MICROBIOLOGICAL REVIEWS, Dec. 1981, p. 502-541
0146-0749/81/120502-40$02.00/0

Vol. 45, No. 4

# Processing of Procaryotic Ribonucleic Acid

PETER GEGENHEIMER†* AND DAVID APIRION

*Department of Microbiology and Immunology, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri 63110*



## INTRODUCTION

The immediate transcription products of procaryotic genes are frequently not identical to the ribonucleic acid (RNA) molecules which are functional in the cell. All of the stable RNA species such as ribosomal RNAs (rRNA's) and transfer RNAs (tRNA's) as well as a few of the messenger RNAs (mRNA's) of *Escherichia coli* and its bacteriophages differ from the primary transcription products of their genes in one or

† Present address: Department of Chemistry, University of California at San Diego, La Jolla, CA 92093.

more aspects. For example, each of the seven rRNA transcription units of *E. coli* is transcribed in the following order: leader-16S rRNA-spacer-23S rRNA-5S rRNA-trailer. Similarly, tRNA genes are frequently clustered and cotranscribed in multimers of up to seven identical or different tRNA's. Moreover, tRNA genes are located in the spacer region of every rRNA transcription unit of *E. coli* and in the trailer regions of some of these gene clusters. Some tRNA's and other small stable RNAs are synthesized as monocistronic precursors possessing additional nucleotide sequences at the 5′ and 3′ termini. Furthermore, the initial transcripts of stable RNA genes lack the modified nucleosides of the mature species, and some tRNA genes coded by bacteriophage genomes do not encode the 3′ terminal $CCA_{OH}$ sequence required for tRNA function.

To produce functional RNA molecules from the immediate products of transcription, procaryotic cells carry out four basic types of RNA maturation reactions: first, precise separation of polycistronic transcripts into monocistronic precursor RNAs; second, accurate recognition of the mature 5′ and 3′ termini and removal of extraneous nucleotides without altering the terminal sequences themselves; third, addition of terminal residues to RNAs lacking them; and fourth, modification of the base or the ribose moiety of the four primary nucleosides in an RNA chain.

Not every RNA molecule is subject to all four of these processes. Certain polycistronic tRNA transcripts do require all four; many—probably most—mRNA's require no processing for their functional expression. This article discusses in detail the first two of these RNA maturation reactions, namely, those endo- and exoribonucleolytic events which reduce the size of primary RNA transcripts.

In the last decade, a number of reviews covering different aspects of RNA maturation have appeared, some of them discussing processing of stable RNA (100) or all RNAs (1, 127) in both procaryotes and eucaryotes, and others describing rRNA (121) or tRNA processing in *E. coli* (5, 6, 34, 159).

Recently, a combination of genetic and enzymological approaches has provided a great deal of new information on in vivo RNA processing in bacterial cells. In this report, we review the enzymology and genetics of RNA processing and then describe the events occurring during endo- and exoribonucleolytic processing of rRNA's, tRNA's, and mRNA's primarily in *E. coli* and the processing of tRNA's and mRNA's of some of the *E. coli* bacteriophages.

We focus primarily on those enzymatic activities which have been characterized biochemically and genetically, reviewing their properties and describing how they interact or are expected to interact in different processing pathways. Whenever possible, in an attempt at coherence and unification, we draw attention to probable identity among activities described independently by different researchers. We have deliberately chosen to describe processing events using the fewest possible number of nucleases, emphasizing their potential versatility. In addition, we seek to draw attention to and stimulate examination of the actual and potential cooperation among enzymes which underlies RNA processing pathways.

We hope that the present review provides an integrative and up-to-date view of the two aspects of RNA processing, endonucleolytic cleavages and exonucleolytic trimming, of all the types of RNA in procaryotic cells. This review cannot be all-inclusive and reflects some of our own biases and limitations; we hope that our sins of omission and commission will be forgiven and forgotten.

## ENZYMOLOGY OF RIBONUCLEIC ACID (RNA) PROCESSING

### Ribonuclease (RNase) P

RNase P was detected and purified as an endonuclease which specifically removes a 41-base-long fragment from the 5′ side of a 128-base-long precursor to *E. coli su3*$^+$ $tRNA_1^{Tyr}$ (4, 8) (Fig. 1), thereby generating the 5′ terminus of the mature tRNA but not affecting the 2 to 3 extra bases present at the 3′ side of the precursor (134). From analysis of tRNA precursors accumulated in temperature-sensitive RNase P mutants, it is evident that RNase P is required for 5′ maturation of most and perhaps all tRNA species of *E. coli* and its bacteriophages (5; also see below). RNase P cleavage does not generate a mature 3′ terminus for any tRNA species thus far examined (5, 6, 132b, 159).

RNase P cleavage generates 5′ phosphoryl and 3′ hydroxyl termini; the activity is optimal at pH 8, requires both monovalent (0.1 M $NH_4^+$ or $K^+$) and divalent (5 mM $Mg^{2+}$ or 1 mM $Mn^{2+}$) cations and is inhibited by 0.1 M NaCl (134). (A brief characterization of RNase P, as well as other RNA-processing enzymes, is given in Table 1.) Cleavage is also inhibited nonspecifically by RNA: bulk transfer or rRNA inhibits RNase P with a $K_i$ about 1% of the $K_m$ for pre-tRNA (165). Substrate recognition involves primarily the mature portion of the tRNA precursor. RNase P will not cleave isolated oligonucleotides, obtained from precursor tRNA, which contain only the sequences surrounding the 5′ terminus of the mature tRNA (151).

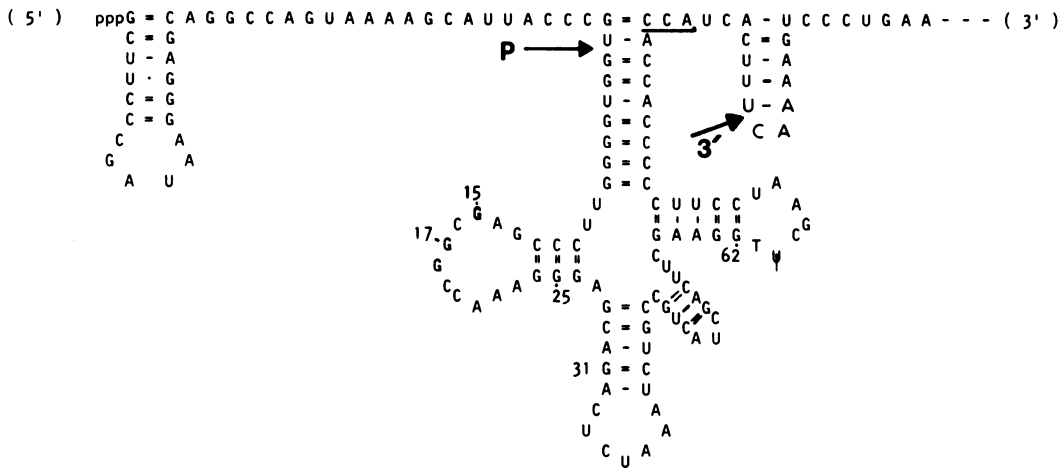



FIG. 1. *tRNA precursor structure. The sequence shown is that of the su3⁺tRNA$_1^{Tyr}$ transcript; transcription in vivo proceeds through a second tRNA$_1^{Tyr}$ gene. Sites of in vitro endonuclease cleavage are shown at the 5' side of the mature tRNA (RNase P) (5, 6, 134) and near the 3' end (155). The mature 3' terminus is underlined. Numbered nucleotide positions are those at which base changes result in reduced RNase P cleavage in vivo (158).*

RNase P processes a spectrum of tRNA precursors which differ widely in primary sequence and in the structure of their precursor-specific regions (74, 75, 143). Furthermore, RNase P from *Bacillus subtilis* will process a tRNA precursor produced by T4-infected *E. coli* (51). RNase P also processes precursors to non-tRNA's. RNase P mutant strains accumulate precursors to the *E. coli* 4.5S RNA (25) and to the T4-encoded species 1 RNA (2). These precursors are processed to their mature forms by RNase P in vitro (2, 25). T4 species 1 RNA can be drawn in a tRNA-like cloverleaf structure (see Fig. 10 of reference 124). Species 1 RNA can be recognized by tRNA nucleotide modification enzymes (125), and, like tRNA's, it undergoes 3' trimming and CCA addition (2, 124). The precursor 4.5S (p4.5S) RNA can potentially form a stem-and-loop structure (25) and may assume a tertiary configuration like that of a tRNA. Another RNA, coded by phage $\phi$80, is also an in vitro substrate for RNase P (26).

Comparison of all of these RNase P substrates indicates that because no primary sequence similarities exist, secondary and tertiary conformations play a major role in determining the substrate specificity of RNase P. This hypothesis is further supported by examination of the effect of tRNA conformational changes on processing.

The tRNA portion of precursor molecules most likely exists in a conformation similar to that of the mature tRNA (97, 103) (Fig. 1). Single-base changes which can alter secondary or tertiary structure in a mature tRNA sequence can impair RNase P processing of the precursor to that tRNA (4, 6, 7, 159). For example, in *E. coli su3*⁺ tRNA$_1^{Tyr}$, mutations which create a single-base mismatch in the acceptor stem, the D-stem, the anticodon stem, or the variable stem also reduce the overall efficiency of RNase P processing in vivo (158; for reviews, see references 6 and 159) or in vitro (7). Interestingly, normal processing can be restored not only by a second-site mutation which restores base pairing but also by single-base changes in other regions of the molecule (6, 158, 159). Moreover, a G-to-A mutation at position 15 in the D-loop also reduces RNase P cleavage in vitro (7) and results in accumulation of uncut precursor tRNA in vivo. This particular change has been found to reduce the stability of the mature tRNA (under physiological conditions) and to alter its in vitro folding kinetics (85). The proposed consequence (85) of this base change is the increase of the separation between the D-loop and the TΨC-loop in the tertiary configuration of the tRNA. Similar effects of base changes on RNase P activity are found in the tRNA's of bacteriophage T4 (6, 66, 159).

RNase P cleavage of pre-tRNA's seems also to require the presence in the precursor of the sequence -CCA-, which is destined to become the 3' terminus of the mature tRNA. Precursors of bacteriophage T4 tRNA's which lack this sequence cannot be processed efficiently by RNase P until after CCA is added (see below). Mutations either in the tRNA sequence or in processing enzymes which serve to prevent CCA addition also block RNase P cleavage (66, 100, 150). Moreover, chemical or enzymatic removal of one or two bases from the CCA sequence of *E. coli* tRNA$_1^{Tyr}$ precursor considerably reduces

TABLE 1. *RNA-processing nucleases in procaryotes*[a]

| Enzyme | Mol wt | Substrate | Product(s) | Ionic optimum | | pH optimum |
|---|---|---|---|---|---|---|
| | | | | Monovalent | Divalent | |
| | | Double-stranded RNA fragments | Random, acid soluble | 0.08–0.2 M | 0.1 mM $Mn^{2+}$; 1–10 mM $Mg^{2+}$ | 9.75 |
| RNase III | 50,000 (2 subunits, 25,000 each) | Secondary cleavage of natural RNAs | Site-specific cleavages | 0.004–0.08 M (inhibitory, >0.09 M) | 1–10 mM $Mg^{2+}$ | ND[b] (probably 7.6–8.0) |
| | | Precursor rRNA's (double-stranded regions) | Site-specific cleavages | 0.15–0.3 M | 1–10 mM $Mg^{2+}$ | ND (probably 7.6–8.0) |
| RNase D | 38,000–40,000 | tRNA-$CCACCC_{OH}$ | tRNA-$CCA_{OH}$ + 3 pC (nonprocessive exonuclease) | None (inhibitory) | 5 mM $Mg^{2+}$; 5 mM $Mn^{2+}$; 5 mM $Co^{2+}$ | 9.1–9.5 |
| RNase E | ~70,000 | *E. coli* 9S rRNA | Pre-5S rRNA + 5′ and 3′ fragments | 0.1–0.2 M | 1 mM $Mn^{2+}$; 5 mM $Mg^{2+}$ | 7.6–8.0 |
| RNase F | ND | T4 p2-species 1 RNA | Species 1 RNA + a 3′ fragment | 0.15–0.2 M | None | 6.8–7.6 |
| RNase M5 | ND (2 subunits: $\beta$, 17,000; $\alpha$, ND) | *B. subtilis* pre-5S rRNA | 5S rRNA + 5′ and 3′ fragments | 0.05 M ($K^+$, $NH_4^+$) | 1 mM $Ca^{2+}$; 5–10 mM $Mg^{2+}$; 10 mM $Mn^{2+}$ (or 0.3 mM spermidine; 10 mM putrescine) | 7.0–7.5 |
| RNase P | Protein, ~17,500; RNA, ~360 bases | Pre-tRNA's | tRNA + a 5′ fragment | 0.1 M ($K^+$, $NH_4^+$; inhibitory, 0.1 M $Na^+$) | 1 mM $Mn^{2+}$; 5 mM $Mg^{2+}$ | 8.0 |

[a] For each processing ribonuclease, enzymatic properties were determined for the substrate cited under the Enzyme column. If properties are known for more than one substrate, the one listed is the one which predominates in vivo or most closely resembles an in vivo substrate. Monovalent cations refer to $Na^+$, $K^+$, or $NH_4^+$ unless otherwise noted. Reactions require only a single cation from those listed. RNases III, E, M5, and P cleave at the 5′ side of a phosphodiester bond; that is, they generate products bearing 5′ phosphoryl and 3′ hydroxyl groups.

[b] ND, Not determined.

the rate of RNase P cleavage in vitro (7).

**Subunit structure.** RNase P is an unusual enzyme in that, as demonstrated by Altman and co-workers, it is composed of both a polypeptide and an RNA subunit. RNase P activity bands in an isopycnic density gradient at 1.7 g/cm$^3$, characteristic of an RNA-protein complex (165). The most highly purified RNase P preparations (purified about 2,000-fold) contain one or two copies of a single polypeptide, termed C5, of about 20,000 molecular weight and one or two closely related RNAs, M1 and M2, each about 360 bases long (79–80). One of these RNAs is similar or identical to the previously described cellular 10S RNA species (84a, 131; "Band IX" of reference 71; Fig. 1).

That an RNA component is required for RNase P function was shown by pretreatment of the enzyme with immobilized pancreatic RNase A or with the $Ca^{2+}$-dependent micrococcal nuclease. After removal of nuclease activity by filtration or dialysis, no cleavage of precursor tRNA was detected (165). Controls ruled out the presence of residual nuclease in the RNase P assay as well as the presence of proteolytic activity in the nuclease preparation. A requirement for both RNA and protein is also supported by genetic analysis (described in some detail below).

RNase P can be dissociated into and reconstituted from inactive RNA and protein constituents (65, 79). Neither protein nor RNA shows any RNase P-like activity (65, 79), suggesting that the RNA component does not simply lend specificity to a nonspecific nuclease activity in the protein component.

RNase P as isolated (79) contains some smaller RNAs in addition to M1/M2, which may be breakdown products of M1 and M2. The largest of these RNAs, when substituted for M1/M2 in a reconstitution experiment, supports partial regeneration of RNase P activity (79). Similarly, large fragments of M1/M2 RNA, produced in vitro by limited alkaline hydrolysis, support the reconstitution of partly active enzyme (79). Larger fragments are more effective than smaller ones, in agreement with another report that the 6S to 10S fraction of RNase P-derived RNA is more active in reconstitution than is unfractionated enzyme-derived RNA (65).

Although the 20,000-molecular-weight polypeptide (5) is the major polypeptide common to RNase P preparations used for reconstitution by the urea-dialysis procedure and thus is likely to be the active polypeptide component of the reconstitution reaction, a definite conclusion awaits its purification to homogeneity.

RNase P isolated from *B. subtilis* is also a ribonucleoprotein with an essential RNA subunit (51). When banded in CsCl, this activity separates into two components, one having the higher density of ribonucleoprotein and the other having the lower density of free protein. Enzymatic activity requires both fractions. Pretreatment of the denser component with insoluble RNase A abolishes enzymatic activity (51). The *B. subtilis* enzyme may thus contain two polypeptides, one of which is tightly bound to an RNA moiety. The *E. coli* enzyme does not dissociate in CsCl (165); its components are presumably more tightly associated.

These results from two diverse species, *E. coli* and *B. subtilis*, indicate that the presence of RNA in RNase P is integral to the mechanism whereby RNase P recognizes, cleaves, or both recognizes and cleaves tRNA precursors. The RNA subunit may be a recognition component, having a sequence and conformation capable of interacting only with tRNA's and structurally similar RNAs. The RNA may also serve as a flexible "backbone" allowing a polypeptide catalytic subunit, bound to a region of the RNA distant from the recognition element (either another protein or a region of the RNA), to be optimally positioned on many different tRNA's having slightly different sizes and conformations.

## RNase III

RNase III was first purified as an endonucleolytic activity which, in vitro, converted high-molecular-weight double-stranded RNA to an acid-soluble form (139). It was found to be active against naturally occurring double-stranded RNAs such as RNA phage replicative forms, as well as synthetic polymeric duplexes such as polyadenylate-uridylate, polyadenylate·polyuridylate, or polyinosinate·polycytidylate (16, 78, 139).

The enzyme is composed of two identical polypeptide subunits of 25,000 molecular weight (40, 139) and probably does not contain an RNA moiety (139). Digestion of synthetic substrates is optimal in 0.1 to 0.2 M monovalent cation ($Na^+$, $K^+$, or $NH_4^+$) and 1 to 10 mM $Mg^{2+}$ (or 0.1 mM $Mn^{2+}$). The rate of synthetic RNA solubilization increases monotonically with increasing pH up to pH 10 (139).

The enzyme cleaves high-molecular-weight double-stranded RNA by making closely spaced, staggered, single-strand breaks (135, 152) at intervals of approximately every 15 bases (range, 10 to 25 bases) (31, 137, 152). These products, which are not further digested, contain 5′ phosphoryl and 3′ hydroxyl termini (31, 137, 139, 152). The reaction is not inhibited by addition of single-stranded RNA (139). Double-stranded

RNAs are cleaved without regard to sequence: there is no nucleotide specificity, at least at the 5′ side of the cleavage point (137), and the narrow size range of products argues against the existence of a unique recognition sequence.

In 1973, two distinctive cellular functions were uncovered for RNase III. First, RNase III was found to be identical to the cellular "sizing factor" which could post-transcriptionally convert an in vitro polycistronic transcript of the bacteriophage T7 early region into discrete monocistronic mRNA's (42). The second physiological role of RNase III was shown to be in rRNA processing. The polycistronic 30S transcript of the rRNA gene cluster (accumulated in RNase III$^-$ strains) can be cleaved by RNase III in vitro to species of the same size as the monocistronic p16 and p23 precursor rRNA molecules detected in vivo (42, 117). Fingerprint and terminal sequence analyses showed that the in vitro RNase III-generated p16 was identical to the in vivo p16 species; the in vitro p23 was similar or identical to authentic in vivo p23 rRNA (27, 62). Other roles have since been proposed for RNase III in the processing of mRNA and tRNA; these are discussed below.

Under certain conditions in vitro, RNase III can also cleave RNA which is essentially single stranded. The factors influencing such cleavage have been studied in some detail (40, 126); at high enzyme/substrate ratios, particularly at monovalent cation concentrations below about 90 mM, cleavages can occur at specific secondary sites which are not attacked at the normal ionic strength (0.15 to 0.30 M monovalent cation) required for double-stranded RNA digestion or RNA processing. Such secondary cleavages probably do not occur in vivo (40, 126). Robertson (133) has proposed that specific RNase III cleavage of nominally single-stranded RNA at low ionic strength and nonspecific cleavage of fully double-stranded RNA at high ionic strength represent two partial reactions of this enzyme, with respect to the site-specific cleavage of duplex RNA that occurs in vivo. Single-strand scission is suggested to result from enzymatic recognition of specific features of RNA structure, predominantly non-Watson-Crick in nature. Nonselective digestion of fully duplex RNA (natural or synthetic) could represent the inherent hydrolytic capability of the enzyme in the absence of such recognition regions in the substrate.

**Structure of RNase III processing regions.** Because *E. coli* rRNA's and T7 early mRNA's are direct products of RNase III cleavage, and because RNase III has a specificity for double-stranded RNA, the structure of in vivo RNase III processing sites is of considerable interest. Figure 2 shows the sequence and secondary structure of duplex regions or stems where RNase III cleavage occurs in the *E. coli* rRNA transcript and in the T7 early mRNA transcript. Some of the details of these processing events are described below. Because of the extensive similarity among the five T7 processing sites, a consensus sequence has also been given in Fig. 2.

Comparison of the sequences shared by the five T7 regions and the rRNA sites (Fig. 2) reveals some areas of homology. The homology among T7 stems is most extensive, consisting of the six-base-pair block

(5′)-A-A-G-G-U-C-(3′)
(3′)-U-U-C-C-G-G-(5′)

(reading from bottom to top of the stem; Fig. 2). Cleavage occurs asymmetrically with respect to this sequence (Fig. 2). The naturally occurring deviations from this consensus sequence which strengthen (or do not disrupt) the structure do not qualitatively affect cleavage in vivo or in vitro. That this block does contain elements recognized by RNase III is demonstrated by a T7 mutant stem in which the third position (as well as the fifth and sixth) is a G-U pair. This mutant stem cannot be cleaved in vivo or in vitro by RNase III (unpublished data of J. J. Dunn, cited in reference 135).

The first three base pairs of the conserved (core) T7 region are shared by T7, λ, and p23 rRNA stems. Furthermore, a -GUG- sequence in the p16 stems is found in all T7 stems, and pGAUU- follows the RNase III cut in three T7 stems and at the 3′ side of p16 rRNA. If these conserved regions are relevant to RNase III recognition, then other RNAs containing them should also be cleaved. An intercistronic region in the *E. coli rpoLJ-rpoBC* operon mRNA (23, 128) resembles the p23 stem and contains most of the core sequence. RNase III cleavage probably occurs in vivo near the center of this stem (23). Knowledge of the exact cleavage site will be of great interest.

One important aspect of RNase III rRNA substrate recognition is that essentially the only cellular duplex RNA lengthy enough to be an in vitro RNase III substrate is that which occurs in rRNA transcripts. The total RNA of wild-type *E. coli* does not contain extensive double-stranded regions (138). From an RNase III$^-$ strain, however, a duplex RNA fraction can be prepared; these RNAs are cleaved in vitro by RNase III (135, 138). Fingerprint analysis of total (135) or fractionated (138) duplex RNA indicates that it is composed predominantly of rRNA processing regions.

