coli* tRNAs examined the CCA terminus of the mature molecule is encoded in the DNA, and is present in the RNA precursor, RNase D [17] activity could be sufficient for 3′-trimming.

6. Processing of messenger RNA

The first clear-cut evidence for processing of RNA in prokaryotes came from a phage rather than a host system. Although the expression of many genes is altered in cells defective in one or more processing enzymes [48], so far no host mRNA has been described whose function is dependent upon a processing event.

6.1. *Host mRNAs*

Analysis of total cell proteins separated in two-dimensional gels revealed that the synthesis of 21 out of 80 individual proteins examined is markedly

reduced in the *rnc rne rnp* strain at 43°C and that this difference is due mainly to the *rnc* and *rne* mutations [48]. One possible interpretation of these results is to assume that some *E. coli* mRNAs require processing for maximal expression. Another possibility is that in wild-type cells RNA processing enzymes can protect certain mRNAs from being inactivated by non-specific nucleases. It is plausible that RNA processing enzymes could bind to ribosome-binding sites of some mRNAs without cutting them and thus protect them from nucleolytic attack. Protection of rRNA by RNase III has been proposed to explain increased degradation of rRNA during carbon starvation in *rnc* cells [49].

In [50] it was shown that RNase III introduces a cleavage into an mRNA from *E. coli* which codes for two ribosomal proteins and the β and β' polypeptides of RNA polymerase. However, the effect of this cut on protein synthesis has not been determined.

6.2. *Bacteriophages T7 and λ mRNAs*

The most thoroughly studied example of mRNA processing in prokaryotes is that of bacteriophage T7. The 'early region' of T7, from 0–~7500 basepairs, contains five genes. In wild-type cells, the RNA transcribed from this region is processed to monocistronic mRNAs by RNase III [25] but the efficiency of only the first (toward the 5'-end) of these cistrons to direct the synthesis of proteins is affected in absence of processing by RNase III [51].

Leftward transcription of induced prophage λ starts at the p_L promoter for the *N* gene. Comparison of RNA transcripts in *rnc*$^+$ and *rnc* isogenic strains suggested that RNase III introduces a cleavage in the P_L message somewhere beyond the coding sequences for the *N* protein [52, 53].

7. General considerations

7.1. *Unity of RNA processing mechanisms*

The prokaryotic cell, as we have seen, employs a very limited number of nucleolytic activities to accomplish the processing of a variety of RNA transcripts. Indeed, each of the three known primary endoribonucleases, RNase III, RNase E and RNase P, plays a role in processing both of ribosomal and of transfer RNA transcripts. Intracellular parasites – the DNA bacteriophages – have evolved so that their RNA transcripts can be processed by host systems. Processing of tRNAs in T-even and related phages [54], as well as of messenger RNAs in T7, λ and probably other phages, is also performed by host enzymes.

7.2. *Conclusions*

1. Transcription of RNA is independent of its processing.
2. Primary processing of rRNA and tRNA occurs during transcription.
3. Most RNA transcripts are processed by more than a single endoribonuclease.
4. The four enzymes responsible for most endonucleolytic processing of RNA transcripts in *E. coli* are RNases III, E, 'F' and P.
5. The efficiency but not the specificity of processing cuts is affected by the size of the substrate; multiple cleavages facilitate efficient processing.
6. Processing endonucleases are highly specific and each performs a unique function. Their recognition sites may be composed of unique combinations of relatively simple yet distinctive features of secondary and tertiary structure.

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