Figure 2 shows that the longest rRNA pro-

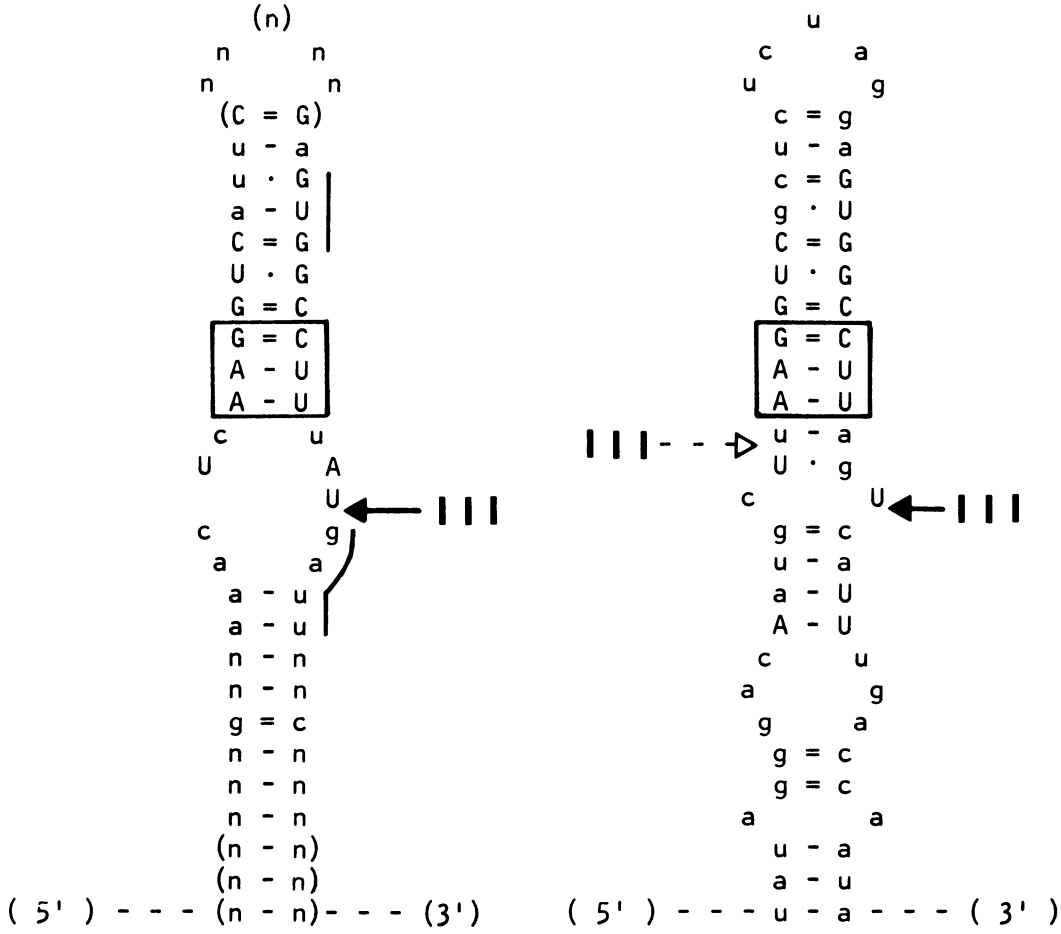
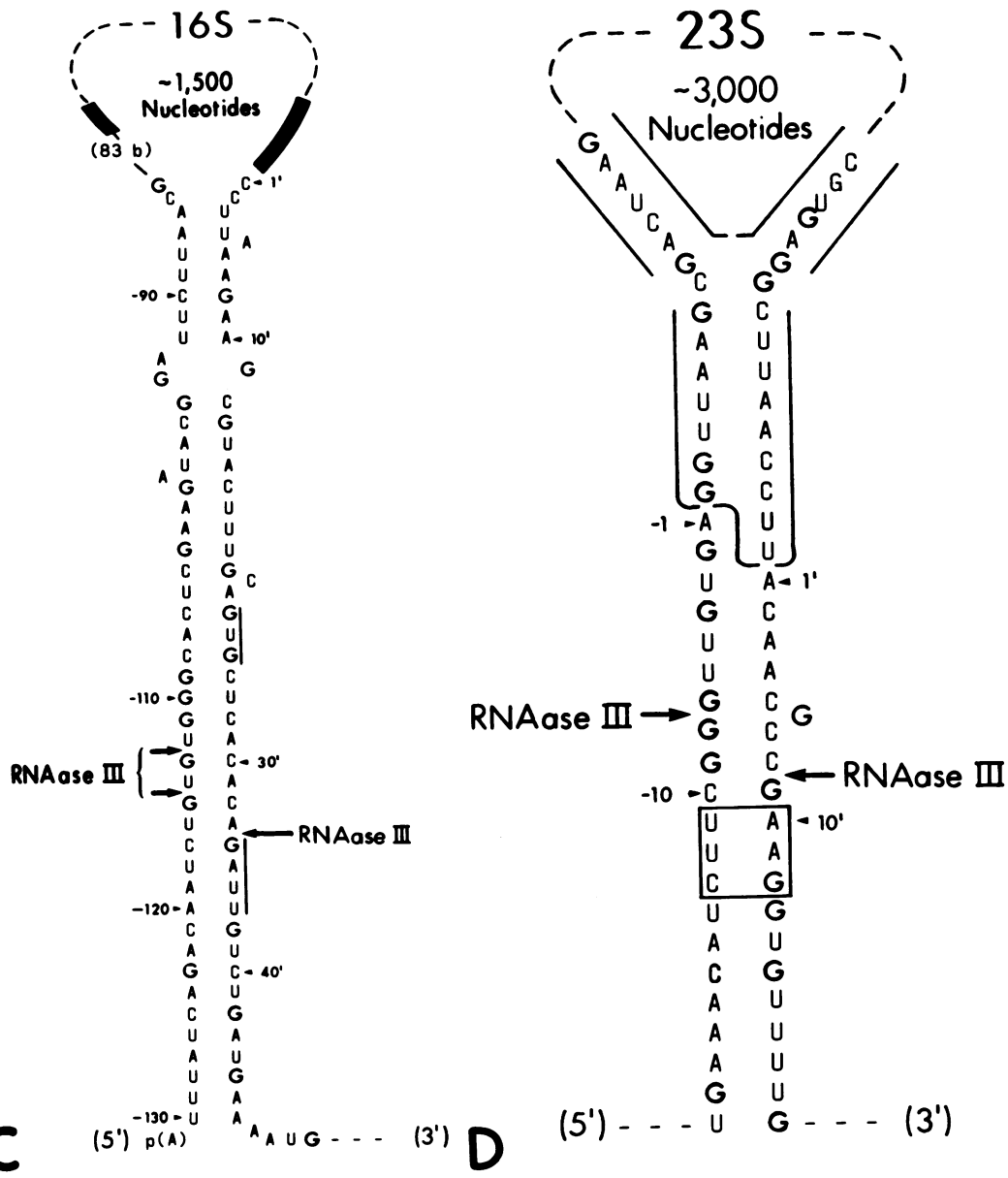



FIG. 2. *RNase III processing sites. (A) Processing sites in bacteriophage T7 early mRNA. The consensus sequence derived from the first four intercistronic regions from the early T7 RNA transcript is shown. Capital letters are sequences identical in all four stems (except for the differences shown). A box surrounds the core sequence common to T7 and E. coli p23 rRNA stems. Vertical lines indicate sequences found in the T7 consensus stem and in E. coli p16 stems (43, 169). (B) Cleavages of the T7 early gene 1.1–1.3 stem. Nucleotides in capital letters are identical to the consensus sequence. The core sequence is boxed. RNase III primary cleavage on the right-hand side of the stem occurs in vivo or in vitro at 0.15 to 0.4 M salt. Secondary cleavage, at the left-hand side, occurs in vitro at low salt concentrations (40). (C) Processing stem for p16 rRNA. The sequence and secondary structure shown were experimentally determined for the p16b rRNA precursor of RNase III⁻ cells (54). Vertical lines indicate sequences shared with the T7 stem (see [A]). The nascent rRNA transcript cleaved by RNase III in wild-type cells additionally includes a 5′ leader sequence with a nucleoside triphosphate terminus. RNase III cleavage sites are from references 55 and 92. Some heterogeneity exists in the 5′ termini produced by RNase III in vitro. Nucleotide positions are numbered downward from the termini of mature 16S rRNA (shown as solid bars). (D) Processing stem for p23 rRNA. The structure is that which was experimentally determined for the p23b precursor of RNase III⁻ cells (54). The core sequence common to p23b and T7 stems is boxed. (Note that although the p23 stem is drawn as inverted with respect to the T7 stems, the 5′ → 3′ polarity of the relationship between the core sequence and RNase III cleavage sites is preserved.) RNase III cleavage site is from reference 27. Sequences of mature 23S rRNA are enclosed within solid lines; nucleotide positions are numbered from the m23 termini.*




cessing stems are 30 to 40 base pairs long. The parts of the stems remaining after RNase III cleavage (14 and 24 or 26 base pairs for p16; 12 and 15 base pairs for p23) (Fig. 2) are of the same size as the in vitro limit digestion products (ca. 15 base pairs) of high-molecular-weight double-stranded RNAs. The size of cleavage products thus seems to be independent of the substrate; however, rRNA stems probably contain additional features which determine the unique cutting points.

Robertson and collaborators (133, 135) have discussed in depth the features of RNAs which may be recognized by processing enzymes, and these researchers have stressed the potentially important role of the tertiary structure of partially or fully double-stranded RNAs. A wealth of non-Watson-Crick base interactions exist which influence RNA conformation and protein-nucleic acid interactions and which may confer uniqueness upon a particular processing signal.

### RNase E

An enzyme which specifically cleaves p5 pre-

cursor rRNA from larger RNA transcripts in *E. coli* has been partially purified (ca. 200-fold) and characterized (109), using as the substrate the p5-containing 9S rRNA (ca. 250 bases long) accumulated in a temperature-sensitive RNase E (*rne*) mutant strain (13, 59). RNase E cleaves 9S RNA to give p5 rRNA (58, 109). The apparent molecular weight of the native enzyme is 70,000; the subunit structure is unknown. The reaction is optimal at 30°C and pH 7.6 to 8.0 in the presence of 0.1 to 0.2 M monovalent cation ($NH_4^+$, $K^+$, or $Na^+$), and 1 mM $Mn^{2+}$ or 5 mM $Mg^{2+}$; cleavage is inhibited by bulk RNA (109).

At early times of the reaction or in the presence of high concentrations of carrier yeast RNA, two main cleavage products are observed, a 4S molecule (ca. 80 bases) representing the 5′ leader portion of 9S and a 7S species containing p5 plus distal sequences at the 3′ end of 9S RNA (58, 109). If cleavage is allowed to proceed to completion, the 7S intermediate is further digested to p5. Analysis of the 4S RNA and p5 rRNA cleaved in vitro from 9S RNA by partially purified RNase E suggests that this enzyme, like other procaryotic processing enzymes, generates 5′ phosphoryl and 3′ hydroxyl termini (58). RNase E also processes p5 rRNA from larger rRNA transcripts which contain p5 and trailer tRNA's (132) as well as p5 rRNA from 25S rRNA which contains p5 and 23S rRNA (60).

The 5′ and 3′ termini of 5S rRNA are base paired (the "molecular stalk" structure referred to in reference 48). Deoxyribonucleic acid (DNA) sequences surrounding the 5S gene (177, 178) indicate that in the primary RNA transcript, this stalk could be extended, after a three-base bulge, by three U-A base pairs. Indeed, such a structure was isolated experimentally (B. Singh and D. Apirion, manuscript in preparation). RNase E cleavages occur near the ends of this region (Fig. 3). The first cleavage takes place between double-stranded and single-stranded RNA, and the second cleavage occurs two nucleotides apart within the helical structure (Fig. 3). These cleavages occur three nucleotides upstream and three nucleotides downstream, respectively, from the 5S rRNA ends (Fig. 3). Both cleavages are made by RNase E, because both fail to occur in an *rne* mutant, and when extracts of such a strain are used, both cleavages are thermolabile (M. K. Roy, B. Singh, B. K. Ray, and D. Apirion, manuscript in preparation). RNase E substrate recognition may then involve the tertiary conformation of mature 5S and a double-stranded stem. Because the region upstream from 5S (nucleotides 1 to 81 in Fig. 3) is almost identical in all seven rRNA genes, we expect that parts of this region participate in the RNase E reaction.

## RNase M5

Three classes of precursors to *B. subtilis* 5S rRNA have been detected which are 240, 179, and 150 bases long (122, 123, 161). The structure of the 179-base precursor ($p5_A$) is shown in Fig. 4. An enzyme, RNase M5, has been substantially purified from *B. subtilis* and accurately processes these molecules to mature 5S rRNA (160). The reaction on $p5_A$ produces simultaneously m5 rRNA plus a 21-base-long 5′ fragment and a 42-base-long 3′ portion (160). No processing intermediates have been detected (122, 160). The optimum pH is 7 to 7.5. The reaction requires a divalent cation (5 to 10 mM $Mg^{2+}$, 10 mM $Mn^{2+}$, or 1 mM $Ca^{2+}$), which can be entirely replaced by polyamines (10 mM putrescine or 0.3 mM spermidine). Cleavage is stimulated by 50 mM $K^+$ or $NH_4^+$ but not by $Na^+$ and is inhibited by all of these cations at concentrations above 100 to 150 mM (160). The activity can be fractionated into two components, $\alpha$ and $\beta$ (160). The $\beta$ component has substrate-binding capability, whereas the $\alpha$ component appears to be the catalytic subunit (160).

Both components are proteins, as judged by their protease sensitivity (160). The $\beta$ subunit is a basic protein of approximately 17,000 molecular weight (D. A. Stahl and N. R. Pace, personal communication). Thermal inactivation studies indicate that inactivation of either component alone is sufficient to inactivate p5 maturation activity and that 5′ and 3′ cleavages are inactivated simultaneously. Both subunits, therefore, are required for cleavage at either side of the precursor.

RNase M5 of *B. subtilis* cleaves not only the three p5 precursors from *B. subtilis* but also at least five closely related p5 species from *Bacillus Q* (166). These precursors are very similar to those of *B. subtilis* in their mature and 5′ precursor regions but differ widely in their 3′ precursor-specific portions. These precursors are also matured by extracts of *Bacillus Q* and *B. licheniformis* (166).

**Substrate recognition.** Substrate recognition and cleavage rate of p5 rRNA are influenced partly by the precursor-specific portion of the molecule but principally by the mature region. In a series of elegant experiments, Pace and his collaborators investigated the effect on cleavage of systematic modifications of the *B. subtilis* $p5_A$ molecule. Substrates were constructed which lacked the 5′ or the 3′ precursor-specific sequences (105) or in which the 5′ sequence was replaced (using RNA ligase) by a synthetic oligonucleotide of defined sequence (106). The role of the mature domain of the molecule was explored by enzymatic excision of various oligo-

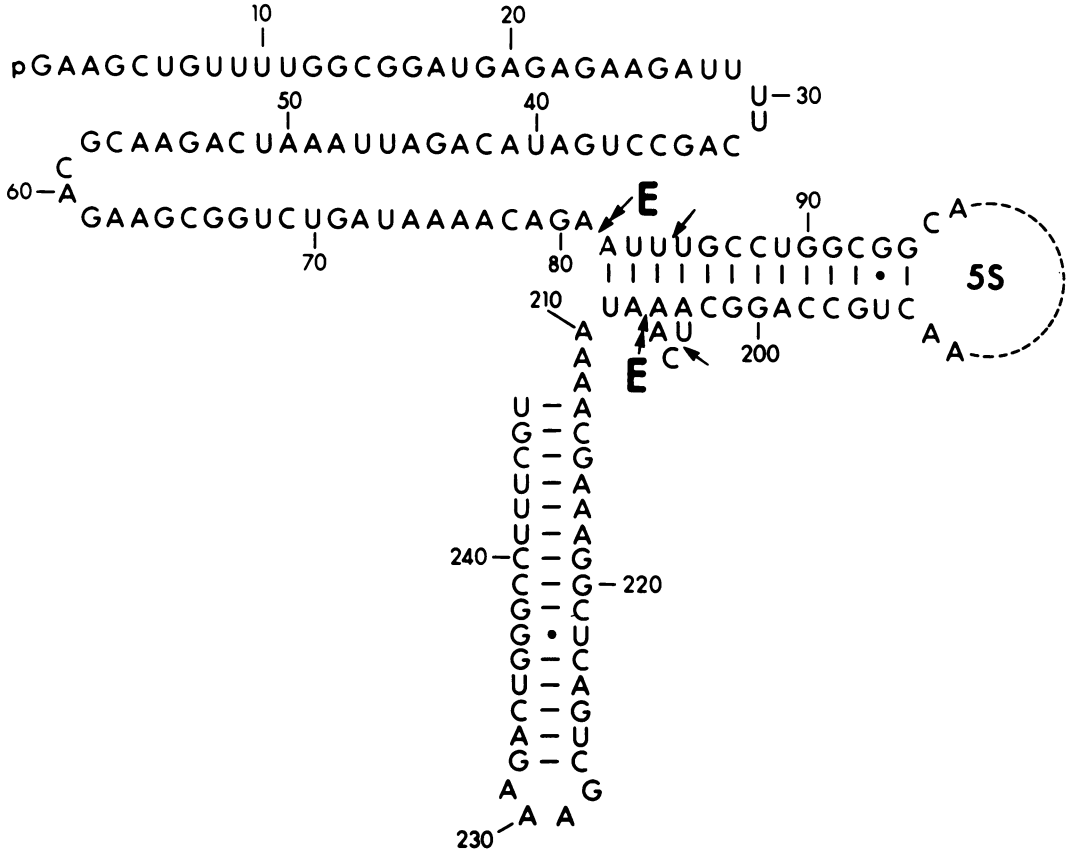



FIG. 3. *Sequence and partial secondary structure of an RNase E substrate, the 9S rRNA of E. coli. The sequence shown is from transcripts of the rrnB gene cluster (29a) (a few base changes are found in the rrnA, rrnE, and rrnG gene clusters). The two duplex structures shown were verified experimentally. Double-headed arrows show the RNase E cleavage sites; single-headed arrows indicate the mature 5′ and 3′ termini of 5S rRNA. The structure shown is transcribed from the four rRNA gene clusters which do not contain trailer tRNA's. An almost identical RNA, up to nucleotide 209, is also transcribed from the other rRNA gene clusters which do contain trailer tRNA's.*

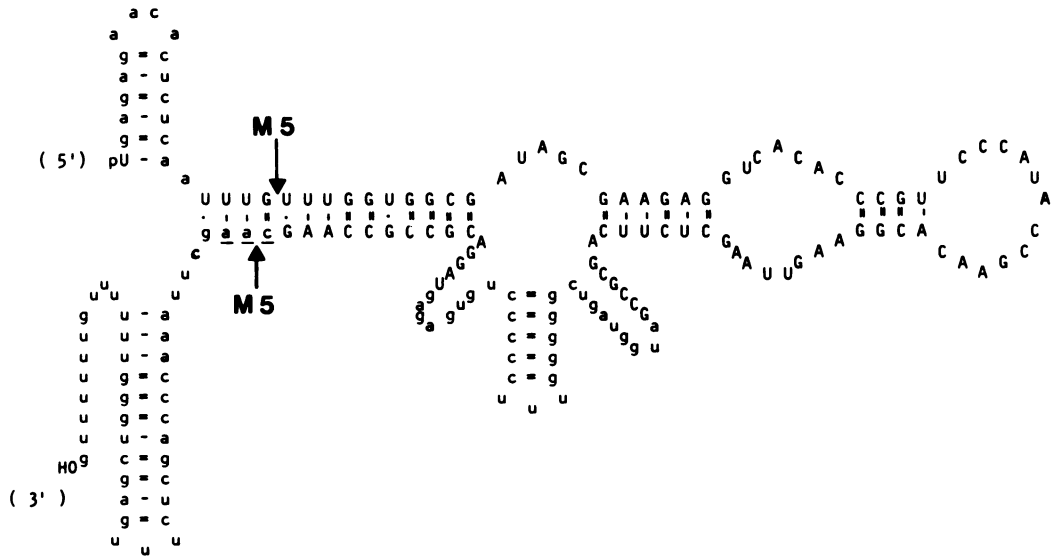



FIG. 4. *Structure of a B. subtilis RNase M5 substrate. The sequence is that of p5A rRNA. Nucleotides shown in capital letters must be present for RNase M5 cleavage of the 5′ precursor-specific segment. Nucleotides in lower case letters are not essential; the presence of the underlined nucleotides increases the reaction rate. RNase M5 cleavage sites which release 5′ and 3′ precursor fragments are indicated (122).*

nucleotides within the m5 sequence (107). These results are described below and summarized in Fig. 4.

In mature 5S rRNA, the 5′ and 3′ termini are hydrogen bonded to form the molecular stalk region, leaving one base unpaired at the 3′ end (48). RNase M5-mediated release of the 3′ precursor segment requires that the nucleotide that will become the 3′ terminus of the m5 sequence be hydrogen bonded to the last nucleotide of the 5′ precursor segment (106). The rate of cleavage is somewhat enhanced if the base pairing extends two additional bases into the precursor region (105, 106), as found in in vivo precursors (161) (Fig. 4). The presence or absence of the remainder of the 5′ segment does not influence release of the 3′ segment (106, 164). Cleavage of this 5′ precursor fragment, on the other hand, does not require any of the 3′ precursor segment (105). Optimal cleavage occurs when the 3′ base of m5 is hydrogen bonded to the 5′ precursor segment, as described above, but cleavage can occur at a low rate in the absence of any base pairing (106, 163).

The structure of the RNase M5 cleavage site has received careful scrutiny. By constructing test substrates in which both the 3′-terminal residue of the 5′ precursor segment and the opposing 3′-terminal base of m5 were systematically replaced with each of the four ribonucleosides (163), the structural requirements for cleavage were determined. A stacked helical configuration in the 5′ precursor-specific segment is absolutely required for RNase M5 processing. A fully duplex structure is most readily cleaved (106). Single-stranded 5′ segments which, by virtue of intrastrand stacking, can extend the helical structure of m5 are cleaved almost as efficiently (163). The 5′ fragments which do not form intrastrand stacks cannot be processed unless they are base paired with the 3′ end of m5 (163). Even so, the rate of cleavage increases considerably if additional base pairing is permitted (106).

Because the last nucleotide of the 5′ precursor segment is required for cleavage of the 3′ fragment but the opposite is not true, the cuts must occur either simultaneously or in the order of 3′ before 5′. An intermediate lacking only the 3′ fragment has not been detected, however, thus favoring a model of simultaneous cleavage.

Whereas only one or at most a few nucleotides of precursor-specific sequences are required for RNase M5 cleavage, the sequence of the mature 5S portion of the substrate must be largely intact for processing to occur. By limited digestion with RNase T1, p5 species can be derived from which specific oligonucleotides have been excised (107). These species are then tested as RNase M5 substrates. This analysis indicates that the only region not essential for RNase M5 processing is the procaryotic loop between positions 70 and 101 of the mature RNA (Fig. 4) (107). Furthermore, the ability of RNase M5 to process an altered precursor whose tertiary configuration is normal except for the positioning of the procaryotic loop (164) reinforces the conclusion that this region is not essential for the reaction. Regions of the $p5_A$ precursor required for proper 5′ cleavage are shown in Fig. 4.

In addition to tertiary structure, RNase M5 may also recognize some aspect of primary sequence, because precursors constructed from *E. coli* m5 rRNA are not processed; the synthetic 5′ oligonucleotides which can be cleaved if ligated in vitro to *B. subtilis* m5 are not cleaved when joined to *E. coli* m5 (122). *E. coli* and bacillus m5 rRNA's have similar sequences and presumably similar conformations (122); they differ in the sequences of the base-paired regions, especially the terminal duplex or molecular stalk (cf. Fig. 3 and 4).

Both RNase M5 of the bacilli and RNase E of *E. coli* process precursors to 5S rRNA. The 5′ end of the RNase E substrates is produced during primary processing of nascent rRNA transcripts by RNase III (13, 27, 58); how the 5′ end of the RNase M5 substrates is derived is unknown. The 3′ ends of both *E. coli* 9S rRNA and *B. subtilis* $p5_A$ rRNA are probably produced by in vivo chain termination events (161; Singh and Apirion, manuscript in preparation) (Fig. 3). RNase M5 produces mature 5S rRNA, whereas RNase E yields a 5S precursor bearing three extra nucleotides at each end (Fig. 3). The extra bases are removed by an unknown enzyme(s) (46, 77). RNases E and M5 may therefore perform analogous roles in the two species. Interestingly, in *E. coli*, the terminal duplex (molecular stalk [48]) of m5 can be extended by three base pairs in the primary rRNA transcript (Fig. 3) (27, 177); RNase E cleavage occurs within this duplex region, though at a comparatively different position than the site of cleavage by *B. subtilis* RNase M5.

It is rather interesting that although all the procaryotic endonucleolytic RNA-processing enzymes studied thus far generate a 5′ phosphoryl and a 3′ hydroxyl group, the yeast enzyme which removes intervening sequences from tRNA precursors generates 3′ phosphoryl and 5′ hydroxyl groups (1). This observation suggests that the yeast enzyme is unrelated to any of the RNA-processing enzymes thus far identified. Therefore, it is not unreasonable to expect to find in procaryotic cells an RNA-processing en-

zyme which generates 3′ phosphoryl and 5′ hydroxyl groups.

## RNase D

Maturation of many monomeric tRNA precursors requires removal of several extra nucleotides distal to the encoded CCA sequence destined to become the mature 3′ end of the tRNA (1, 6, 100, 159). Although a plethora of possible 3′ exonucleases was suggested for this role (see below), no activity was characterized that fulfilled the requirements for a true processing enzyme, largely because of the unavailability of a suitable substrate. To systematically search for such a maturation enzyme, Deutscher and colleagues constructed a semisynthetic tRNA precursor substrate, "tRNA-CCACC[C]," by adding two (37) or three (32) [$^{14}$C]cytosine residues to the normal tRNA $CCA_{OH}$ terminus of bulk *E. coli* tRNA. Another substrate, "tRNA-CU," was constructed as an analog of phage tRNA precursors in which the CCA terminus is (partially) replaced by other sequences. The terminal CA residues were removed from bulk tRNA by limited exonuclease digestion, and a single [$^{3}$H]uridine residue was added, using nucleotidyltransferase.

Using this substrate, these investigators purified to homogeneity an enzyme, RNase D, which has the required characteristics of an authentic in vivo 3′ maturation activity. RNase D efficiently removes nucleotides before the mature 3′-terminal CCA sequence, thus generating amino acid acceptor activity, but is inefficient at hydrolysis of the CCA sequence itself (32, 61).

RNase D is a monomeric protein of 38,000 molecular weight. Its processing activity against tRNA-CCACCC substrate requires $Mg^{2+}$ or $Mn^{2+}$. Monovalent cations $Na^{+}$ or $K^{+}$ are not required. Processing occurs at pH 7.5 (32) to 10 (61). Optimal assay conditions appear to vary somewhat with the substrate tested.

The enzyme is a 3′ exonuclease which releases mononucleoside 5′-phosphates (32). Its mode of action is random (nonprocessive). Because digestion of mature terminal CCA nucleotides proceeds slowly, probably as a result of steric hindrance (32), RNase D-treated precursor tRNA's can be essentially fully amino acylated (32, 61). The enzyme can process semisynthetic precursors to at least 15 different tRNA species, as well as the in vitro precursor to *su3*$^{+}$ $tRNA_1^{Tyr}$ (32). RNase D can also remove 3′-terminal residues from tRNA-CU, but with much lower efficiency (10- to 15-fold) than from tRNA-CCACC substrates (61). After infection of *E. coli* with T4, a small protein is added to RNase D (32a), but it is not known whether the modified RNase D plays any role in the maturation of T4 tRNA.

Although RNase II (a 3′ exonuclease) (157) is capable of removing terminal nucleotides from tRNA precursors (147), it is clear that RNase D is a different and far more selective activity. First, RNase II and RNase D are different proteins which are completely separated during their purification to homogeneity (32, 61). Polyadenylate, an excellent RNase II substrate, is not attacked by RNase D (61). Second, RNase II is a processive exonuclease which continues digestion into the mature portion of the tRNA, and amino acid acceptor activity is not produced (32, 61). RNase D, therefore, is most likely an authentic in vivo 3′ tRNA maturation enzyme. (Other, less well-defined activities are discussed below.)

## Other Activities Implicated in RNA Processing

**3′ Endonuclease activities.** Endonucleases other than RNase P are known to be required for tRNA maturation in at least two roles. First, transcription of the $tRNA_1^{Tyr}$ gene cluster and of the tRNA genes in rRNA transcriptional units continues for a considerable distance past the 3′ terminus of the last tRNA gene (45, 84, 115, 178), whereas the corresponding cellular monomeric tRNA precursors found in temperature-sensitive RNase P strains contain only a few extra bases (8, 74, 143), or none (74, 143, 173), at their 3′ termini. The postulated in vivo 3′ cleavage activity(ies) for ribosomal operon tRNA's was generically denoted RNase "F" (11, 55). In a cell-free extract, Sekiya et al. (155) detected cleavage of an in vitro transcript of a synthetic *su3*$^{+}$ $tRNA_1^{Tyr}$ gene at a position seven bases past the mature 3′ terminus (Fig. 1). Similarly, Bikoff et al. (24) indicated that a cellular supernatant fraction was capable of converting a large in vitro transcript of the *su3*$^{+}$ $tRNA_1^{Tyr}$ gene into a smaller molecule. Second, it was inferred that some tRNA precursors encoded by bacteriophage T4 are cleaved in vivo near their 3′ termini by endonuclease activity (49, 100a).

An activity which can perform such cleavages was identified in an RNase III$^{-}$, temperature-sensitive RNase E, temperature-sensitive RNase P strain. This activity, called RNase F, was partially purified from *E. coli* cells (173a; M. Gurevitz, N. Watson and D. Apirion, manuscript in preparation). It can cleave in a specific site a precursor RNA molecule which contains species 1 RNA and the 3′-termination end. (Species 1 RNA is a small stable RNA which is 140 nucleotides long and is derived from the distal part of the T4 tRNA gene cluster [see below] [1, 2, 49, 50].) The cleavage is near the 3′ end of the

mature sequences of species 1. The products of the reaction are very similar to RNA molecules found in the cell. The enzyme is stimulated by monovalent cations ($NH_4^+$, $K^+$, or $Na^+$) but not by divalent cations and has an optimum pH of 6.8 to 7.6. It is active at high temperatures up to 60°C, and the activity is very resistant to irreversible heat inactivation; it can withstand heating at 90°C for 30 min. The enzyme has a high degree of specificity and is only somewhat inhibited by yeast RNA. It does not act on substrates which are specifically cleaved by the other three processing endoribonucleases of *E. coli*: RNases III, E, and P. RNase F introduces a specific cut at the 3′ end of species 1 T4 RNA, which is in a precursor molecule. Because species 1 RNA resembles a tRNA molecule (124), it is possible that this enzyme introduces a cleavage near the 3′ ends of tRNA molecules and other RNAs which could resemble tRNA in their three-dimensional structure. However, because mutants deficient in this activity have not been isolated, the physiological role of this enzyme remains unknown.

**RNase P2 and O activities.** Cleavage products from multimeric tRNA transcripts are observed in vivo in temperature-sensitive RNase P strains at the nonpermissive temperature (74, 143, 146, 147) and in vitro after processing with heat-inactivated temperature-sensitive RNase P extracts (142). The $tRNA_1^{Tyr}$ precursor can also be isolated from temperature-sensitive RNase P strains in a form containing just 9 or 16 bases of the 41-base leader segment (7, 146). The endonuclease activities responsible for such cleavages in cells lacking RNase P were named RNase P2 (146) and RNase O (142). Each was subsequently partially purified and shown to be capable of processing some multimeric transcripts to smaller monomeric tRNA precursors (147, 156). Digestion of these multimers with RNase P was facilitated by addition of RNase P2 (75, 147) or O (143, 156). Relatively few tRNA multimers are RNase O substrates (143).

RNase O has chromatographic properties (156) resembling those of RNase III (139). The two activities copurify, and inhibitors of RNase III activity seem to inhibit the tRNA cleavage activity of RNase O preparations (156), as do salt concentrations which inhibit "secondary" cleavages of single-stranded RNA by RNase III (40). It must be kept in mind that for two well-characterized substrates, RNase III secondary cleavages occur infrequently, if at all, in vivo (40, 126).

The chromatographic properties reported for RNase P2 (147) are compatible with those of RNase E (109; unpublished data of M. K. Roy, T. K. Misra, and D. Apirion), although it is not known whether these two processing activities copurify.

**3′ Exonucleolytic trimming activities.** Numerous activities have been detected in different laboratories which could remove precursor-specific nucleotides from the 3′ end of tRNA precursors. Apart from RNase D, none of these in vitro activities has been sufficiently purified or characterized to permit definite conclusions to be drawn regarding its role in tRNA metabolism in vivo. Bikoff et al. (24) reported an activity, RNase P III, which could be separated from RNase II and which has chromatographic and enzymatic properties resembling those of RNase D. RNase P III, like RNase D, restores amino acid acceptor activity to RNase P-cleaved pre-$tRNA_1^{Tyr}$ (24). On the other hand, Schedl et al. (147) partially purified an activity which could remove nucleotides from the 3′ termini of tRNA precursors and which appeared to be identical to RNase II. Shimura et al. also fractionated a similar activity, "Q" (156), which again contained RNase II activity (as judged by substrate specificity, pH optimum, and ionic requirements and inhibition). As mentioned previously, it is unlikely that RNase II is involved in tRNA maturation in vivo.

**RNase BN.** Certain phage-encoded tRNA precursors lack the 3′-terminal CCA sequence and contain instead one to three extra bases. These residues must be removed and CCA must be added by nucleotidyltransferase for the tRNA to be functional (148, 154). McClain and collaborators (149, 154) have identified and partially purified an activity (RNase "BN") which can remove the 3′ termini of immature T4 $tRNA^{Pro}$, $tRNA^{Ser}$, and $tRNA^{Ile}$. As an analog of immature T4 $tRNA^{Pro}$, an in vivo BN substrate whose -CCA sequence is replaced by -CU, the semisynthetic precursor tRNA-CU was generated from mature tRNA with exonuclease and nucleotidyltransferase (149). Several host activities were detected which could release the terminal $^3H$-$U_{OH}$ residue. One of these, which appeared to be absent from extracts of the BN mutant, has been partially purified (149). This activity can accurately remove in vitro the 3′-terminal -$U_{OH}$ from immature $tRNA^{Pro}$; the 3′ termini of pre-$tRNA^{Ser}$ (-UAA) and pre-$tRNA^{Ile}$ (-UAU) are also removed, although very inefficiently. RNase BN has an apparent molecular weight of 35,000 and requires 2 mM $Mg^{2+}$ but has no monovalent cation requirement. These properties are essentially identical to those determined for RNase D (32, 61). RNase D, like RNase BN, can release terminal residues from tRNA-CU, but only very slowly (61).

An *E. coli* mutant, BN, has been isolated (95) which is selectively defective in removing the 3′

termini from immature forms of T4 $tRNA^{Pro}$, $tRNA^{Ser}$, and $tRNA^{Ile}$ (154). This mutation does not affect growth of the host cell (95) and appears to affect only the maturation of T4 tRNA's. Furthermore, no other BN-like activities against exonuclease-treated tRNA could be resolved from RNase II or D (32, 61). Curiously, however, the BN mutant is not defective in RNase D activity when assayed with tRNA-CCACCC as a substrate (32). The suggestion has been made (32), therefore, that the BN mutant may be defective in a component of RNase D activity directed against tRNA-CU substrates.

**Secondary maturation activities.** The first detectable products of rRNA biosynthesis in wild-type *E. coli* are p16, p23, and p5 precursors which contain extra terminal sequences and which are found in ribonucleoprotein particles (76, 86–88, 98, 99, 121). Additional processing steps, carried out by postulated enzymes designated RNases M16, M23, and M5, are therefore required to form the mature rRNA species. Conversion of p16 and p23 precursors to their mature m16 and m23 forms, respectively, requires removal of polynucleotide segments from both the 5′ and the 3′ termini of the precursors (27, 88, 179) (see Fig. 2 and 7). The termini of m23 rRNA are base paired (27–29, 54) so that they may be generated by a single cleavage. (RNase III itself does not produce m23 termini [27, 54, 62].) The termini of m16, however, are not paired in the p16 molecule; hence, separate 5′- and 3′-specific cleavage activities can be envisioned.

*E. coli* RNase M23 and RNase M5 activities have not been detected in vitro, but RNase M16, an activity(ies) which can convert the p16 RNA found in precursor particles to mature-size m16 rRNA, has been described and partially purified (67, 68, 104). An RNase M16 or M16-like enzyme was subsequently partially purified by Dahlberg et al. (33). Using as the substrate an in vivo processing intermediate with a mature 3′ terminus, the activity introduces an endonucleolytic cleavage at a position zero to three bases before the 5′ terminus of m16 rRNA sequences. The ability of this activity to generate the mature 3′ terminus of m16 rRNA has not been tested. Interestingly, ribosomes in vivo may contain one or two additional bases at the 5′ side of m16 without gross alteration of their functioning (47). This raises the possibility that some 5′ trimming is normally performed after RNase M16 cleavage. This in vitro maturation activity recognizes only RNA in a ribonucleoprotein particle; it does not recognize naked precursor RNA.

The analysis of these enzymatic activities is insufficient to permit a critical comparison. Moreover, since no mutants have been isolated which are defective in these activities, it is not clear whether any of them is used in vivo. Any of these activities may represent a nonspecific nuclease(s) which attacks the exposed precursor-specific RNA sequences more readily than the mature 16S sequences, which are protected by 30S ribosomal subunit proteins (175).

Maturation of the termini of m5 rRNA also occurs in *E. coli* because the primary processing yields a molecule possessing three extra nucleotides at both ends (76, 77) (Fig. 3). Thus, whereas in *E. coli* there are at least two enzymes which are directly involved in the maturation of 5S rRNA, *B. subtilis* contains a single enzyme for this function (cf. Fig. 3 and 4).

## GENETICS OF RNA PROCESSING

### Mutants Defective in RNA Processing

Genetic analysis of RNA processing reactions has provided numerous benefits, in part because the role assigned to particular enzymes in vitro can be verified in vivo and also because isolation of mutant strains defective in a particular processing step has frequently allowed detection of RNA precursor species which are not detectable in wild-type cells and which can then be used as substrates in the purification of novel processing enzymes and in the investigation of processing reactions.

The first major steps toward delineating pathways and mechanisms of RNA processing were taken in 1973 with the isolation of two mutant strains: one lacked the activity of RNase III (78), and the other was defective in RNase P (145). Subsequently, screening of colonies derived from mutagenized cells led to the detection of several other mutants with defects in rRNA or tRNA biosynthesis which could be attributed to lesions in specific RNase functions (13, 21, 33, 95, 144). The isolation and properties of each of these mutants are described below.

#### *rnp* Mutants

**Identification.** To identify enzymes required for tRNA biosynthesis in vivo, Schedl and Primakoff (145) screened temperature-sensitive mutants which at the restrictive temperature were unable to express the amber-suppressing *su3*$^+$ $tRNA_1^{Tyr}$ after infection with bacteriophage $\phi$80 carrying this gene. Several such mutants were isolated, one of which, A49, proved upon further analysis to be defective in RNase P. Cell-free extracts from the mutant strain showed a greatly reduced specific activity of RNase P (80) and could not process the *su3*$^+$ $tRNA_1^{Tyr}$ precursor to its mature form (at 30 or 42°C), whereas the parental extract was fully active in such reactions (145).

Ozeki and collaborators chose a different approach, devising an ingenious selection procedure in which expression of the amber-suppressive *su3*$^+$ tRNA gene is lethal (144). The parental cells were resistant to infection by the virulent phages T6 and BF23 by virtue of amber mutations in genes coding for the corresponding phage receptor proteins. The parental cells also carried a *lacZ* amber mutation and a thermoinducible λ prophage. After mutagenesis, cells were infected at 42°C with λ *psu3*$^+$ and with bacteriophages T6 and BF23. (The infecting λ represses the prophage.) Cells which expressed the *su3* gene as well as those which were not infected with λ *psu3*$^+$ were hence killed. Survivors were then screened for the ability to express mRNA genes but not tRNA genes at 43°C. Strains were chosen which could express *lacZ* at 43°C after infection with φ80 *plac* (using tRNA's made at 30°C) but were unable to suppress the chromosomal *lacZ* amber mutation after infection with φ80 *psu3*$^+$ at 42°C. This procedure yielded six mutants in which RNase P activity was thermolabile in vivo and in vitro (74, 143, 144). Another temperature-sensitive RNase P mutant was isolated from a strain initially characterized as defective in rRNA processing (21) (see below). The conditional lethality conferred by these RNase P mutations demonstrated that RNase P cleavage is an essential requirement for the functioning of at least some tRNA species.

Genetic analysis indicates that some of these mutations fall into two loci, *rnpA* at 82.5 min of the *E. coli* chromosome (10) and *rnpB* between 68.5 and 69 min (21, 155a). These loci are shown in Fig. 5. Since complementation tests were not carried out, one cannot conclude that all known *rnp* mutants lie in two genes.

Mutations at the *rnpA* locus affect the poly-

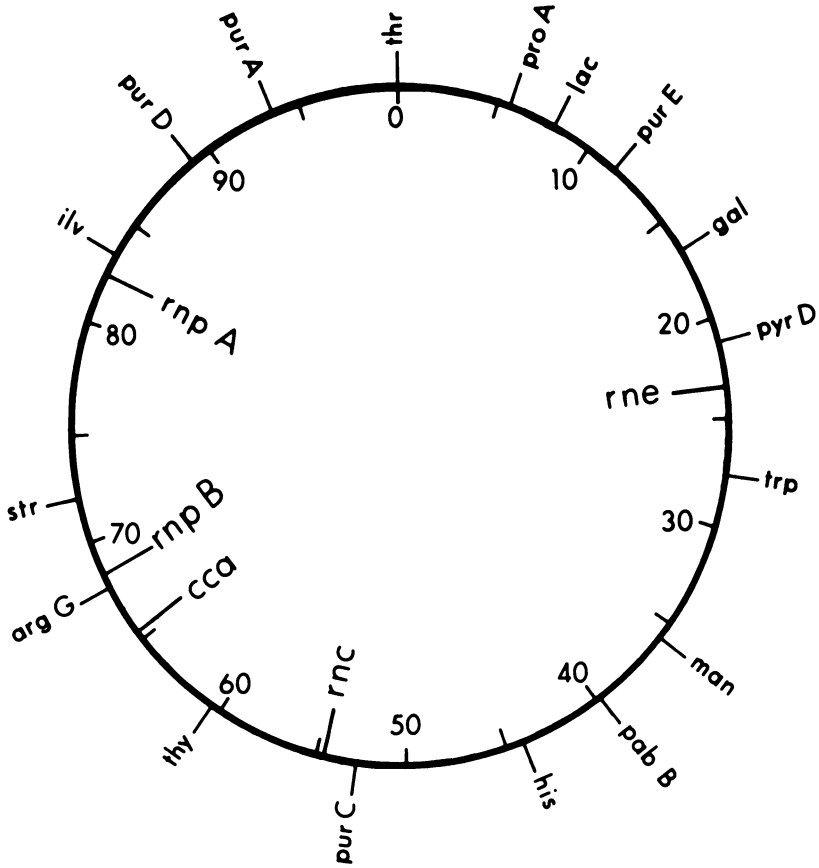



FIG. 5. *Genetic map of RNA processing mutations in E. coli K-12. The RNA processing genes are inside the circle. The loci are discussed in the text. rnc, RNase III (18, 168); rne, RNase E (9); rnp, RNase P (10, 21, 155a); cca, nucleotidyltransferase (38, 47a). A mutation which affects RNase D (rnd) was mapped at 39.8 min (M. Deutscher, personal communication).*

peptide component (see above), and those at the *rnpB* locus affect the RNA component of RNase P (80). In vitro reconstitution experiments with enzyme prepared from cells grown at 30°C demonstrated that RNA extracted only from *rnpB709* RNase P or protein derived only from the *rnpA241* enzyme could confer thermolability on the reconstituted RNase P (80). RNA from *rnpA* RNase P and protein from the *rnpB* enzyme complement each other to regenerate wild-type enzymatic activity.

Enzymes purified from an *rnpA49* strain and from a $ts^+$ revertant have altered isoelectric points, suggestive of amino acid substitutions (80). Similarly, the RNA prepared from an *rnpB709* strain grown at 30°C shows decreased amounts of M1 RNA, although retaining normal levels of M2 RNA (80).

Although it is tempting to suggest that the *rnpA* locus codes for the RNase P protein component and that the *rnpB* locus codes for the RNase P RNA component, the available data are insufficient for drawing such a conclusion. The fingerprints of M1 and M2 RNAs show that M2 but not M1 (80) is identical with the 10S RNA identified in the *E. coli* cell (84a, 131). Although only M1 RNA is required for RNase P activity in vitro (79a), M2 RNA copurifies with the enzyme and thus may participate in RNase P action in vivo. The 10S RNA hybridizes to three to four loci in *E. coli* (22), which would complicate observation of direct mutations in one of these genes if 10S can be transcribed from all of them. Therefore, although it is certain that an RNA moiety is required for RNase P action, many more details must be worked out before the picture can be clarified.

RNase P may be an RNA-protein complex like the ribosome for which the RNA genes are reiterated but the protein genes are not. Indeed, such a relationship may be mandatory for the maintenance of equivalent amounts of RNA and protein, because the product of protein genes is increased at two successive levels of gene expression (transcription and translation), whereas the product of structural RNA genes is amplified only once, by transcription.

**Transfer RNA (tRNA) precursors accumulated in *rnp* strains.** The RNA species accumulated in various *rnp* mutants have been examined by several groups: *rnpA49* was studied by Schedl and co-workers (146, 147) and by Ilgen et al. (75), and exhaustive catalogs of small RNA were prepared by Sakano and Shimura (143) for *rnpA241* and by Ikemura et al. (74) for *rnpB709*. These results can be summarized as follows. A large group of RNAs are about 4S in size. These small monomeric tRNA precursors bear short stretches of extra nucleotides at their 5′ termini. These species apparently have undergone some processing in vivo, because they do not contain 5′ triphosphoryl termini. In addition, only a few residues, or none at all, remain at their 3′ termini. Another group of RNAs (ca. 4.5S and 5S) are large monomeric precursors, some of which do contain 5′ triphosphates and hence represent primary transcripts unprocessed at least at their 5′ ends. Many of these species have also undergone some 3′ cleavages, because tRNA's known to be transcribed as much longer precursors or whose genes are known to include additional 3′ sequences are found in transcripts bearing only a few extra trailing bases. The prototype of this class is the $tRNA_1^{Tyr}$ precursor, which has 41 extra 5′ bases starting with pppG and only one to three extra 3′ nucleotides (8), although its transcription unit continues for a considerable distance past its 3′ end (84).

Perhaps the most interesting species produced by *rnp* mutants, however, are the multimeric tRNA transcripts, which may contain up to four or five individual tRNA species (6, 74, 75, 116, 143, 147, 159). Some of these precursors are derived from reiterated genes for a single tRNA species, and others are derived from heterogenous tRNA gene clusters. Many of these species contain 5′ triphosphoryl termini. Some of the monomers and dimers observed in the *rnp* strain are apparently subsets cleaved from these larger species (143). This phenomenon of residual pre-tRNA cleavage in temperature-sensitive RNase P cells suggested the involvement of additional endonucleolytic tRNA processing activities apart from RNase P (142, 146) (see above). The effects of combining *rnp* mutations with other RNA processing mutations are described below.

### *rnc* Mutants

As a means of facilitating in vitro studies on the replicative intermediates of RNA bacteriophages, Kindler and co-workers (78) screened extracts of heavily mutagenized *E. coli* cells for the inability to solubilize $^3$H-labeled, double-stranded replicative forms of phage M13. One such mutant, AB301-105, proved to be unconditionally defective in the activity of RNase III. The ability of extracts from the mutant strain to solubilize labeled synthetic ribohomopolymer duplexes was <1% that of the parental strain (78). The apparent "residual" activity detected by such assays cannot be attributed to RNase III, however, because it has a different optimum pH and different (more stable) thermal inactivation kinetics (15, 16). (This residual activity is probably not double strand specific [15, 16].) Furthermore, in RNase $III^-$ strains, no RNase III processing activity can be detected in vivo (54) (see below).

Genetic analysis of strain AB301-105 revealed the presence of at least seven mutations other than the RNase III lesion, confirming the original indication that it was not isogenic with the parental strain (17). For study of the effect of the RNase III mutation *rnc-105* by itself, the mutation was first mapped at 54.5 min (18, 168) (Fig. 5), and isogenic *rnc*$^+$/*rnc-105* pairs were then constructed by transducing the *rnc-105* mutation into a different genetic background (18) which allowed further study of the physiological role of RNase III (see below).

**Ribosomal RNA metabolism in RNase III$^-$ cells.** Electrophoretic analysis of labeled RNAs from an *rnc-105* strain demonstrates a dramatic effect on rRNA production. Figure 6 illustrates that in addition to the normal m16 and m23 rRNA's, the mutant synthesizes RNAs of 30S, 25S, and 18S (11, 42, 57, 117), whose presence is solely dependent upon the *rnc-105*

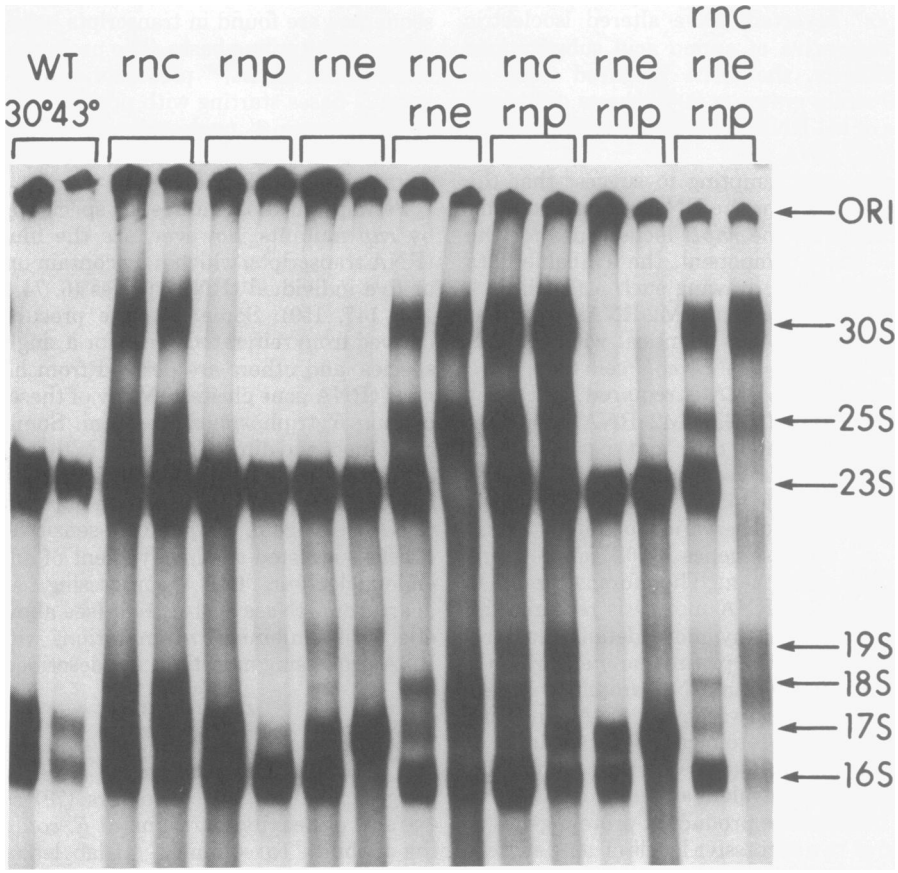



FIG. 6. *rRNA's produced in different RNA processing mutants. For each of the genotypes indicated, cultures of the corresponding strain were labeled with [*$^{32}$*P]orthophosphate at 30 or 43°C, and nucleic acids were fractionated on a 3% polyacrylamide gel (details as in reference 53). For each strain, the left lane represents RNA from cells grown at 30°C, and the right lane represents RNA from cells labeled at 43°C. Whereas 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 (59), and therefore the patterns observed at 30 and 43°C are similar. The rnpA49 mutation used here is apparently defective in the synthesis but not the function of RNase P (80), and certain features of this mutation are already expressed at temperatures at which the mutant can grow (10). The rne and rne rnp strains used here contain an additional ts mutation(s) which blocks protein synthesis (9), and therefore 16S 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, 16S rRNA appears. Whereas the difference between p16 and m16 is substantial (~150 nucleotides) and can be easily detected in gels, the difference between p23 and m23 is only 15 nucleotides (37, 54) and cannot be observed in the gel (see Fig. 2C and D). The size differences between p16a and p16b and between p23a and p23b (see text) are also not readily observed in gels.*

allele (16). The normal monomeric rRNA precursors p16a (17S) and p23a of wild-type cells are replaced by slightly larger p16b and p23b precursors which extend beyond the RNase III cleavage sites (54). Competition hybridization analysis of 30S RNA indicated the presence of m16 and m23 sequences (42, 117). Analysis of its RNase III cleavage products revealed that the 30S species was an intact primary transcript of the rRNA gene cluster, beginning with an initial 5′ purine triphosphate terminus and containing sequences of p16, p23, and p5 rRNA's (62). The 25S RNA species contains both 23S and 5S rRNA sequences (60); the 18S contains 5′ leader segments and p16 sequences (53, 55). Both the 18S and 30S species are unprocessed at their 5′ ends because they contain 5′-terminal nucleoside triphosphates (53, 55, 62, 92). RNase III cleavage of 30S in vitro yields authentic p16 and (probably) p23 rRNA's; the majority of p5 RNA sequences are embedded in a 300-base-long RNA product (62).

Intact polycistronic transcripts of rRNA operons were first detected by Pettijohn and coworkers in a cell-free transcription system. They detected them by studying elongation and termination of rRNA molecules in isolated *E. coli* nucleoids and by studying the RNA polymerase molecules which initiated rRNA synthesis in vivo (127a). Hybridization analysis (127a; D. E. Pettijohn, personal communication) indicated that the in vitro rRNA's corresponded closely to the 30S and 25S rRNA's accumulated in vivo in an RNase III$^-$ strain.

Detailed analysis of specific activity and labeling kinetics demonstrated that all the rRNA's appearing in an RNase III$^-$ cell, with the exception of mature m16 and m23, were produced by cleavages occurring during rather than after transcription (52, 57). Little if any contribution to p16 and p23 rRNA's was made by 30S and 25S species, which are probably degraded nonproductively instead (52, 57). Electron micrographic visualization of ribosomal DNA transcription in wild-type and RNase III$^-$ cells (70) demonstrated that in the mutant, some nascent rRNA transcripts are uncleaved and reach a length corresponding to 30S chains, whereas other transcripts are cleaved in the intercistronic spacer region and reach lengths of p16 or p23 rRNA.

rRNA gene clusters also contain tRNA genes located in the spacer region between 16S and 23S genes (72, 91, 94, 114, 115, 176). Endonucleolytic cleavage of these spacer tRNA sequences by RNase P could thus be partially responsible for production of 18S and 25S RNAs in the *rnc-105* cell (57). This was confirmed by the demonstration that *rnc-105 rnpA49* double mutants accumulate, at the expense of 18S, a new 19S rRNA containing the sequences of 18S RNA linked to spacer tRNA's (53, 55) (Fig. 6 and 7). RNase III cleavage of 19S RNA gives 5′ leader RNAs initiated with nucleoside 5′ triphosphates, p16 rRNA, and 3′ segments from which RNase P can subsequently release mature tRNA molecules (55).

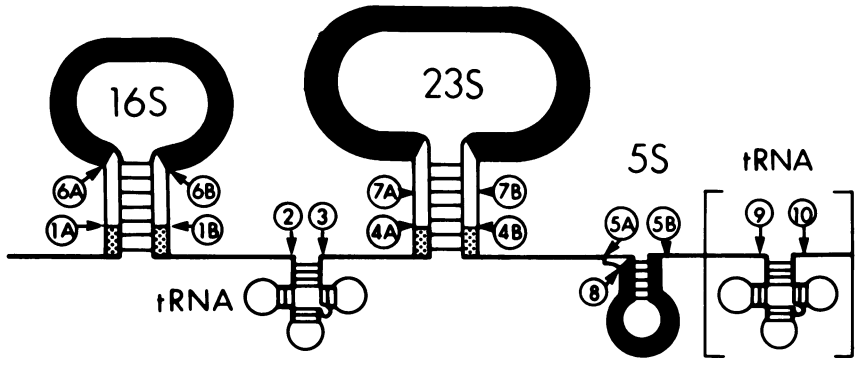
Comparison of *rnc-105* with *rnc*$^+$ strains also indicated the involvement of RNase III in the processing of host and bacteriophage mRNA transcripts and in the processing of tRNA precursors in *E. coli* and bacteriophage T4. These studies are described below.

### *rne* Mutants

In an attempt to obtain mutants defective in rRNA-processing enzymes other than RNase III, Apirion and Lassar (13) mutagenized RNase III$^-$ cells and, after enrichment for temperature sensitivity, screened the mutants for defects in the pattern of radioactively labeled rRNA species by electrophoresis in polyacrylamide gels. One such mutant was detected which at the nonpermissive temperature failed to produce 23S and 5S rRNA's and accumulated instead a 25S RNA species (13) containing both 23S and 5S (60). Transduction of the temperature-sensitive mutation into an RNase III$^+$ genetic background yielded a strain in which 23S production was normal but in which 5S rRNA still did not appear at the restrictive temperature; several new low-molecular-weight RNAs, on the other hand, were detected (9, 59). This mutation, *rne-3071*, is a recessive single-point mutation which maps at 24 min of the *E. coli* genome (9) (Fig. 5). Combination of the *rnc-105* and *rne-3071* mutations yields a phenotype similar to that of the original isolate (Fig. 6 and 7) (9). When an *rne* mutant is shifted to the nonpermissive temperature, growth as well as macromolecule synthesis continues linearly, but cell division is promptly blocked (63a).

A major new RNA detected in an *rne* strain is a 9S species (ca. 250 bases long), containing the entire sequence of p5 rRNA as well as extra sequences at both ends (58). From 5′ to p5, 9S contains about 80 bases (the "4S" fragment) which correspond to DNA sequences starting 9 bases past the 3′ end of the 23S rRNA gene; following the 123 bases of p5 sequences, 9S continues for about 40 bases (Fig. 2 and 3). The 3′ end of 9S contains a stem-and-loop structure (Singh and Apirion, manuscript in preparation) similar to structures near known transcription termination sites (3, 177). A product very similar

A




Cuts 1, 4. RNase III 2,9. RNase P 3,10. RNase F 5. RNase E
6. RNase M16 7. RNase "M23" 8. RNase "M5"

B

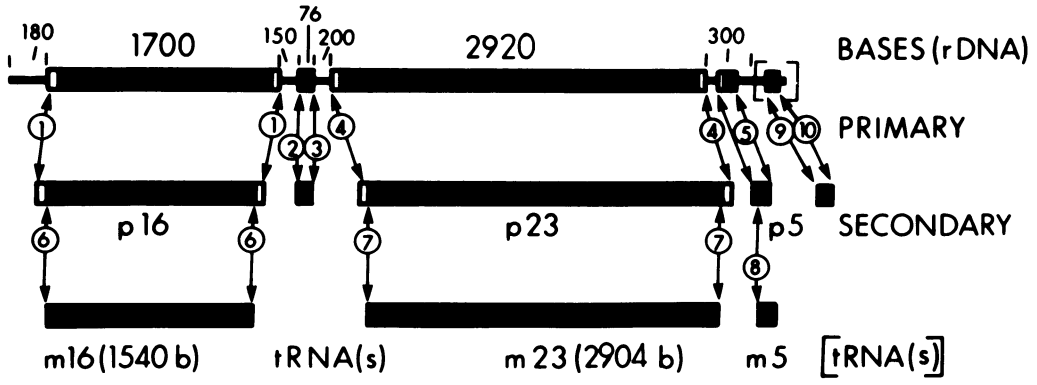



FIG. 7. *Structure and processing map of rRNA transcripts. (A) Structure and cleavage sites of the rRNA primary transcript (not to scale). Derived from data from a number of laboratories (11, 57). Distal (trailer) tRNA's are bracketed because not all rDNA's contain them. Transcripts may contain one or two spacer tRNA's, and no, one, or two trailer RNAs. Arrows indicate endonucleolytic cleavage sites. Each cutting event is given a separate number, referring to the enzyme involved; A and B indicate that two (or more) separate cuts may be required. (Cut 8 is now known to be composed of two cuts or trimming events on either side of the 5S rRNA). 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 tRNA's) represent nonconserved 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, drawn approximately to scale. Distances in bases are between vertical bars above the map. The primary and secondary cuts, numbered as in (A), are shown above the products they generate. Open and solid segments are as in (A).*

to 9S RNA is produced by cleavage of 30S RNA with RNase III in vitro (62).

In *rne* cells at 43°C, no 5S RNA sequences could be detected in the 5S region of polyacrylamide gels (59). Moreover, RNase E partially purified from an *rne* strain was found to be thermolabile in vitro (110). The *rne-3071* mutation, therefore, determines a structurally altered RNase E. The $rne^+$ gene has been cloned in a λ vector (130). Cells infected with this λ *rne* phage have elevated levels of RNase E activity, thus providing evidence that RNase E is indeed the product of the *rne* gene.

Although 9S RNA contains no stable cellular RNA other than p5 rRNA, an *rne rnp* double-mutant strain accumulates two novel RNAs consisting of p5 rRNA joined to $tRNA_1^{Asp}$ or p5 plus $tRNA_1^{Asp}$ and $tRNA^{Trp}$ (132). (The p5-$tRNA_1^{Asp}$ precursor comigrates with a previously described $tRNA_3^{Gly}$ trimeric precursor [75, 143, 146]; these are most likely two separate species.) This confirms previous observations that genes coding for $tRNA_1^{Asp}$ and $tRNA^{Trp}$ are found near and are cotranscribed with the p5 cistrons of some rRNA gene clusters (113, 115, 177).

Another of the novel 8S to 10S RNAs appearing in an *rne* single mutant has recently been shown to be a dimeric tRNA precursor containing sequences of $tRNA_1^{Leu}$, $tRNA_1^{His}$, and a 100-base-long 3′ trailer segment (132a). Processing of this molecule in vitro requires both partially purified RNase E and RNase P as well as another endonuclease activity supplied by extracts of RNase $P^-$, of RNase $E^-$, or of RNase $III^-$ cells. It appears that these cleavages are cooperative and must be performed synchronously for complete processing to occur. Recently, monomeric precursors for $tRNA^{Ser}$ and $tRNA^{Asn}$ have been detected in an *rne* strain (132b). These molecules contain only a few extra sequences past the 5′ and 3′ ends of the mature tRNA. For RNase P to release the 5′ precursor segment in vitro, a 3′ precursor fragment must first be removed (132b). This was accomplished by a partially purified RNase E preparation. However, since the RNase E preparation used was not pure, the possibility that the cleavage was introduced by an enzyme different from RNase E cannot be excluded.

### A Putative RNase M16 Mutant

A mutant strain has been described in which maturation of the 5′ terminus of p16 rRNA is altered (33). This slower-growing but viable strain has a low rate of p16 maturation and accumulates two intermediates; the 3′ terminus of each intermediate is the same as that of m16 rRNA, but the 5′ terminus is longer than that of m16 rRNA. One of these, a 16.3S molecule containing 50 extra 5′ nucleotides, is found in 30S ribosomal subunits on polysomes and may be competent in protein synthesis (33). These 30S particles or the 70S ribosomes containing them can be matured in vitro. This supports the suggestion that 27S precursor particles are first associated with polysomes and converted to 30S particles before the precursor p16 rRNA is cleaved to m16 rRNA (86, 87, 99, 172). A few extra nucleotides at the 5′ end of m16 rRNA do not affect ribosome function (47), but whether the presence of 50 nucleotides is deleterious or whether this extra fragment is removed before or after the 30S subunit becomes functional is not known. Also unknown is the mechanism whereby m16 rRNA is ultimately formed in this mutant strain, which seems to lack an activity capable of p16 maturation in vitro (33). No genetic analysis of this mutation(s) was carried out.

### The BN Mutant

*E. coli* strain BN (a B strain) is a viable mutant (95) which after infection with bacteriophage T4 accumulates several phage-coded tRNA's with extra sequences at their 3′ ends (154). The precursors detected are precursors to Pro, Ser, and Ile tRNA's. In these tRNA's, part or all of the mature $CCA_{OH}$ terminus is not initially encoded; instead, the DNA sequence at the CCA position consists of one to three other bases (49). Surprisingly, these tRNA's are found in two dimeric precursors in the mutant: Pro-Ser and Thr-Ile. The mutant strain is apparently defective in an activity which removes these 3′ nucleotides (154). This activity, denoted RNase BN, may be related to one of the activities of RNase D (see above). The role of this activity in tRNA maturation is discussed below.

## PROCESSING OF RIBOSOMAL RNA

The p16 and p23 precursor rRNA's are the direct products of cleavage by RNase III (27, 62), which has a specificity for double-stranded RNA (139) (see above). RNase III cleavages were first proposed to occur in duplex structures formed by base pairing between regions of the rRNA transcript flanking the 5′ and 3′ ends either of 16S or of 23S sequences (56, 57, 176). DNA sequences surrounding 16S and 23S genes in several different rRNA operons have been determined by several groups (cited in reference 54), and in each case, the expected sequence complementarity was found. rRNA precursors from RNase $III^-$ cells have been isolated, and their duplex regions have been studied (54). The sequence and secondary structure of the p16 and

p23 RNase III processing regions is shown in Fig. 2. rRNA transcripts in the cell thus take on the form of giant ribonucleoprotein loops held together by duplex stems; p16 and p23 rRNA precursors arise by cleavage within these stems (Fig. 8).

### Processing in Wild-Type Cells

The detailed sequence of processing events discussed here is illustrated by the models presented in Fig. 7 and 8. An RNA polymerase molecule which initiates transcription of an rRNA gene cluster (Fig. 8) continues to synthesize the various components of the polycistronic rRNA primary transcript, that is, leader sequences, p16 rRNA, spacer sequences containing tRNA's, p23 rRNA, p5 rRNA, and trailer sequences which in some operons contain tRNA's. 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 from which m16 sequences loop out. As Fig. 7 and 8 show, the stem thus formed is susceptible to endonucleolytic processing cleavage by RNase III, which cuts within it (Fig. 7, cuts 1A and B) to release p16 precursor rRNA plus a 5′ leader fragment from the growing RNA chain. As the spacer region is synthesized, tRNA's are removed by endonucleolytic cleavage (RNase F) at or near the 3′ side and at the 5′ end by RNase P (Fig. 7, cuts 2 and 3). Trimming of the 3′ end, perhaps by RNase D, may also be required to produce mature spacer

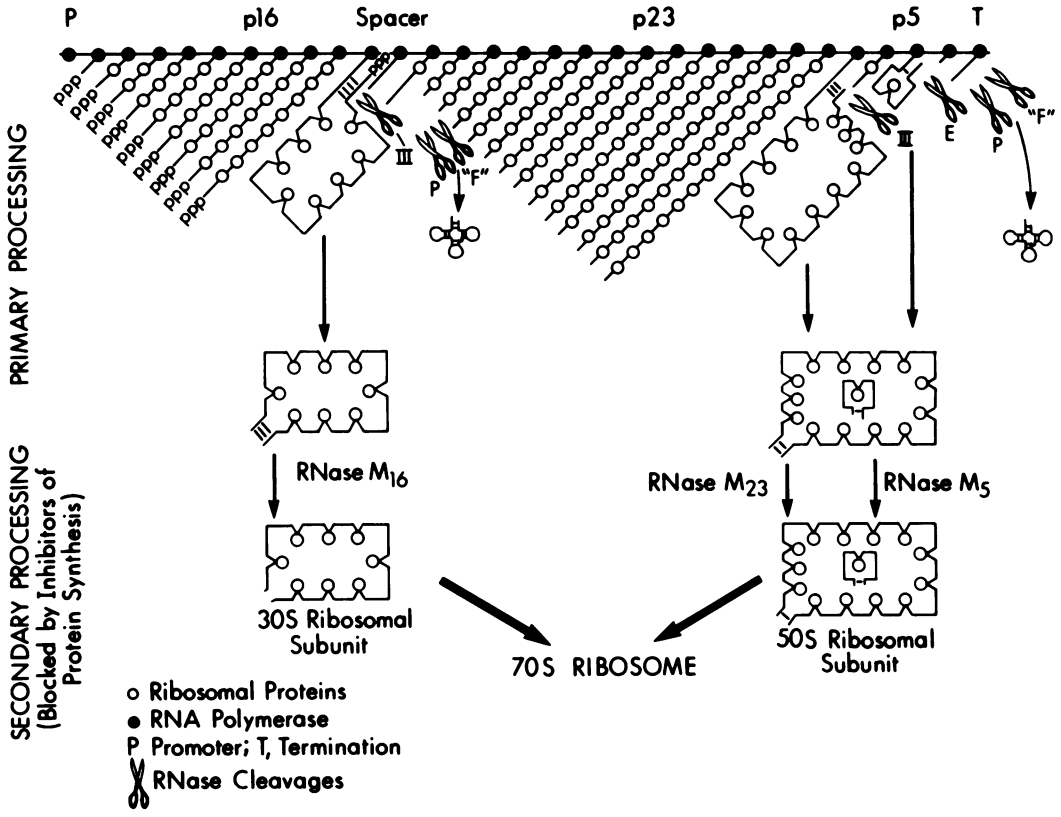



FIG. 8. *E. coli processing of rRNA. Maturation and assembly of ribosomes. Each nascent chain of ribosomal ribonucleoprotein represents a successive point in time in the transcription of an rRNA operon by an RNA polymerase molecule. Many ribosomal proteins attach sequentially to the nascent rRNA (30), and endonuclease cleavages by RNases III and E can occur only after the 3′-terminal region of their respective substrates has been synthesized and hydrogen bonded to the 5′-terminal region. The spacer and trailer tRNA's are not drawn in the nascent transcript, but they too must assume their mature conformation before being cleaved by RNases P and F. Secondary maturation of p16, p23, and p5 rRNA's 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.*

tRNA's. As the RNA polymerase completes transcription of 23S genes, RNase III excises p23 sequences, again by cleaving in the double-stranded stem formed by complementary sequences surrounding m23 (Fig. 7, cuts 4A and B). Transcription of the distal portion of the gene cluster now proceeds into the 5S gene (Fig. 7 and 8). As soon as p5 rRNA sequences are formed and have folded into the appropriate conformation, they are excised by RNase E (Fig. 7, cuts 5A and B), and distal or trailer tRNA's are removed by RNase P (Fig. 7, cut 9) and another activity (Fig. 7, cut 10) possibly identical to RNase F.

That p23 and p5 rRNA's are excised from rRNA transcripts before transcription termination is evidenced by the failure to detect 25S rRNA (p23 plus p5) in wild-type strains and by the demonstration that no material in the 9S region of wild-type cells contains p5 sequences (59). It is clear, then, that the primary processing cleavages by RNase III and RNase E are exceedingly rapid events: virtually all sites are cleaved within seconds after synthesis of the sequences which comprise them. (The rate of chain elongation of RNA polymerase on rRNA operons is about 85 nucleotides per s at 37°C [39]. The distance between p23 and p5 is 81 bases, and that between p5 and the termination signal is 39 bases in four of the seven rRNA operons which do not contain trailer tRNA's [Singh and Apirion, manuscript in preparation]; it is somewhat longer in the other three which do contain trailer tRNA's [177].)

There is an interesting major difference between processing of rRNA in procaryotic and eucaryotic cells. Whereas, as emphasized here, most of the rRNA processing occurs during transcription in procaryotic cells, in all eucaryotic systems studied thus far, most of the rRNA species are transcribed in a single molecule which is processed post-transcriptionally (127).

## Processing in Mutant Strains

The origin of rRNA species seen in mutants defective in RNA-processing enzymes (Fig. 6) can readily be described by reference to the model shown in Fig. 7. In strains lacking the processing endonuclease RNase III, scission of nascent rRNA transcripts is initiated by RNase P and another enzyme(s) which cuts in the spacer region to remove the tRNA sequences (Fig. 7, cuts 2 and 3). The single-stranded region between cut 2 and the p16 stem is removed, possibly by an enzyme(s) such as RNase II or polynucleotide phosphorylase, giving rise to 18S RNA. Subsequently, the 5′ leader sequence is removed from 18S RNA in a slower process by single-strand-specific (endo)nucleases which leave intact the duplex stem (Fig. 7). (Note that RNase III is probably the only double-strand-specific RNase in *E. coli* [16, 78, 138]. However, there is another enzyme, RNase N, which can degrade single- and double-stranded RNA [108]. This activity does not contribute much to the degradation of double-stranded RNA in extracts [16, 78].) The final rRNA product, a p16b molecule which contains the entire duplex stem and is thus slightly larger than normal p16a (54), is converted to normal m16 rRNA (162) by the maturation enzyme(s) RNase(s) M16 (Fig. 7, cuts 6A and B). RNase E cleavage in the distal portion of the nascent rRNA transcript generates p5 rRNA and a p23-like molecule, the latter containing extra single-stranded spacer sequences extending from cleavages 3 to 5A, which could be rapidly and nonspecifically removed, giving rise to the p23b rRNA of the *rnc* strain (54). This p23b is further processed to mature p23a rRNA via the maturation enzyme(s) RNase(s) "M23" (Fig. 7, cuts 7A and B).

When RNase E is inactivated in the *rne* mutant, p5 rRNA is not removed from the distal portion of the transcript, which instead accumulates as 9S RNA (Fig. 7, cuts 4B to 9). If RNase P is also inactivated, trailer tRNA's are found linked to 5S rRNA (131) (Fig. 7, cuts 4B to 10). Failure to perform RNase E and RNase III cleavages in the *rnc rne* strain at the nonpermissive temperature yields p5 sequences linked to p23 rRNA via the intermediate spacer RNA. A 25S RNA thus accumulates at the expense of p23 and p5 (Fig. 6 and 7). In these *rnc rne* double-mutant cells, unlike in *rnc* single-mutant strains, no p23 rRNA is detected.

Spacer tRNA's are linked to 18S RNA, thus giving 19S RNA, if RNase P is inactivated in an *rnc rnp* strain (Fig. 7, cut 3). Each 19S 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 (55). Production of 19S RNAs therefore requires endonucleolytic cleavage of nascent rRNA transcripts at a site near the 3′ end of each spacer tRNA sequence (Fig. 7, cut 3). In an *rnc rne rnp* mutant, some 19S- and 25S-sized species are still produced (Fig. 6). It appears that a processing activity(ies), distinct from RNase III, E, or P, does exist which can cut in the rRNA spacer region. At present, we refer to such activity(ies) as RNase F.

## Efficiency and Order of Processing Steps

Inasmuch as RNase $III^-$ cells are viable and form normal, functional mature m16, m23, and m5 rRNA's, the physiological usefulness of

RNase III may well be questioned. One answer may lie in the observation that the doubling time of an RNase III$^-$ cell is 40% longer (at 37°C) than that of an isogenic RNase III$^+$ cell (15). This observation can best be explained by the lower efficiency of rRNA processing in RNase III$^-$ cells. For example, 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 30S and partially cleaved 25S molecules, few if any of which contribute significantly to the pool of mature rRNA species (52, 57). To clarify this point and to shed light on the possible order of processing events, a further discussion of processing efficiencies is required.

RNase P cleavage of spacer tRNA's is less efficient when p16 sequences are not previously removed by RNase III because uncleaved 30S transcripts are detected in RNase III$^-$ RNase P$^+$ strains (Fig. 6), even though RNase P can cleave tRNA sequences in purified 30S RNA (93). Similarly, tRNA 3′ endonuclease action (RNase F) is impaired by the presence of p16 sequences linked to spacer tRNA, because, as seen in Fig. 6, relatively more 30S RNA is found in the RNase III$^-$ RNase P$^-$ strain than in the strain lacking only RNase III. Again, because the 25S species is seen in RNase III$^-$ single-mutant strains even though RNase E is active, it can be concluded that the addition of p23 sequences to nascent 9S transcripts severely impairs the efficiency of p5 excision by RNase E.

This pattern of impaired processing may result from a steric hindrance. Indeed, since ribosomal proteins attach to rRNA during transcription (30), it is likely that the uncleaved nascent rRNA exists as a ribonucleoprotein particle which restricts access to the 5S and tRNA processing recognition sites (Fig. 8).

### Sequential Nature of Processing Reactions

The order in which processing steps are performed influences their relative efficiencies, as the preceding discussion points out. Conversely, examination of cleavage efficiencies can be used to determine the order of processing steps.

Since the presence of p16 sequences impairs the efficiency of RNase P and 3′ endonuclease cleavages in the spacer region of nascent rRNA transcripts, it can be inferred that these tRNA processing cleavages normally occur on RNA chains lacking p16 structures and that removal of p16 rRNA therefore precedes processing of spacer tRNA's. For the same reason, RNase III-mediated release of p23 must normally precede RNase E excision of p5 rRNA. As previously discussed, RNase III cleavage of a growing transcript is completed during the time required for the polymerization by RNA polymerase of only a few hundred more bases; excision of p16 rRNA will usually have occurred by the time the RNA polymerase has moved into the 23S gene (30; Pettijohn, personal communication).

Since RNase III and RNase P in vitro can each cleave 19S or 30S RNA independently of the other (11, 55, 93), it may be assumed that the order of rRNA processing cleavages in vivo is determined partly by the linearity of rRNA transcription and partly by the relative intrinsic efficiency of competing enzymatic reactions. In a complementary fashion, the high overall efficiency of processing in wild-type cells may arise from the particular order observed in or imposed upon primary cleavage events.

### Other Processing Enzymes and Secondary Maturation

All available data indicate that the four major primary rRNA-processing endonucleases of *E. coli* are RNases III, E, F, and P. (Once a transcript has been cut, nonconserved sequences are degraded. The nature of the degradative enzymes is unknown; the nonspecific endonuclease RNase N [108, 111] is one candidate for such an activity.)

The primary processing events described here give rise to precursor forms of 16S, 23S, and 5S rRNA's, whose maturation occurs in ribonucleoprotein particles (Fig. 8). The processing of p16 to m16 by RNase(s) M16 (33, 68, 104) is probably endonucleolytic (Fig. 7, cuts 6A and B). Similarly, RNase(s) M23 (Fig. 7, cuts 7A and B) is proposed to mature p23 to m23 rRNA, and *E. coli* p5 rRNA may be trimmed to m5 by RNase M5 (Fig. 7, cut 8).

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 and 7), then, does not alter the accuracy of cleavages which give rise to m16 and m23.

## PROCESSING OF tRNA

### *Escherichia coli* tRNA

The immediate transcriptional products of *E. coli* tRNA genes are molecules containing extra nucleotides at both their 5′ and their 3′ termini. Reflecting the nature of tRNA gene organization, many transcripts are polycistronic, contain-

ing as many as four or five different or identical tRNA species (74, 75, 116, 143, 147). Because these intact transcripts are not detected in wild-type cells, endonucleolytic processing cleavages must begin to occur before transcription is terminated.

**Enzymes involved.** All the information gathered so far on tRNA processing in vivo, including processing of ribosomal spacer tRNA's, leads to the conclusion that tRNA species of *E. coli* are the products of posttranscriptional processing by four, and probably only four, endonucleases. These are RNases P, E, and III and at least one distinct 3′ endonuclease (which could be RNase F). (As described previously, RNase E may be identical to RNase P2, and RNase III may be identical to RNase O. Because RNases E and III have been defined genetically as well as enzymologically, we shall discuss processing in terms of these activities.) To illustrate the participation of these enzymes in tRNA maturation, the effect of *rnc*, *rne*, and *rnp* mutations on tRNA production in vivo has been examined (11). Figure 9 summarizes this information, which demonstrates that tRNA maturation is more severely restricted in strains lacking RNase E or RNases E and III, as well as RNase P, compared with the single RNase P mutant. Figure 9a is a two-dimensional gel pattern of tRNA's from a wild-type strain labeled at 43°C. The tRNA's of the *rnc* strain, which are displayed in Fig. 9b, are all apparently normal. The *rne* strain (Fig. 9c) shows a fairly normal pattern but lacks mature 5S rRNA and a few tRNA's. The molecules accumulating in the 5S region contain monomeric tRNA precursors (132b). It is noteworthy that the *rnp* strain (Fig. 9d), even when incubated for 40 min at 43°C before being labeled, makes an appreciable amount of mature or almost-mature-size tRNA molecules, as compared with wild-type cells. These molecules probably represent the previously described small monomeric tRNA precursors (74, 143) which are produced by endonucleases other than RNase P. The 4S to 5S RNAs of the *rnc rne* strain are very similar to those of the single *rne* strain (Fig. 9e). Combination of the *rnc* allele with *rnp* does not significantly change the pattern of tRNA accumulation from that of the single *rnp* strain, as seen in Fig. 9f. Most dramatically, however, an *rnp rne* double mutant shows a drastic reduction in mature tRNA species and a number of new 5S-sized species, compared with the single *rnp* mutant (Fig. 9g).

A further decrease in tRNA maturation is found upon combining all three processing mutations. The *rnp rnc rne* mutant accumulates few if any mature-size tRNA molecules (Fig. 9h). Again, although little difference was detected between *rnp* and *rnp rnc* strains, the enhanced deficiency in tRNA maturation observed upon introduction of the *rnc* mutation into an *rnp rne* strain indicates that RNase III is involved in tRNA maturation in *E. coli*.

Most of the RNA species (16 of 22 tested) in the 4S-to-6S region which accumulate in an *rnc rne rnp* strain at the nonpermissive temperature are primary transcription products initiated with adenosine 5′-triphosphate or guanosine 5′-triphosphate (127b). The *rnc rne rnp* mutant also accumulates large (>6S) precursors to most cellular tRNA's (11); these precursors can be processed with a wild-type cell extract to give a pattern of small RNAs indistinguishable from that shown in Fig. 9a (127b).

Apart from RNases III, E, and P and a 3′ endonuclease, one additional activity, presumably an exonuclease, is required for trimming extra sequences from the 3′ termini. Because in all *E. coli* tRNA's examined, the CCA terminus of the mature molecule is encoded in the DNA and is present in the RNA precursor, only the activity of RNase D would be required for 3′ trimming. It has been suggested, however, that RNase D trimming in vivo or in vitro may require prior RNase P cleavage at the 5′ side of the precursor $tRNA_i^{Tyr}$ (61, 142). In a temperature-sensitive RNase P strain, this and many other pre-tRNA's are found to bear extra sequences past their mature 3′ termini (6, 159). Other tRNA precursors examined, however, possess fully matured 3′ termini, even though extra sequences are present at the 5′ end (74, 173). Thus, either RNase D trimming stops at different sites on different precursors in the absence of RNase P cleavage or another 3′ exonuclease is also involved in tRNA maturation.

**Sequence of processing events.** After an RNA polymerase moves downstream of a monomeric tRNA gene, the tRNA sequences are released from the nascent transcript by one or more endonucleases. As soon as the mature portion of the molecule has assumed its final secondary and tertiary configuration, it can be recognized by RNase P, whose cleavage generates the mature tRNA 5′ terminus. RNase D trimming, if necessary, then exposes the mature 3′ terminal $CCA_{OH}$.

Processing of transcripts from polycistronic tRNA gene clusters is somewhat more complex. The processing endonucleases RNase E and RNase III may also cleave between adjacent tRNA sequences to produce smaller multimers and monomeric substrates from which RNase P can rapidly remove 5′ precursor-specific se-

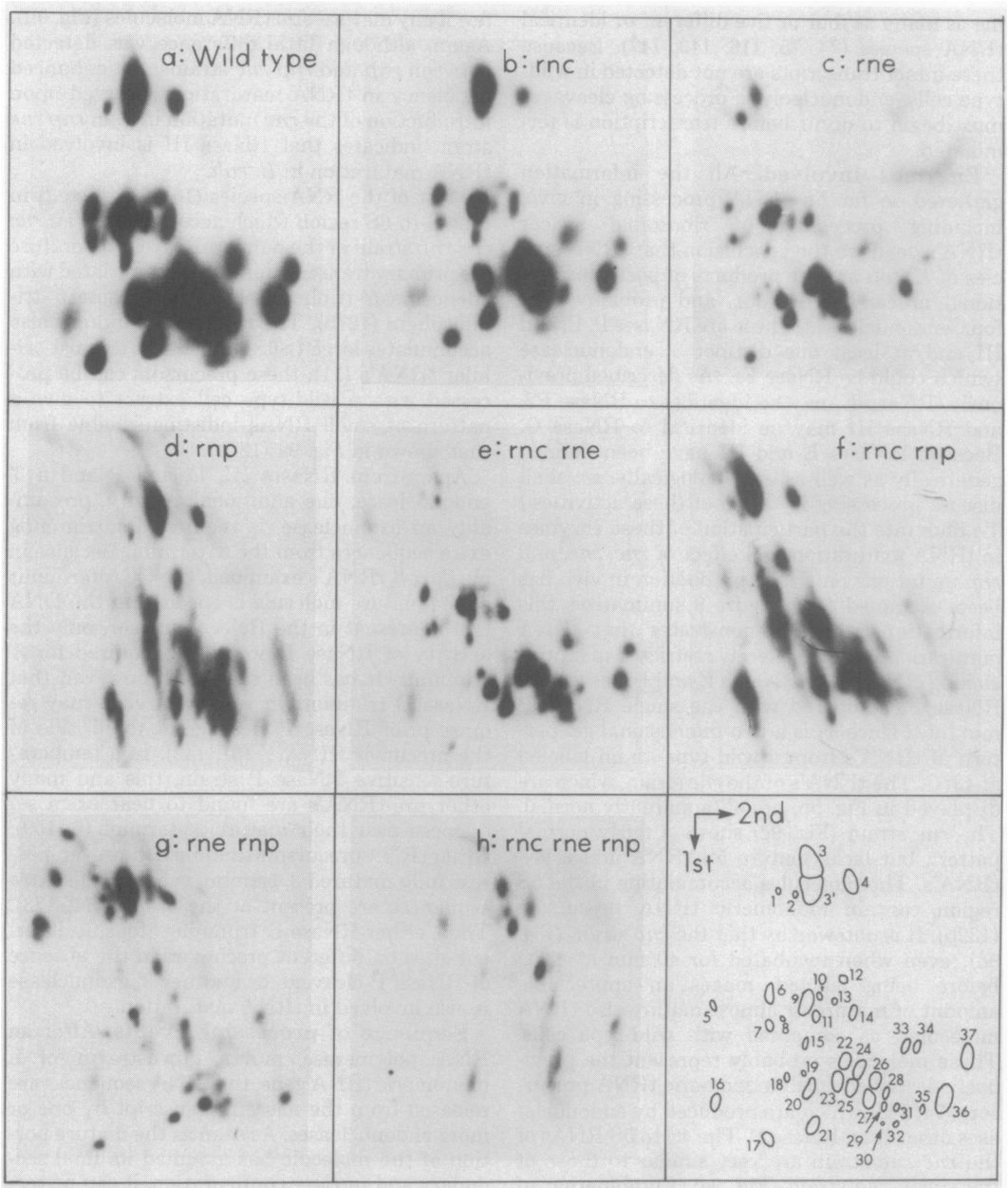



FIG. 9. *tRNA's and 5S rRNA produced in different RNA processing mutants. Cultures of strains with the indicated genotypes were labeled with [$^{32}$P]orthophosphate for 60 min after 40 min of incubation at 43°C. RNA was fractionated on 10% → 20% two-dimensional polyacrylamide gels. Spots 3, 3', and 4 are 5S rRNA isomers; the other spots are tRNA; e.g., spot 17 is tRNA$_2^{Glu}$ (see last panel of this figure).*

quences. Again, 3′ trimming by RNase D is probably necessary to expose the terminal sequence of the functional tRNA species.

It is clear that in the absence of RNases E and III (Fig. 9e), most tRNA's continue to be processed correctly but that in the absence of RNase P, very few if any mature 5′ termini are produced. RNase P, therefore, performs the final 5′ maturation of tRNA molecules and this cleavage cannot be performed by another enzyme, whereas RNase F or an equivalent enzyme performs most of the 3′ endonucleolytic cleavages.

In cells lacking one or more processing nucleases, various tRNA precursors can be detected, and it is by studying the effects in vivo of inactivating one or more enzymes, as well as by comparing their relative efficiencies in vitro, that one can begin to establish a hierarchy of processing events. Cleavage by the other endonucleases can clearly precede RNase P processing, because monomeric tRNA precursors accumulated in vivo in the absence of RNase P have already been cleaved at or near their 3′ termini. Conversely, RNase P can cleave some multimeric pre-tRNA transcripts in vitro, although inefficiently, without other endonucleolytic cuts (75, 143, 147). Three precursors have been identified, however, for which RNase P action is absolutely dependent upon prior cleavage by a 3′ endonuclease. Cleavage between ribosomal spacer tRNA's $tRNA_1^{Ile}$ and $tRNA_{1B}^{Ala}$ by an activity other than RNase III or P is required before RNase P can cleave at the 5′ side of $tRNA_1^{Ile}$ (55). RNase P processing of monomeric precursors to $tRNA_1^{Ser}$ and $tRNA^{Asn}$ requires prior 3′ processing (132b). Apparently, in all of these precursors, the RNase P cleavage site is masked by secondary or tertiary structure. The action of RNases III and E is not essential for proper maturation of all tRNA's, because *rnc* strains are viable, and *rnc rne* mutants also accumulate many mature-size tRNA's (Fig. 9e). The role of endonucleases such as RNases III and E may be largely to facilitate RNase P action (75, 143, 147).

In the absence of RNases E and III, therefore, RNase P and RNase F cleavages can still produce the normal pattern of most mature-size tRNA's. In the absence of RNases P and III, RNase E and 3′ endonucleases produce small tRNA monomers and tRNA precursors. In the absence of RNases P and E, RNase III and 3′ endonuclease action can produce a more limited number of large tRNA monomers. Strains lacking RNases III, E, and P accumulate very few 4S- to 6S-size RNA species almost all of which contain purine nucleoside 5′ triphosphoryl termini and thus represent 5′-unprocessed primary transcripts (127b).

## Bacteriophage T4 tRNA

Infection of *E. coli* with bacteriophage T2, T4, T5, T6, BF23, or RB69 results in shutoff of host RNA synthesis and the appearance of new phage-encoded tRNA species (73, 112). Because processing of tRNA has been best studied in phage T4, we will describe that system in some detail. The T4 stable RNA transcription unit is about 3,000 nucleotides long (49, 50, 100a) and includes a proximal segment of about 1,000 bases, which apparently contains a gene coding for a protein. Next follows a cluster of seven tRNA genes coding for (5′ to 3′) Gln, Leu, Gly, Pro, Ser, Thr, and Ile tRNA's and another cluster containing $tRNA^{Arg}$ and two stable RNAs (species 2 [or B] and 1 [or A]) of unknown function. The two clusters are about 600 nucleotides apart (1, 49). The CCA terminus is encoded only in $tRNA^{Arg}$, $tRNA^{Leu}$, $tRNA^{Gly}$, and $tRNA^{Thr}$ (49, 66). Of the other tRNA's, $tRNA^{Gln}$ contains only the first C residue, and in the primary transcripts of $tRNA^{Pro}$, $tRNA^{Ser}$, and $tRNA^{Ile}$, the CCA position is occupied by the nucleotides CU, UAA, or UAU, respectively (49). Most of the late processing steps leading to the formation of mature tRNAs are understood, but details of the earliest stages are not known.

Initial processing of T4 tRNA's occurs during transcription, because large transcripts have been detected in vitro (34) but not in vivo, and cleavage products are seen very early after infection (66). The largest detectable products are mature Arg and Gly tRNA's, species 1 and 2 RNAs, and dimeric precursors containing Gln-Leu, Pro-Ser, and Thr-Ile tRNA's (66). A cleavage is made prior to $tRNA^{Gln}$ (49) by an endonuclease which seems to be RNase III, because mature $tRNA^{Gln}$ is not observed in an RNase $III^-$ host (101, 129; J. Olah and W. Sauerbier, personal communication) but is instead found as part of a larger transcript containing $tRNA^{Leu}$ (129, 129a). As Gln and Leu tRNA sequences are synthesized, an endonuclease cleaves at the 3′ terminus of $tRNA^{Leu}$, and RNase P cleaves at its 5′ terminus, releasing mature $tRNA^{Leu}$ and precursor $tRNA^{Gln}$. Very rarely, this RNase P cut is not made, and a dimeric Gln-Leu tRNA precursor can be detected in low amounts (66). Mature $tRNA^{Gly}$ is excised by RNase P and a 3′ endonuclease from the nascent transcript. Endonuclease cleavage following the terminus of $tRNA^{Ser}$ releases a dimeric Pro-Ser precursor, and, finally, RNase P and a 3′ endonuclease excise a Thr-Ile precursor and $tRNA^{Arg}$.

The Arg-species 2-species 1 cluster can be processed directly to monomers, because a CCA terminus is encoded in both $tRNA^{Arg}$ and species 2 (49, 100a). Endonuclease cleavage can probably occur before the 5′ end of Arg (49); RNase P and a 3′ endonuclease release Arg with one extra 3′ nucleotide which can be removed by RNase D. Another 3′ cut releases mature species 2 (plus one additional 3′ nucleotide); a third 3′ cleavage excises species 1 RNA as a precursor containing six extra nucleotides at its 5′ end (2). Species 1 RNA probably has a tRNA-like configuration (124). The 3′ end is converted to $CCA_{OH}$ in vivo, presumably by RNase D (or BN activity) and

nucleotidyltransferase. The mature 5′ terminus is apparently generated by RNase P cleavage (2).

The dimeric precursors Pro-Ser and Thr-Ile accumulate because $tRNA^{Ser}$ and $tRNA^{Ile}$ lack an encoded ..CCA.. sequence which is apparently a prerequisite for RNase P cleavage (150, 153). Therefore, the 3′ terminus must first be removed by an exonucleolytic activity (the BN enzyme) and the $CCA_{OH}$ terminus be added by nucleotidyltransferase before RNase P cleavage at the 5′ terminus of the distal tRNA moiety (Ser or Ile). The first RNase P cut in the Pro-Ser precursor thus releases mature $tRNA^{Ser}$ plus an immature $tRNA^{Pro}$ species with 5′ and 3′ extensions. RNase P-catalyzed maturation of $tRNA^{Pro}$ is again dependent upon terminal trimming and CCA addition (102, 150). Maturation of $tRNA^{Gln}$ requires only $CCA_{OH}$ addition and RNase P cleavage.

RNase P processing of the Thr-Ile precursor gives mature $tRNA^{Ile}$ plus a $tRNA^{Thr}$ from which an extra 3′ nucleotide must be trimmed. Moreover, cleavage between $tRNA^{Thr}$ and $tRNA^{Ile}$ can be accomplished by another endonuclease in the absence of RNase P (2). The identity of the 3′ endonuclease(s) whose products are detected in vivo has not been ascertained; its cleavages occur in an RNase $III^-$ host (Olah and Sauerbier, personal communication). This 3′ endonuclease(s) is unlikely to be encoded by T4 itself. (Normal T4 tRNA precursors are accumulated in a temperature-sensitive RNase P host infected with λ phage carrying a cloned DNA fragment containing the first seven T4 tRNA's [49]. Because this fragment contains only 5,160 bases of T4 DNA [50], the likelihood that processing nucleases are T4 encoded is very low.) This activity could be RNase F, which was isolated as an enzyme that cuts a precursor of species 1 at the 3′ end of the species 1 sequence (see above).

Analysis of T4 tRNA metabolism in RNase $III^+$ and RNase $III^-$ strains by using T4Δ27 (a mutant which contains an internal deletion in the tRNA cluster that leaves intact only the first two tRNA's, $tRNA^{Gln}$ and $tRNA^{Leu}$, and the last RNA, species 1) suggests that there is an enzyme which separates the messenger part of the transcript from the nonmessage. This unknown enzyme introduces a cleavage about 60 nucleotides upstream from the first tRNA in the cluster, $tRNA^{Gln}$, followed by an RNase III cleavage six nucleotides upstream from $tRNA^{Gln}$ (129). This is indicated by the accumulation of an RNA molecule, 10.5S, which is about 450 nucleotides long and containing $tRNA^{Gln}$, $tRNA^{Leu}$, and species 1; $tRNA^{Gln}$ is preceded by about 60 nucleotides. In an RNAse $III^+$ strain, a smaller molecule, 10.1S, can be observed which is missing these 60 nucleotides. The 10.1S RNA can be derived from 10.5S by RNAse III, and it contains six nucleotides prior to $tRNA^{Gln}$. RNase III is not required for the maturation of 10.1S RNA to the three final products, $tRNA^{Gln}$, $tRNA^{Leu}$, and species 1 RNA, whereas RNase III is required for maturation when the substrate is 10.5S RNA. In the absence of RNAse III, the production of $tRNA^{Gln}$ is greatly reduced.

These findings suggest that the RNA region which precedes the tRNA cluster has to be removed by RNase III before further processing can take place. In the absence of RNase III, another nonspecific nuclease can degrade this region, often cleaving within $tRNA^{Gln}$, which explains why $tRNA^{Gln}$ is underproduced in the absence of RNAse III. When wild-type T4 infects an RNase $III^-$ mutant of *E. coli*, only the production of $tRNA^{Gln}$ is affected (101); therefore, it seems that RNase III has only a single cleavage site in the T4 tRNA cluster.

Interestingly, as in the above-discussed case of processing of the host rRNA, the enzyme RNAse III seems to provide the first cleavage in the processing pathway, enabling other enzymes to mature smaller RNA substrates. In all cases studied thus far, RNase III is not introducing final maturation cleavages but is rather involved in an early processing step. Thus, it is perhaps useful to think of RNAse III as a coarse processing enzyme which is followed by fine-tuning enzymes which mold the final shape of the RNA molecule.

## Summary

Our present understanding of the function, efficiency, and order of RNase III, E, and P cleavages in rRNA processing may be used to assemble a coherent picture of the involvement of these enzymes in tRNA processing. Because most processing of *E. coli* tRNA probably occurs during transcription, the availability of processing sites is governed by the linearity of transcription as well as by the three-dimensional structure assumed by the newly synthesized sequences. The endonucleolytic cleavages of tRNA transcripts are performed primarily by the 5′ endonuclease RNase P, which generates the mature 5′ terminus, and by a 3′ endonuclease(s) which (alone or followed by the 3′ trimming activity of RNase D or a similar enzyme) exposes the mature 3′ terminus. This basic mode of tRNA processing is graphically visualized in Fig. 10A, which illustrates the nucleolytic processing of nascent transcripts from the *E. coli* $tRNA_1^{Tyr}$ doublet gene cluster (6, 45, 84). In this simple situation, only a 5′ endonuclease (RNase P), a 3′ endonuclease, and a 3′ exonuclease

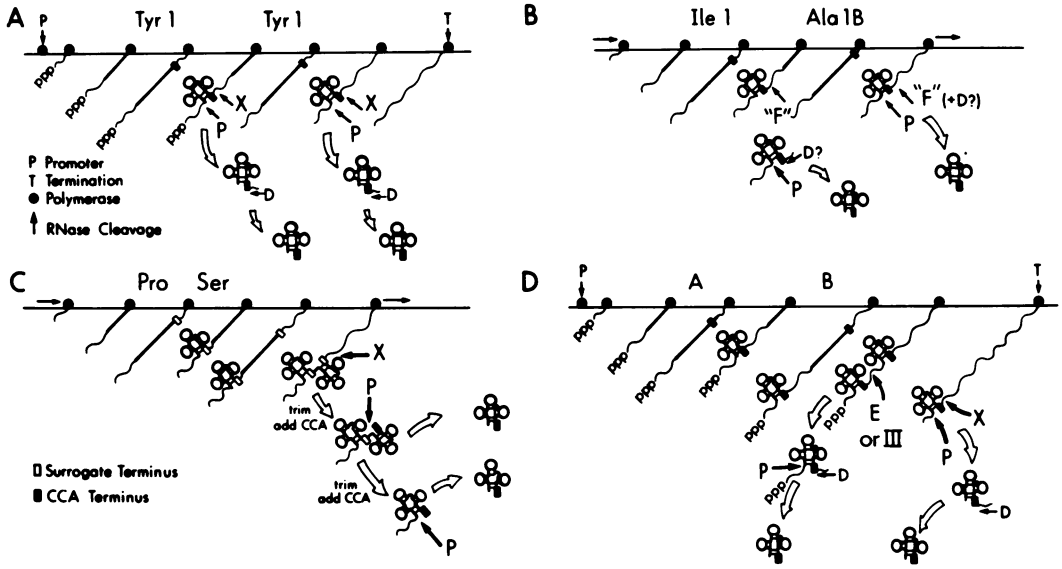



FIG. 10. *Processing of tRNA. Several different schema for tRNA processing are illustrated. Successively elongated nascent tRNA transcripts are shown. Thick straight lines represent tRNA sequences not in their mature conformation, and thin wavy lines are leader and spacer sequences. The first processing events occur during transcription but only after tRNA has been assumed its mature conformation, a process which is depicted graphically. Endonuclease cleavages by RNases P and E and 3' endonuclease activities (F and X) are shown, as well as 3' exonuclease trimming by RNase D. Details are discussed in the text. (A) Processing of the E. coli* $tRNA_1^{Tyr}$ *doublet transcript. (B) Processing of* $tRNA_1^{Ile}$ *and* $tRNA_{1B}^{Ala}$ *from the spacer region of the E. coli rRNA genes rrnA, rrnD, and rrnF. RNase F cleavage must precede RNase P cleavage of* $tRNA_1^{Ile}$*. The involvement of RNase D is conjectural. (C) Sequential processing of the* $tRNA^{Pro}$*-*$tRNA^{Ser}$ *dimeric transcript of phage T4. Prerequisites for RNase P action are 3' trimming by the BN activity and CCA addition. (D) Processing of a hypothetical dimeric tRNA transcript in E. coli. In this instance, RNase P action requires a prior 3' cleavage by RNase E or III. RNase X in (A), (C), and (D) could be RNase F.*

(RNase D) are required to produce mature-size tRNA's. The endonuclease cuts are not strictly ordered, but both precede RNase D trimming.

An example of strict ordering of endonucleolytic cleavage events is shown in Fig. 10B, which demonstrates processing of $tRNA_1^{Ile}$ and $tRNA_{1B}^{Ala}$ from the spacer region of rRNA transcripts (53, 55, 93, 178). In this transcript, endonuclease cleavage at the 3' side of the $tRNA_1^{Ile}$ is a prerequisite for cleavage at the 5' side by RNase P. Not ordered are 5' and 3' cleavages of $tRNA_{1B}^{Ala}$ (55, 93). A dependence of endonuclease cleavage upon post-transcriptional alteration of the primary sequence is exemplified by the T4 Pro-Ser precursor; Fig. 10C illustrates an ordering of RNase P 5' cleavages based on the presence or absence of the $CCA_{OH}$ 3' terminus (102, 107). Conversely, CCA addition to $tRNA^{Pro}$ requires prior RNase P cleavage of $tRNA^{Ser}$, and all these steps are dependent upon endonuclease scission at the 3' end of $tRNA^{Ser}$.

Other enzymes also play roles in tRNA processing. RNase III can participate in but is not absolutely required for production of mature tRNA's in vivo; likewise, the necessity for RNase E cleavage in wild-type cells has been demonstrated so far only for a very few tRNA precursors (132a, 132b). The cellular function of RNases III and E in tRNA biosynthesis is probably to convert large nascent multimeric tRNA transcripts into smaller substrates for which RNase P cleavage is more efficient.

A hypothetical example of such a mode of tRNA processing is given in Fig. 10D, which extends the simple situation depicted in Fig. 10A by including an obligatory RNase E-mediated cleavage between two tRNA sequences. In other transcripts, RNase III may cut between two tRNA's. Cleavage by RNase III, RNase E, or both may also be performed in the leader preceding the first tRNA sequence.

The sequence of the *E. coli supB-E* tRNA operon, recently determined by Ozeki and collaborators (116), contains seven tRNA genes in the order $Met_m$-$Leu$-$Gln_1$-$Gln_1$-$Met_m$-$Gln_2$-$Gln_2$. The gene sequences are separated by—and in one instance surrounded by—regions of secondary structure which may be substrates for RNase III, E, or F. In particular, the postulated RNase III core recognition sequence (Fig. 2) and A-U-

rich stems are both present. The largest polycistronic transcripts of this gene cluster detected in a temperature-sensitive RNase P cell in vivo have already been processed (in a region lacking secondary structure) into a proximal tetramer and a distal trimer. These transcripts are further processed, in the RNase P temperature-sensitive cell, to smaller precursors. The presence of endonuclease activities other than RNase P is thus again indicated.

It is noteworthy that temperature-sensitive RNase P single mutants accumulate some tRNA precursors which are in vitro substrates for other processing activities as well as for RNase P (75, 143, 147). This phenomenon is indicative of the order and cooperativity which exist between different phases of tRNA processing.

The conclusion, then, from analysis of host and phage tRNA metabolism in vivo and in vitro, is that cooperation exists among all tRNA processing events. Studies with processing mutants indicate that although RNases III and E assist in tRNA processing, many RNase P cleavages appear to be made in the absence of RNase III and RNase E activity. From the available data on tRNA processing, four conclusions can be drawn. First, most processing of tRNA occurs during transcription or shortly thereafter. Second, based on genetic evidence, RNase E performs a role in vivo compatible with that attributed to P2 activity, and O activity is identical to the secondary cleavage activity of RNase III (and is hence a dispensable function). Third, some transcripts may be cleaved near tRNA 3′ termini by RNase F or by yet another endonuclease(s). Fourth, RNase P performs the final 5′ maturation of tRNA molecules, and this cleavage cannot be performed by any other enzyme. All of these endonucleases act synergistically to process mature tRNA species from nascent tRNA gene transcripts.

## PROCESSING OF MESSENGER RNA

The first clear-cut evidence for processing of mRNA in procaryotes came from a phage rather than a host system. Subsequently, considerable attention was focused on the possibility of processing of host mRNA's. At least one host mRNA appears to be cleaved in vivo, and expression of many genes is altered in cells defective in one or more processing enzymes. So far, however, no host or phage mRNA has been described whose function is absolutely dependent upon a processing event. Additionally, mutations in the enzyme RNase III, E, or P do not seem to affect the stability of host mRNA (12).

### Host Messenger RNAs

Direct evidence has been presented for processing of one host mRNA, the transcript of the *E. coli rplJL-rpoBC* operon, which encodes ribosomal proteins L10 and L7/12 and RNA polymerase subunits $\beta$ and $\beta'$. Total cellular RNA was examined for its ability to hybridize with and protect from nuclease S1 cleavage a restriction endonuclease fragment of DNA spanning the *rplL-rpoB* intercistronic region. RNA from wild-type cells allows the probe to be cut into two pieces, whereas RNA from *rnc-105* cells completely protects the probe (23). In *rnc-105* cells, therefore, mRNA from this intercistronic region is intact, but in wild-type cells, it is cleaved in a region about 200 bases beyond the 3′ end of the *rplL* message (23). The sequences in this region can be depicted in a stem-and-loop structure (23, 128) similar to the p23 rRNA stem and containing a short conserved core sequence which may be involved in RNase III recognition (Fig. 2). Actual RNase III cleavage of one or both strands in this region, however, remains to be demonstrated in vitro. Again, because the *rnc-105* strain is viable, processing in this region is not required for the qualitative expression of either proximal or distal mRNA's. Processing may, however, play a role in the complex quantitative regulation of this operon (23, 36).

Studies with isogenic $rnc^+/rnc^-$ strains have failed to detect a requirement for RNase III in *lac* gene expression at 30°C (19). The RNase $III^-$ strain AB301-105 (78) appears to be defective in initiation of translation of *lac* mRNA (171), but the involvement of RNase III cannot be evaluated because this mutant contains a number of mutations in addition to the RNase III lesion (17, 18).

Most recently, however, both RNase III and RNase E have been found to be required at 43°C for full expression not only of the *lac* operon but also of a significant number of other proteins in *E. coli* (63). The differential induction of the *lacZ* gene as well as the amount of $\beta$-galactosidase protein is reduced in *rnc-105* or *rne-3071* strains at 43°C, compared with 30°C. In the double *rnc rne* mutant, this reduction is even more pronounced. Moreover, analysis of total cell protein separated in two-dimensional gels reveals that synthesis of 21 of 80 individual proteins examined is markedly decreased in the *rnc rne rnp* strain at 43°C and that this difference is caused mainly by the *rnc* and *rne* mutations (63).

Another interesting and at present unexplained effect of the *rnc-105* mutation is that *rnc-105* strains are nonmotile even at 30°C be-

cause they are defective in the production of flagella (20). Whether this results from a failure to process a specific mRNA or simply from the known sensitivity of flagellar production to a variety of physiological perturbations has not been determined. These results, although not demonstrating mRNA processing, do open up such a possibility.

Although these intriguing observations could be due to processing of mRNA, other possibilities, such as effects on synthesis, stability, or utilization of mRNA, were not ruled out.

### Bacteriophage T7 Messenger RNAs

The most thoroughly studied example of mRNA processing in procaryotes is that of bacteriophage T7. The early region of T7, from 0 to about 7,500 base pairs, which is transcribed by the host RNA polymerase, contains a transcribed leader region ("*I*") and genes designated *0.3*, *0.7*, *1.0*, *1.1*, and *1.3*. In wild-type cells, the earliest detectable transcription products are monocistronic mRNA's (41, 167, 169). Using purified RNA polymerase and T7 DNA in vitro or after T7 infection of an RNase III$^-$ host, the early region is transcribed into a single 7,500-base-long polycistronic transcript. When a "sizing factor" was included in the in vitro transcription system, only the wild-type-size mRNA's were detected (41). Subsequently, it was found that posttranscriptional treatment of in vitro or in vivo polycistronic transcripts with purified RNase III produces monomer-sized mRNA's (41, 42) and that the sizing factor is identical to RNase III (137). Comparison of the terminal sequences of T7 early mRNA's produced in vivo with those of the RNAs generated by in vitro RNase III cleavage of the polycistronic transcript demonstrated that T7 mRNA's are the direct products of RNase III cleavage in vivo (83, 141) see (Fig. 2). Some of the mRNA's transcribed from the late region of the T7 genome are also the products of RNase III cleavage (43). RNase III processing of T3 mRNA's has also been suggested (69, 96).

The rationale for production of monomeric T7 mRNA's is not fully understood. Although the efficiency of plating of phage T7 and its burst size are reduced about threefold in an *rnc-105* host, as compared with an isogenic *rnc*$^+$ host (18), the uncleaved polycistronic transcript is translated in vitro with roughly the same efficiency as cleaved mRNA's (44). One difference has been noted, however: in the absence of prior RNase III cleavage, the gene *0.3* message is poorly translated in vivo or in vitro (44). This may be because sequences surrounding the *0.3* mRNA initiation codon can base pair with complementary sequences in the preceding leader RNA (169), thus hindering translation of the *0.3* message. RNase III cleavage in vivo or in vitro between the leader and gene *0.3* mRNA permits normal translation efficiency of the gene *0.3* mRNA (44, 169). Although this phenomenon does not explain why most other T7 early mRNA's are monocistronic, the presence of intercistronic RNase III cleavage sites is unlikely to be fortuitous, in light of the exceptional structural similarity among them (118, 136, 140, 169; J. J. Dunn, personal communication). Primary cleavages occur in vivo in the 3′ half of the hairpins, within single-stranded bubbles (118, 140; Dunn, personal communication) (Fig. 2). At low salt concentrations in vitro, a second staggered cleavage is made in the 5′ half of the intercistronic hairpin between genes *1.1* and *1.3* (Fig. 2B). The seeming disparity between cleavage of rRNA and synthetic double-stranded RNA on the one hand and cleavage of T7 mRNA on the other led Robertson and Barany (135) to propose that T7 cleavage bubbles present no interruption in the surrounding helical region because they can be folded in such a way as to simulate a helix. Bacteriophage T7 processing sites were thus proposed to result from a process of evolutionary mimicry of host RNA processing sites (135).

### Bacteriophage λ Leftward Transcript

Leftward transcription of induced prophage λ starts at the $p_L$ promoter for the N gene and, in the presence of N-gene product, reads through the $t_L$ termination signal and continues to the *att* region (81, 82, 90, 170). Processing of the leftward transcript was first indicated by the observation that the 36S $p_L$-promoted transcript isolated after infection of wild-type hosts did not contain mRNA sequences corresponding to the N gene (81). Because RNase III was known to process T7 mRNA, and because λ, like T7, plated with lower efficiency on an RNase III$^-$ host (17), the possible role of RNase III in λ RNA metabolism was investigated. Transcriptional mapping experiments were performed in which the 36S λ message was hybridized to DNA from a series of deletion mutants. The results demonstrated that in an RNase III$^+$ host, the apparent start point for the leftward transcript ($l_3$ RNA) is in the region immediately following the N gene, whereas in an RNase III$^-$ host, the leftward transcript ($l'_3$ RNA) contains sequences hybridizing to the N gene and defining an origin at the $p_L$ promoter (89, 174). These experiments demonstrated that RNase III cleavage in vivo releases N-gene message from the rest of the leftward transcript. (The nucleotide sequences

at this RNase III site have not been determined.)

Further cleavages at the 5′ end of the $p_L$-promoted N-gene transcript release two 4S to 5S leader RNAs called $l_1^\circ$ and $l_1$ (90). The rest of the N-gene transcript prior to $t_L$ is rapidly degraded and cannot be detected in wild-type cells (89, 174); in RNase III$^-$ hosts, the entire N-gene message ($p_L$ to $t_L$) decays at the same slower rate as the total 36S leftward transcript to which it is attached (174). The sequence of the 5′-terminal portion of the $p_L$ transcript has been determined (90). The region cleaved in vivo can assume a stem-and-loop structure quite similar to that found between the T7 gene *1.1* and gene *1.3* mRNA's (Fig. 2B). Cleavage in vivo, possibly by RNase III, gives $l_1^\circ$ RNA, a 17-base-long hairpin fragment, and $l_1$ RNA (90) in much the same fashion as the T7 gene *1.1–1.3* intercistronic region is cleaved in vitro to *1.1* mRNA, F5 fragment, and *1.3* mRNA's (Fig. 2B).

Because functional N protein is produced in RNase III$^-$ hosts (i.e., N-dependent antitermination at $t_L$ occurs), N-gene message is translatable whether or not it is attached to the remainder of the leftward transcript. RNase III cleavage does, however, result in increased lability of N message. Rapid turnover of N-gene mRNA is one factor which, coupled with lability of the N protein, could control λ gene expression by making leftward transcription dependent upon continued transcription of the N gene.

Recently, it became evident that RNase III participates also in the synthesis of the λ *int* protein, a protein required for the integration and excision of λ DNA. When *int* message is transcribed from the $p_L$ promoter, it continues beyond the 3′ end of the *int* gene, and a site is created for RNase III cleavage. After the cut by RNase III, degradation of the *int* message follows in the 3′-to-5′ direction, which is perhaps analogous to what happens to the N-protein message in the presence of RNAse III. However, in this case, no *int* protein is detected. The *int* protein can be synthesized when transcription starts from the $p_I$ promoter. Because of the rearrangement of the λ DNA between the free and lysogenic stages, the *int* gene in the prophage state is followed by host DNA, and therefore the transcribed message is not subjected to this type of degradation, which is initiated by RNase III. This mode of gene regulation is referred to as retroregulation (D. Schindler and H. Echols; D. Court, T. F. Huang, and A. Oppenheim; U. Schmeissner, K. McKenney, D. Court, and M. Rosenberg, personal communications).

RNase III also plays a role in late λ transcription, because it seems to introduce a cleavage beyond the 6S RNA, which is extended by the antitermination action of the Q gene product (D. L. Daniels, H. Lozeron, and F. R. Blattner, personal communication). The situation here seems to be rather similar to that described for the λ transcript which originates from the $p_L$ promoter (see above).

### Processing and Attenuation

In two mRNA transcripts which are subject to regulated rho-mediated termination of transcription between two cistrons, the site of attenuation is located near a region for RNase III cleavage (23, 82, 89). A proposed RNase III cleavage site lies just downstream of an attenuator in the *rplL-rpoB* intercistronic region of the *E. coli rplJL-rpoBC* operon mRNA (23), and the site of RNase III cleavage following N-gene message in the major leftward transcript of bacteriophage λ is in the region where rho protein causes transcription termination in the absence of functional N protein (81, 89). RNA sequences in the vicinity of termination or attenuation sites can form multiple stem-and-loop structures (3, 119), but, at least at the *rplL-rpoB* attenuator, these structures are probably not themselves cleaved by RNase III (23).

It is noteworthy that these termination signals near RNase III processing regions both occur in intercistronic spacer regions of an mRNA transcript rather than in a 5′ leader region. The mechanism of attenuation in leader RNAs (119) could be somewhat different from that in spacer regions. RNase III cleavage might be a general phenomenon which occurs near attenuation signals in intercistronic spacer regions. Termination or attenuation followed by endonucleolytic cleavage in an intercistronic spacer region may be important in the maintenance of complex transcription and posttranscriptional regulatory patterns. It could, for instance, ensure that the size of the messages produced from this region of the genome will remain the same whether or not termination takes place.

Genetic analysis of *rnc rho* double mutants (14) indicated that the presence or absence of RNase III does not influence suppression by *rho* mutants of a polar mutation in the *lacZ* gene. Moreover, no obvious alterations in the RNA molecules were observed in the double mutant (14). These observations suggest that *rho* and RNase III do not recognize the same sequences.

## GENERAL CONSIDERATIONS

### Unity of RNA Processing Mechanisms

The procaryotic cell, as we have seen, employs a very limited number of nucleolytic activities to accomplish the primary processing of a variety

of RNA transcripts and a comparable number of activities for the secondary maturation steps. Indeed, each of the three known primary endoribonucleases of *E. coli*—RNases III, E, and P—plays a role in the processing of both rRNA and tRNA transcripts. Intracellular parasites, such as the DNA bacteriophages, have evolved to the point that their RNA transcripts can be processed by host systems. Processing of tRNA's and other stable RNAs in T-even and related phages, as well as processing of mRNA's in T3, T7, and λ, is performed by host enzymes.

## Function of Polycistronic Transcription and Processing

As Pace originally suggested (121), one explanation of precursor-specific sequences in monomeric rRNA precursors is that they are remnants of the recognition sites for the enzymes which generate those precursors. This consideration could be true for tRNA precursors as well. The larger question, however, is the physiological necessity or utility of polycistronic transcription of stable RNAs.

Four primary advantages for polycistronic transcription of stable RNAs can be considered. First, production of equimolar amounts of functionally interdependent molecules, such as rRNA's, is achieved. Second, cotranscription of rRNA's allows coordinated and orderly sequential assembly of the complex ribonucleoprotein structure of the ribosomal subunits. Third, the termini of RNA species are protected during transcription and until the molecule is functional. As an example, endonucleolytic cleavage of rRNA transcripts releases p16 and p23 rRNA precursors (33, 62) whose 5′ and 3′ termini are paired in a duplex stem which is resistant to degradative cellular nucleases (54, 138). Because the 3′-terminal portion of m16 RNA, for example, must carry out a crucial function in translation, protection of p16 from exonuclease attack is beneficial until the m16 portion plus ribosomal proteins can assume the mature, nuclease-resistant (175) conformation of the ribosome, at which time a specific maturation endonuclease(s) can safely expose the functional ends. Likewise, precursor tRNA's may be protected from nonspecific degradation—in particular, irreparable excessive removal of 3′-terminal sequences—by being present in a long (nascent) transcript until the mature portion of the tRNA has assumed its correct (RNase-resistant) configuration, which is recognized by the processing enzymes as well as by (for example) aminoacylating enzymes. Fourth, processing of RNA molecules provides the possibility for regulation of stable RNA gene expression at the posttranscriptional level by a physiologically controlled balance between productive processing and decay. Processing versus degradation of tRNA precursors has been described and discussed elsewhere (6, 7, 66, 159).

One potentially exciting role for precursor-specific sequences could be to actively regulate RNA conformational folding (164). Study of *B. subtilis* p5 rRNA by Pace and co-workers has shown that the first six bases of the precursor are hydrogen bonded to an immediately adjacent precursor-specific sequence. These latter sequences would otherwise base pair with an internal region of the m5 portion of the precursor, resulting in an abnormal conformation for the RNA (164). This fact in itself, however, does not explain why any precursor-specific sequences should interact with mature sequences. Because isolated m5 rRNA can assume an apparently normal conformation without the aid of precursor-specific sequences, the precise involvement of precursor regions in the regulation of conformational folding remains to be clarified. Further work, perhaps with mutants affecting RNA structure, will be required to elucidate the roles of processing and precursor-specific sequences. Similar studies of mutations in mature tRNA sequences have yielded important information on tRNA structure and processing (reviewed in references 6, 7, 66, 100, 158, and 159).

## Specificity and Efficiency of RNA Processing Reactions

What specific structural elements are recognized by RNA-processing enzymes? Since the role of processing events is ultimately to produce mature functional RNA molecules, we would expect that recognition of the mature RNA portion or domain of the precursor transcript is required. The mature domain contains information indicative not only of the generic species of RNA but also of its readiness to be productively processed, as determined by revealing whether or not it has assumed its final and functional configuration. RNase P and *B. subtilis* RNase M5 have been shown to recognize predominantly the mature domain of their respective substrates (6, 107). A similar recognition mechanism can be predicted for *E. coli* RNase E. Likewise, maturation of p16 rRNA precursor by RNase(s) M16 appears to require a properly assembled rRNA-protein complex (33, 47, 104). Because RNase III cleavage of rRNA transcripts does not generate functional termini, it need not recognize the mature domain, but because it generates base-paired precursor termini, processing by RNase III requires an intact duplex structure.

At present, however, little can be said about such structure-function relationships in processing of phage or host mRNA's. The rationale of processing may well be regulatory, but much more remains to be learned. It is attractive, nonetheless, to imagine that processing enzymes recognize and cleave a particular configuration or that one of several alternative configurations adopted in a region of an mRNA molecule is involved in regulation of its own translation or transcription.

All of the processing endonucleases known thus far, RNases III, E, and P and *B. subtilis* RNase M5, recognize their substrates only after the 5′ and 3′ termini are base paired. In addition, RNase P, which removes tRNA 5′ precursor-specific sequences, requires also the presence of the 3′-terminal CCA sequence of the tRNA. The double-stranded nature of these processing substrates may serve the purpose of ensuring that endonuclease cleavages which could otherwise occur during transcription do not take place until transcription of the entire substrate is completed and proper RNA folding has occurred. This also applies to some extent to the yeast enzyme which removes intervening sequences from precursor tRNA molecules. In this case, the substrate for this enzyme contains the aminoacyl stem which is comprised from nucleotides upstream and downstream in relation to the nucleotides which are excised (1).

From these considerations, we anticipate that cleavages which produce mature termini or which are required for their stability during processing cannot be bypassed, whereas other cleavages can be bypassed or performed at slightly different locations by other enzymes. All available experimental evidence supports this view: for example, inactivation of RNase P is a lethal event, because mature tRNA 5′ termini are not formed. Inactivation of RNase III is not lethal, however, since nascent rRNA transcripts are cleaved by the remaining enzymes (RNases E, F, and P), and p16b and p23b rRNA precursors are formed which can be processed successfully to mature m16 and m23 rRNA's. Because inactivation of RNase III does, however, drastically reduce the efficiency of the remaining RNA processing steps, a second point becomes clear: cleavages which do not directly yield mature termini and those which convert polycistronic transcripts to monocistronic precursors act in concert with other cleavages to enhance the overall efficiency of an RNA processing pathway. The order of such cleavages is largely determined by the order in which their substrates become available, during transcription, in a conformation recognized by the appropriate enzyme.

Another point that should be mentioned is that the transcription machinery of the bacterial cell does not seem to require feedback signals from processed RNAs because transcription of precursors for rRNA's and tRNA's continues unabated in the absence of processing by RNases III, P, and E (Fig. 6) (11, 127b).

Bearing these considerations in mind, then, we can offer a general principle governing enzymatic recognition and processing of RNA transcripts. A relatively limited number of processing enzymes, principally endonucleases, with stringent but not sequence-specific substrate specificities, acting on a modest repertoire of variations on a few fundamentally distinctive processing signals, may be capable of carrying out a broad range of distinct, highly specific, and potentially well-ordered processing steps. By their concerted, synergistic action, these enzymes accomplish with utmost efficiency the processing of RNA transcripts.

## FINAL POINTS

**(i)** All "stable" RNAs and some messages in *E. coli* require processing.

**(ii)** Transcription of RNA is independent of its processing.

**(iii)** Most of the processing of rRNA and tRNA occurs during transcription.

**(iv)** Most stable RNA transcripts are processed by more than a single endoribonuclease.

**(v)** The enzymes responsible for most processing of stable RNA transcripts in *E. coli* are four endoribonucleases: RNases III, E, F, and P, and at least one exonuclease, RNase D.

**(vi)** The efficiency but not the specificity of processing cuts is affected by the size of the substrate; multiple cleavages facilitate efficient and accurate processing, but not all steps are essential.

**(vii)** Some processing steps are completely dependent on the occurrence of an earlier step.

**(viii)** All procaryotic processing endoribonucleases studied thus far create polynucleotide chains with 5′ phosphoryl and 3′ hydroxyl termini.

**(ix)** A given RNase, by and large, fulfills either a degradative or a processing function.

**(x)** Processing endonucleases are highly specific, and each performs a unique function. Their recognition sites may be composed of unique combinations of secondary and tertiary structures.

**(xi)** In some processing events, the substrate is not naked RNA but RNA in ribonucleoprotein particles.

**(xii)** The order of processing steps is governed primarily by the order in which substrate recognition signals become available. A certain

amount of flexibility exists in the order of initial processing events, but the final steps, which generate the mature RNA termini, must be preserved. These final cleavages are performed by enzymes which recognize the mature domain of the substrate in its final conformation.

## ACKNOWLEDGMENTS

We thank those colleagues who communicated their results before publication.

Research performed in our laboratory and described here was supported by grants GM-25890, GM-19821, and CA-24727 from the National Institutes of Health.

## LITERATURE CITED

1. **Abelson, J.** 1979. RNA processing and the intervening sequence problem. Annu. Rev. Biochem. **48**:1035–1069.
2. **Abelson, J., K. Fukada, P. Johnson, H. Lamfrom, D. P. Nierlich, A. Otsuka, G. V. Paddock, T. C. Pinkerton, A. Sarabhai, S. Stahl, J. H. Wilson, and H. Yesian.** 1975. Bacteriophage T4 tRNAs: structure, genetics, and biosynthesis. Brookhaven Symp. Biol. **26**: 77–88.
3. **Adhya, S., and M. Gottesman.** 1978. Control of transcription. Annu. Rev. Biochem. **47**:967–996.
4. **Altman, S.** 1971. Isolation of tyrosine tRNA precursor molecules. Nature (London) New Biol. **229**:19–21.
5. **Altman, S.** 1975. Biosynthesis of transfer RNA in *Escherichia coli*. Cell **4**:21–29.
6. **Altman, S.** 1978. Transfer RNA biosynthesis. Int. Rev. Biochem. **17**:19–44.
7. **Altman, S., A. L. M. Bothwell, and B. C. Stark.** 1975. Processing of *E. coli* $tRNA^{Tyr}$ precursor *in vitro*. Brookhaven Symp. Biol. **26**: 12–25.
8. **Altman, S., and J. D. Smith.** 1971. Tyrosine tRNA precursor molecule polynucleotide sequence. Nature (London) New Biol. **233**:35–39.
9. **Apirion, D.** 1978. Isolation, genetic mapping, and some characterization of a mutation in *Escherichia coli* that affects the processing of ribonucleic acid. Genetics **90**:659–671.
10. **Apirion, D.** 1980. Genetic mapping and some characterization of the *rnpA49* mutation of *Escherichia coli* that affects the RNA processing enzyme ribonuclease P. Genetics **94**:291–299.
11. **Apirion, D., B. K. Ghora, G. Plautz, T. K. Misra, and P. Gegenheimer.** 1980. Processing of rRNA and tRNA in *Escherichia coli*: cooperation between processing enzymes, p. 139–154. *In* D. Söll, J. Abelson, and P. Schimmel (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Apirion, D., and D. Gitelman.** 1980. Decay of RNA in RNA processing mutants of *E. coli*. Mol. Gen. Genet. **177**:139–154.
13. **Apirion, D., and A. B. Lassar.** 1978. A conditional lethal mutant of *Escherichia coli* which affects the processing of ribosomal RNA. J. Biol. Chem. **253**:1738–1742.
14. **Apirion, D., J. Neil, T. S. Ko, and N. Watson.** 1978. Metabolism of ribosomal RNA in mutants of *Escherichia coli* doubly defective in ribonuclease III and the transcription termination factor rho. Genetics **90**:19–35.
15. **Apirion, D., J. Neil, and N. Watson.** 1976. Consequences of losing ribonuclease III on the *Escherichia coli* cell. Mol. Gen. Genet. **144**: 185–190.
16. **Apirion, D., J. Neil, and N. Watson.** 1976. Revertants from RNase III negative strains of *Escherichia coli*. Mol. Gen. Genet. **149**:201–210.
17. **Apirion, D., and N. Watson.** 1974. Analysis of an *Escherichia coli* strain carrying physiologically compensating mutations one of which causes an altered ribonuclease III. Mol. Gen. Genet. **132**:89–104.
18. **Apirion, D., and N. Watson.** 1975. Mapping and characterization of a mutation in *Escherichia coli* that reduces the level of ribonuclease III specific for double-stranded ribonucleic acid. J. Bacteriol. **124**:317–324.
19. **Apirion, D., and N. Watson.** 1975. Unaltered stability of newly-synthesized RNA in strains of *Escherichia coli* missing a ribonuclease specific for double-stranded RNA. Mol. Gen. Genet. **136**:317–326.
20. **Apirion, D., and N. Watson.** 1978. Ribonuclease III is involved in motility of *Escherichia coli*. J. Bacteriol. **133**:1543–1545.
21. **Apirion, D., and N. Watson.** 1980. A second gene which affects the RNA processing enzyme ribonuclease P of *Escherichia coli*. FEBS Lett. **110**:161–163.
22. **Bailey, S. C., and D. Apirion.** 1979. Repetitive DNA in *Escherichia coli*: multiple sequences complementary to small stable RNAs. Mol. Gen. Genet. **172**:339–343.
23. **Barry, G., C. Squires, and C. L. Squires.** 1980. Attenuation and processing of RNA from the *rplJL-rpoBC* transcription unit of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:3331–3335.
24. **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.
25. **Bothwell, A. L. M., R. L. Garber, and S. Altman.** 1976. Nucleotide sequence and *in vitro* processing of a precursor molecule to *Escherichia coli* 4.5S RNA. J. Biol. Chem. **251**: 7709–7716.
26. **Bothwell, A. L. M., B. C. Stark, and S. Altman.** 1976. Ribonuclease P substrate specificity: cleavage of a bacteriophage $\phi$80-induced RNA. Proc. Natl. Acad. Sci. U.S.A. **73**:1912–1916.
27. **Bram, R. J., R. A. Young, and J. A. Steitz.** 1980. The ribonuclease III site flanking 23S sequences in the 30S ribosomal precursor RNA of *E. coli*. Cell **19**:393–401.

28. **Branlant, C., J. Sri Widada, A. Krol, and J.-P. Ebel.** 1976. Extensions of the known sequences at the 3′ and 5′ ends of 23S ribosomal RNA from *Escherichia coli*, possible base pairing between these 23S RNA regions and 16S ribosomal RNA. Nucleic Acids Res. **3**:1671–1687.

29. **Brosius, J., T. J. Dull, and H. F. Noller.** 1980. Complete sequence of a 23S ribosomal RNA gene from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:201–204.

29a. **Brosius, J., T. J. Dull, D. D. Sleeter, and H. F. Noller.** 1981. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. J. Mol. Biol. **148**:107–127.

30. **Cowgill de Narvaez, C., and H. W. Schaup.** 1979. *In vivo* transcriptionally coupled assembly of *Escherichia coli* ribosomal subunits. J. Mol. Biol. **134**:1–22.

31. **Crouch, R. J.** 1974. Ribonuclease III does not degrade deoxyribonucleic acid-ribonucleic acid hybrids. J. Biol. Chem. **249**:1314–1316.

32. **Cudny, H., and M. P. Deutscher.** 1980. Apparent involvement of ribonuclease D in the 3′ processing of tRNA precursors. Proc. Natl. Acad. Sci. U.S.A. **77**:837–841.

32a. **Cudny, H., P. Roz, and M. P. Deutscher.** 1981. Alteration of *Escherichia coli* RNase D by infection with bacteriophage T4. Biochem. Biophys. Res. Commun. **98**:337–345.

33. **Dahlberg, A. E., J. E. Dahlberg, E. Lund, H. Tokimatsu, A. B. Rabson, P. C. Calvert, F. Reynolds, and M. Zahalak.** 1978. Processing of the 5′ end of *Escherichia coli* 16S ribosomal RNA. Proc. Natl. Acad. Sci. U.S.A. **75**:3598–3602.

34. **Daniel, V.** 1981. Biosynthesis of transfer RNA. Crit. Rev. Biochem. **9**:253–292.

35. **de Boer, H., and M. Nomura.** 1979. *In vivo* transcription of rRNA operons in *Escherichia coli* initiates with purine nucleoside triphosphates at the first promoter and with CTP at the second promoter. J. Biol. Chem. **254**:5609–5612.

36. **Dennis, P. P., and N. P. Fiil.** 1979. Transcriptional and post-transcriptional control of RNA polymerase and ribosomal protein genes cloned on composite colE1 plasmids in the bacterium *Escherichia coli*. J. Biol. Chem. **254**:7540–7547.

37. **Deutscher, M. P., and R. K. Ghosh.** 1978. Preparation of synthetic tRNA precursors with tRNA nucleotidyl transferase. Nucleic Acids Res. **5**:3821–3829.

38. **Deutscher, M. P., and R. H. Hilderman.** 1974. Isolation and partial characterization of *Escherichia coli* mutants with low levels of transfer ribonucleic acid nucleotidyl transferase. J. Bacteriol. **118**:621–627.

39. **Dougan, A. H., and D. A. Glaser.** 1974. Rate of chain elongation of ribosomal RNA molecules in *Escherichia coli*. J. Mol. Biol. **87**:775–795.

40. **Dunn, J. J.** 1976. RNase III cleavage of single-stranded RNA. Effect of ionic strength on the fidelity of cleavage. J. Biol. Chem. **251**:3807–3814.

41. **Dunn, J. J., and F. W. Studier.** 1973. T7 early RNAs are generated by site-specific cleavages. Proc. Natl. Acad. Sci. U.S.A. **70**:1559–1563.

42. **Dunn, J. J., and F. W. Studier.** 1973. T7 early RNAs and *Escherichia coli* ribosomal RNAs are cut from large precursor RNAs *in vivo* by ribonuclease III. Proc. Natl. Acad. Sci. U.S.A. **70**:3296–3300.

43. **Dunn, J. J., and F. W. Studier.** 1975. Processing, transcription, and translation of bacteriophage T7 messenger RNAs. Brookhaven Symp. Biol. **26**:267–276.

44. **Dunn, J. J., and F. W. Studier.** 1975. Effect of RNase III cleavage on translation of bacteriophage T7 messenger RNAs. J. Mol. Biol. **99**:487–499.

45. **Eagan, J., and A. Landy.** 1978. Structural analysis of the $tRNA_1^{Tyr}$ gene of *Escherichia coli*. A 178 base pair sequence that is repeated 3.14 times. J. Biol. Chem. **253**:3607–3622.

46. **Feunteun, J., B. R. Jordan, and R. Monier.** 1972. Study of the maturation of 5S RNA precursors in *Escherichia coli*. J. Mol. Biol. **70**:465–474.

47. **Feunteun, J., R. Rosset, C. Ehresmann, P. Stiegler, and P. Fellner.** 1974. Abnormal maturation of precursor 16S RNA in a ribosomal assembly defective mutant of *E. coli*. Nucleic Acids Res. **1**:141–147.

47a. **Foulds, J., R. H. Hilderman, and M. P. Deutscher.** 1974. Mapping of the locus for *Escherichia coli* transfer ribonucleic acid nucleotidyl transferase. J. Bacteriol. **118**:628–632.

48. **Fox, G. E., and C. R. Woese.** 1975. 5S RNA secondary structure. Nature (London) **256**:505–507.

49. **Fukada, K., and J. Abelson.** 1980. DNA sequence of a T4 transfer RNA gene cluster. J. Mol. Biol. **139**:377–391.

50. **Fukada, K., A. Otsuka, and J. Abelson.** 1980. A restriction map of the T4 transfer RNA gene cluster. J. Mol. Biol. **137**:191–211.

51. **Gardiner, K., and N. R. Pace.** 1980. RNase P of *Bacillus subtilis* has a RNA component. J. Biol. Chem. **255**:7507–7509.

52. **Gegenheimer, P., and D. Apirion.** 1975. *Escherichia coli* ribosomal ribonucleic acids are not cut from an intact precursor molecule. J. Biol. Chem. **250**:2407–2409.

53. **Gegenheimer, P., and D. Apirion.** 1978. Processing of rRNA by RNAase P: spacer tRNAs are linked to 16S rRNA in an RNAase P RNAase III mutant strain of *Escherichia coli*. Cell **15**:527–539.

54. **Gegenheimer, P., and D. Apirion.** 1980. Precursors to 16S and 23S ribosomal RNA from a ribonuclease $III^-$ strain of *Escherichia coli* contain intact RNase III processing sites. Nucleic Acids Res. **8**:1873–1891.

55. **Gegenheimer, P., and D. Apirion.** 1980. Struc-

tural characterization and *in vitro* processing of *Escherichia coli* ribosomal RNA transcripts containing 5′ triphosphates, leader sequences, 16S-RNA and spacer tRNAs. J. Mol. Biol. **143**: 227–257.

56. **Gegenheimer, P., N. Watson, and D. Apirion.** 1976. Processing of ribosomal RNA in *E. coli*, p. 405–410. *In* D. P. Nierlich, W. J. Rutter, and C. F. Fox (ed.), ICN/UCLA Symposium on Molecular Mechanisms in the Control of Gene Expression. Academic Press, Inc., New York.
57. **Gegenheimer, P., N. Watson, and D. Apirion.** 1977. Multiple pathways for primary processing of ribosomal RNA in *Escherichia coli*. J. Biol. Chem. **252**:3064–3073.
58. **Ghora, B. K., and D. Apirion.** 1978. Structural analysis and *in vitro* processing to p5 rRNA of a 9S RNA molecule isolated from an *rne* mutant of *E. coli*. Cell **1015**:1055–1066.
59. **Ghora, B. K., and D. Apirion.** 1979. Identification of a novel RNA molecule in a new RNA processing mutant of *Escherichia coli* which contains 5S rRNA sequences. J. Biol. Chem. **254**:1951–1956.
60. **Ghora, B. K., and D. Apirion.** 1979. 5S ribosomal RNA is contained within a 25S ribosomal RNA that accumulates in mutants of *Escherichia coli* defective in processing of ribosomal RNA. J. Mol. Biol. **127**:507–513.
61. **Ghosh, R. K., and M. P. Deutscher.** 1978. Purification of potential 3′ processing nucleases using synthetic tRNA precursors. Nucleic Acids Res. **5**:3831–3842.
62. **Ginsburg, D., and J. A. Steitz.** 1975. The 30S ribosomal precursor RNA from *Escherichia coli*. A primary transcript containing 23S, 16S, and 5S sequences. J. Biol. Chem. **250**:5647–5654.
63. **Gitelman, D. R., and D. Apirion.** 1980. The synthesis of some proteins is affected in RNA processing mutants of *Escherichia coli*. Biochem. Biophys. Res. Commun. **96**:1063–1070.

63a. **Goldblum, K., and D. Apirion.** 1981. Inactivation of the RNA processing enzyme ribonuclease E blocks cell division. J. Bacteriol. **146**: 128–132.

64. **Griffin, B. E., and D. L. Baillie.** 1973. Precursors of stable RNA accumulated in a mutant of *E. coli*. FEBS Lett. **34**:273–279.
65. **Guthrie, C., and R. Atchison.** 1980. Biochemical characterization of RNAse P: a tRNA processing activity with protein and RNA components, p. 83–97. *In* D. Söll, J. Abelson, and P. Schimmel (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
66. **Guthrie, C., J. G. Seidman, M. M. Comer, R. M. Bock, F. J. Schmidt, B. G. Barrell, and W. H. McClain.** 1975. The biology of bacteriophage T4 transfer RNAs. Brookhaven Symp. Biol. **26**:106–123.
67. **Hayes, F., and M. Vasseur.** 1974. *In vitro* maturation of a 16S RNA precursor. FEBS Lett. **46**:364–367.
68. **Hayes, F., and M. Vasseur.** 1976. Processing of the 17S *Escherichia coli* precursor RNA in the 27S pre-ribosomal particle. Eur. J. Biochem. **61**:433–442.
69. **Hercules, K., M. Schweiger, and W. Sauerbier.** 1974. Cleavage by RNase III converts T3 and T7 early precursor RNA into translatable message. Proc. Natl. Acad. Sci. U.S.A. **71**:840–844.
70. **Hoffman, S., and O. L. Miller, Jr.** 1977. Visualization of ribosomal ribonucleic acid synthesis in a ribonuclease III-deficient strain of *Escherichia coli*. J. Bacteriol. **132**:718–722.
71. **Ikemura, T., and J. E. Dahlberg.** 1973. Small ribonucleic acids of *Escherichia coli*. I. Characterization by polyacrylamide gel electrophoresis and fingerprint analysis. J. Biol. Chem. **248**:5024–5032.
72. **Ikemura, T., and M. Nomura.** 1977. Expression of spacer tRNA genes in ribosomal RNA transcription units carried by hybrid col E1 plasmids in *E. coli*. Cell **11**:779–793.
73. **Ikemura, T., and H. Ozeki.** 1975. Two-dimensional polyacrylamide-gel electrophoresis for purification of small RNAs specified by virulent coliphages T4, T5, T7, and BF23. Eur. J. Biochem. **51**:117–127.
74. **Ikemura, T., Y. Shimura, H. Sakano, and H. Ozeki.** 1975. Precursor molecules of *Escherichia coli* transfer RNAs accumulated in a temperature-sensitive mutant. J. Mol. Biol. **96**: 69–86.
75. **Ilgen, C., L. L. Kirk, and J. Carbon.** 1976. Isolation and characterization of large transfer ribonucleic acid precursors from *Escherichia coli*. J. Biol. Chem. **251**:922–929.
76. **Jordan, B. R., J. Feunteun, and R. Monier.** 1970. Identification of a 5S RNA precursor in exponentially growing *Escherichia coli* cells. J. Mol. Biol. **50**:605–615.
77. **Jordan, B. R., B. G. Forget, and R. Monier.** 1971. A low molecular weight ribonucleic acid synthesized by *Escherichia coli* in the presence of chloramphenicol: characterization and relation to normally synthesized 5S ribonucleic acid. J. Mol. Biol. **55**:407–421.
78. **Kindler, P., T. U. Keil, and P. H. Hofschneider.** 1973. Isolation and characterization of a ribonuclease III deficient mutant of *Escherichia coli*. Mol. Gen. Genet. **126**:53–69.
79. **Kole, R., and S. Altman.** 1979. Reconstitution of RNase P activity from inactive RNA and protein. Proc. Natl. Acad. Sci. U.S.A. **76**:3795–3799.

79a. **Kole, R., and S. Altman.** 1981. Properties of purified RNase P from *Escherichia coli*. Biochemistry **20**:1902–1906.

80. **Kole, R., M. F. Baer, B. C. Stark, and S. Altman.** 1980. *E. coli* RNase P has a required RNA component *in vivo*. Cell **19**:881–887.
81. **Kourilsky, P., M. F. Bourguignon, M. Bouquet, and F. Gros.** 1970. Early transcription controls after induction of prophage λ. Cold Spring Harbor Symp. Quant. Biol. **35**:305–314.

82. **Kourilsky, P., M. F. Bourguignon, and F. Gros.** 1971. Kinetics of viral transcription after induction of prophage, p. 647–666. *In* A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

83. **Kramer, R. A., M. Rosenberg, and J. A. Steitz.** 1974. Nucleotide sequences of the 5′ and 3′ termini of bacteriophage T7 early messenger RNAs synthesized *in vivo*: evidence for sequence specificity in RNA processing. J. Mol. Biol. **89**:767–776.

84. **Küpper, H., T. Sekiya, M. Rosenberg, J. Egan, and A. Landy.** 1978. A rho-dependent termination site in the gene coding for tyrosine tRNA $su_3$ of *Escherichia coli*. Nature (London) **272**:423–428.

84a. **Lee, S. Y., S. C. Bailey, and D. Apirion.** 1978. Small stable RNAs from *Escherichia coli*: evidence for the existence of new molecules and for a new ribonucleoprotein particle containing 6S RNA. J. Bacteriol. **133**:1015–1023.

85. **Leon, V., S. Altman, and D. M. Crothers.** 1977. Influence of the A15 mutation on the conformational energy balance in *E. coli* $tRNA^{Tyr}$. J. Mol. Biol. **113**:253–265.

86. **Lindahl, L.** 1973. Two new ribosomal precursor particles in *E. coli*. Nature (London) New Biol. **243**:170–172.

87. **Lindahl, L.** 1975. Intermediates and time kinetics of the *in vivo* assembly of *Escherichia coli* ribosomes. J. Mol. Biol. **92**:15–37.

88. **Lowry, C. V., and J. E. Dahlberg.** 1971. Structural differences between the 16S ribosomal RNA of *E. coli* and its precursor. Nature (London) New Biol. **232**:52–54.

89. **Lozeron, H. A., P. J. Anevski, and D. Apirion.** 1977. Antitermination and absence of processing of the leftward transcript of coliphage lambda in the RNAase III-deficient host. J. Mol. Biol. **109**:359–365.

90. **Lozeron, H. A., J. E. Dahlberg, and W. Szybalski.** 1976. Processing of the major leftward mRNA of coliphage lambda. Virology **71**:262–277.

91. **Lund, E., and J. E. Dahlberg.** 1977. Spacer transfer RNAs in ribosomal RNA transcripts of *E. coli*: processing of 30S ribosomal RNA *in vitro*. Cell **11**:247–262.

92. **Lund, E., and J. E. Dahlberg.** 1979. Initiation of *Escherichia coli* ribosomal RNA synthesis *in vivo*. Proc. Natl. Acad. Sci. U.S.A. **76**:5480–5484.

93. **Lund, E., J. E. Dahlberg, and C. Guthrie.** 1980. Processing of spacer tRNAs from ribosomal RNA transcripts of *E. coli*, p. 123–137. *In* D. Söll, J. Abelson, and P. Schimmel (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

94. **Lund, E., J. E. Dahlberg, L. Lindahl, S. R. Jaskunas, P. P. Dennis, and M. Nomura.** 1976. Transfer RNA genes between 16S and 23S rRNA genes in rRNA transcription units of *E. coli*. Cell **7**:165–177.

95. **Maisurian, A. N., and E. A. Buyanovskaya.** 1973. Isolation of an *Escherichia coli* strain restricting bacteriophage suppressor. Mol. Gen. Genet. **120**:227–229.

96. **Majumder, H. K., S. Bishayee, P. R. Chakraborty, and U. Maitra.** 1977. Ribonuclease III cleavage of bacteriophage T3 RNA polymerase transcripts to late T3 mRNAs. Proc. Natl. Acad. Sci. U.S.A. **74**:4891–4894.

97. **Manale, A., C. Guthrie, and D. Colby.** 1979. S1 nuclease as a probe for the conformation of a dimeric tRNA precursor. Biochemistry **18**: 77–83.

98. **Mangiarotti, G., D. Apirion, D. Schlessinger, and L. Silengo.** 1968. Biosynthetic precursors of 30S and 50S ribosomal particles in *Escherichia coli*. Biochemistry **7**:456–472.

99. **Mangiarotti, G., E. Turco, A. Ponzetto, and F. Altruda.** 1974. Precursor 16S RNA in active 30S ribosomes. Nature (London) **247**:147–148.

100. **Mazzara, G. P., G. Plunkett, and W. H. McClain.** 1980. Maturation events leading to transfer RNA and ribosomal RNA, p. 439–545. *In* L. Goldstein and D. M. Prescott (ed.), Cell biology: a comprehensive treatise, vol. 3. Academic Press, Inc., New York.

100a. **Mazzara, G. P., G. Plunkett, and W. H. McClain.** 1981. DNA sequence of the transfer RNA region of bacteriophage T4: implications for transfer RNA synthesis. Proc. Natl. Acad. Sci. U.S.A. **78**:889–892.

101. **McClain, W. H.** 1979. A role for ribonuclease III in synthesis of bacteriophage T4 transfer RNAs. Biochem. Biophys. Res. Commun. **86**: 718–724.

102. **McClain, W. H.** 1977. Seven terminal steps in a biosynthetic pathway leading from DNA to transfer RNA. Acc. Chem. Res. **10**:418–425.

103. **McClain, W. H., and J. G. Seidman.** 1975. Genetic perturbations that reveal tertiary conformation of tRNA precursor molecules. Nature (London) **257**:106–110.

104. **Meyhack, B., I. Meyhack, and D. Apirion.** 1974. Processing of precursor particles containing 17S rRNA in a cell free system. FEBS Lett. **49**:215–219.

105. **Meyhack, B., B. Pace, and N. R. Pace.** 1977. Involvement of precursor-specific segments in the *in vitro* maturation of *Bacillus subtilis* precursor 5S ribosomal RNA. Biochemistry **16**:5009–5015.

106. **Meyhack, B., B. Pace, O. Uhlenbeck, and N. R. Pace.** 1978. Use of T4 RNA ligase to construct model substrates for a ribosomal RNA maturation endonuclease. Proc. Natl. Acad. Sci. U.S.A. **75**:3045–3049.

107. **Meyhack, B., and N. R. Pace.** 1978. Involvement of the mature domain in the *in vitro* maturation of *Bacillus subtilis* precursor 5S ribosomal RNA. Biochemistry **17**:5804–5810.

108. **Misra, T. K., and D. Apirion.** 1978. Characterization of an endoribonuclease, RNase N, from *Escherichia coli*. J. Biol. Chem. **253**:5594–5599.

109. **Misra, T. K., and D. Apirion.** 1979. RNase E, an RNA processing enzyme from *Escherichia*

*coli.* J. Biol. Chem. **254**:11154–11159.

110. **Misra, T. K., and D. Apirion.** 1980. Gene *rne* affects the structure of the ribonucleic acid processing enzyme ribonuclease E of *Escherichia coli.* J. Bacteriol. **142**:359–361.
111. **Misra, T. K., S. Rhee, and D. Apirion.** 1976. A new endoribonuclease from *Escherichia coli.* Ribonuclease N. J. Biol. Chem. **251**:7669–7674.
112. **Moen, T. L., J. G. Seidman, and W. H. McClain.** 1978. A catalogue of transfer RNA-like molecules synthesized following infection of *Escherichia coli* by T-even bacteriophages. J. Biol. Chem. **253**:7910–7917.
113. **Morgan, E. A., T. Ikemura, L. Lindahl, A. M. Fallon, and M. Nomura.** 1978. Some rRNA operons in *E. coli* have tRNA genes at their distal ends. Cell **13**:335–344.
114. **Morgan, E. A., T. Ikemura, and M. Nomura.** 1977. Identification of spacer tRNA genes in individual ribosomal RNA transcription units of *Escherichia coli.* Proc. Natl. Acad. Sci. U.S.A. **74**:2710–2714.
115. **Morgan, E. A., T. Ikemura, L. E. Post, and M. Nomura.** 1980. tRNA genes in ribosomal RNA operons of *Escherichia coli,* p. 259–266. *In* D. Söll, J. Abelson, and P. Schimmel, (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
116. **Nakajima, N., H. Ozeki, and Y. Shimura.** 1981. Organization and structure of an *E. coli* tRNA operon containing seven tRNA genes. Cell **23**:239–249.
117. **Nikolaev, N., L. Silengo, and D. Schlessinger.** 1973. Synthesis of a large precursor to ribosomal RNA in a mutant of *Escherichia coli.* Proc. Natl. Acad. Sci. U.S.A. **70**:3361–3365.
118. **Oakley, J., and J. Coleman.** 1977. Structure of a promoter for T7 RNA polymerase. Proc. Natl. Acad. Sci. U.S.A. **74**:4266–4270.
119. **Oxender, D. L., G. Zurawski, and C. Yanofsky.** 1979. Attenuation in the *Escherichia coli* tryptophan operon: role of RNA secondary structure involving the tryptophan codon region. Proc. Natl. Acad. Sci. U.S.A. **76**:5524–5528.
120. **Pace, B., R. L. Peterson, and N. R. Pace.** 1970. Formation of all stable RNA species in *Escherichia coli* by posttranscriptional modification. Proc. Natl. Acad. Sci. U.S.A. **65**:1097–1104.
121. **Pace, N. R.** 1973. Structure and synthesis of the ribosomal RNA of prokaryotes. Bacteriol. Rev. **37**:562–603.
122. **Pace, N. R., B. Mayhack, B. Pace, and M. L. Sogin.** 1980. The interaction of RNase M5 with a 5S ribosomal RNA precursor, p. 155–171. *In* D. Söll, J. Abelson, and P. Schimmel (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
123. **Pace, N. R., M. K. Pato, J. McKibbin, and C. W. Radcliffe.** 1973. Precursors of 5S ribosomal RNA in *Bacillis subtilis.* J. Mol. Biol. **75**:619–631.
124. **Paddock, G. V., and J. Abelson.** 1975. Nucleotide sequence determination of bacteriophage T4 species I ribonucleic acid. J. Biol. Chem. **250**:4185–4206.
125. **Paddock, G. V., and J. Abelson.** 1975. Nucleotide sequence determination of bacteriophage T2 and T6 species I ribonucleic acids. J. Biol. Chem. **250**:4207–4219.
126. **Paddock, G. V., K. Fukada, J. Abelson, and H. D. Robertson.** 1976. Cleavage of T4 species I ribonucleic acid by *Escherichia coli* ribonuclease III. Nucleic Acids Res. **3**:1351–1372.
127. **Perry, R. P.** 1976. Processing of RNA. Annu. Rev. Biochem. **45**:605–629.

127a. **Pettijohn, D. E., O. G. Stonington, and C. R. Kossman.** 1970. Chain termination of ribosomal RNA synthesis *in vitro.* Nature (London) **228**:235–239.

127b. **Plautz, G., and D. Apirion.** 1981. Processing of RNA in *Escherichia coli* is limited in the absence of ribonuclease III, ribonuclease E and ribonuclease P. J. Mol. Biol. **149**:813–819.

128. **Post, L. E., G. D. Strycharz, M. Nomura, H. Lewis, and P. P. Dennis.** 1979. Nucleotide sequence of the ribosomal protein gene cluster adjacent to the gene for RNA polymerase subunit $\beta$ in *Escherichia coli.* Proc. Natl. Acad. Sci. U.S.A. **76**:1697–1701.
129. **Pragai, B., and D. Apirion.** Processing of bacteriophage T4 tRNAs: the role of RNAase III. J. Mol. Biol., in press.

129a. **Pragai, B., T. S. Ko, and D. Apirion.** 1980. Processing of bacteriophage T4 tRNAs: an RNase III$^-$ strain contains a precursor for $tRNA^{Gln}$ and $tRNA^{Leu}$. Biochem. Biophys. Res. Commun. **95**:1431–1436.

130. **Ray, A., and D. Apirion.** 1980. Cloning the gene for ribonuclease E, an RNA processing enzyme. Gene **12**:87–94.
131. **Ray, B. K., and D. Apirion.** 1979. Characterization of 10S RNA: a new stable RNA molecule from *Escherichia coli.* Mol. Gen. Genet. **174**: 25–32.
132. **Ray, B. K., and D. Apirion.** 1980. Identification of RNA molecules which contain 5S ribosomal RNA and transfer RNA in an RNAase E$^-$ RNAase P$^-$ double mutant strain of *Escherichia coli.* J. Mol. Biol. **139**:329–348.

132a. **Ray, B. K., and D. Apirion.** 1981. Transfer RNA precursors are accumulated in *Escherichia coli* in the absence of RNase E. Eur. J. Biochem. **114**:517–524.

132b. **Ray, B. K., and D. Apirion.** 1981. RNAase P is dependent on RNAase E action in processing monomeric RNA precursors that accumulate in an RNAase E$^-$ mutant of *Escherichia coli.* J. Mol. Biol. **149**:599–617.

133. **Robertson, H. D.** 1977. Structure and function of RNA processing signals, p. 549–568. *In* H. J. Vogel (ed.), Nucleic acid-protein recognition. Academic Press, Inc., New York.
134. **Robertson, H. D., S. Altman, and J. D. Smith.** 1972. Purification and properties of a specific *Escherichia coli* ribonuclease which cleaves a tyrosine transfer ribonucleic acid precursor. J.

Biol. Chem. **247**:5243–5251.

135. **Robertson, H. D., and F. Barany.** 1979. Enzymes and mechanisms in RNA processing, p. 285–295. *In* Proceedings of the 12th FEBS Congress. Pergamon Press, Oxford.
136. **Robertson, H. D., E. Dickson, and J. J. Dunn.** 1977. A nucleotide sequence from a ribonuclease III processing site in bacteriophage T7 RNA. Proc. Natl. Acad. Sci. U.S.A. **74**:822–826.
137. **Robertson, H. D., and J. J. Dunn.** 1975. Ribonucleic acid processing activity of *Escherichia coli* ribonuclease III. J. Biol. Chem. **250**: 3050–3056.
138. **Robertson, H. D., E. G. Pelle, and W. H. McClain.** 1980. RNA processing in an *Escherichia coli* strain deficient in both RNase P and RNAse III, p. 107–122. *In* D. Söll, J. Abelson, and P. Schimmel (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
139. **Robertson, H. D., R. E. Webster, and N. D. Zinder.** 1968. Purification and properties of ribonuclease III from *Escherichia coli.* J. Biol. Chem. **243**:82–91.
140. **Rosenberg, M., and R. A. Kramer.** 1977. Nucleotide sequence surrounding a ribonuclease III processing site in bacteriophage T7 RNA. Proc. Natl. Acad. Sci. U.S.A. **74**:984–988.
141. **Rosenberg, M., R. A. Kramer, and J. A. Steitz.** 1974. T7 early messenger RNAs are the direct products of ribonuclease III cleavage. J. Mol. Biol. **89**:777–782.
142. **Sakano, H., and Y. Shimura.** 1975. Sequential processing of precursor tRNA molecules in *Escherichia coli.* Proc. Natl. Acad. Sci. U.S.A. **72**:3369–3373.
143. **Sakano, H., and Y. Shimura.** 1978. Characterization and *in vitro* processing of transfer RNA precursors accumulated in a temperature-sensitive mutant of *Escherichia coli.* J. Mol. Biol. **123**:287–326.
144. **Sakano, H., S. Yamada, T. Ikemura, Y. Shimura, and H. Ozeki.** 1974. Temperature sensitive mutants of *Escherichia coli* for tRNA synthesis. Nucleic Acids. Res. **1**:355–371.
145. **Schedl, P., and P. Primakoff.** 1973. Mutants of *Escherichia coli* thermosensitive for the synthesis of transfer RNA. Proc. Natl. Acad. Sci. U.S.A. **70**:2091–2095.
146. **Schedl, P., P. Primakoff, and J. Roberts.** 1975. Processing of *E. coli* tRNA precursors. Brookhaven Symp. Biol. **26**:53–76.
147. **Schedl, P., J. Roberts, and P. Primakoff.** 1976. In vitro processing of *E. coli* tRNA precursors. Cell **8**:581–594.
148. **Schmidt, F. J.** 1975. A novel function of *Escherichia coli* nucleotidyl transferase. Biosynthesis of the C-C-A sequence in a phage T4 transfer RNA precursor. J. Biol. Chem. **250**: 8399–8403.
149. **Schmidt, F. J., and W. H. McClain.** 1978. An *Escherichia coli* ribonuclease which removes an extra nucleotide from a biosynthetic intermediate of bacteriophage T4 proline transfer RNA. Nucleic Acids Res. **5**:4129–4139.
150. **Schmidt, F. J., and W. H. McClain.** 1978. Transfer RNA biosynthesis. Alternate orders of ribonuclease P cleavage occur *in vitro* but not *in vivo.* J. Biol. Chem. **253**:4730–4738.
151. **Schmidt, F. J., J. G. Seidman, and R. M. Bock.** 1976. Transfer ribonucleic acid biosynthesis. Substrate specificity of ribonuclease P. J. Biol. Chem. **251**:2440–2445.
152. **Schweitz, H., and J.-P. Ebel.** 1971. A study of the mechanism of action of *E. coli* ribonuclease III. Biochimie **53**:585–593.
153. **Seidman, J. G., and W. H. McClain.** 1975. Three steps in conversion of large precursor RNA into serine and proline transfer RNAs. Proc. Natl. Acad. Sci. U.S.A. **72**:1491–1495.
154. **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.
155. **Sekiya, T., R. Contreras, T. Takeya, and H. G. Khorana.** 1979. Total synthesis of a tyrosine suppressor transfer RNA gene. XVII. Transcription, *in vitro*, of the synthetic gene and processing of the primary transcript to transfer RNA. J. Biol. Chem. **254**:5802–5816.

155a. **Shimura, Y., H. Sakano, S. Kubokawa, F. Nagara, and H. Ozeki.** 1980. tRNA precursors in RNase P mutants, p. 43–88. *In* D. Söll, J. Abelson, and P. Schimmel (ed.), Transfer RNA: biological aspects. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

156. **Shimura, Y., H. Sakano, and F. Nagawa.** 1978. Specific ribonucleases involved in processing of tRNA precursors of *Escherichia coli.* Eur. J. Biochem. **86**:267–281.
157. **Singer, M. F., and G. Tolbert.** 1965. Purification and properties of a potassium-activated phosphodiesterase (RNAase II) from *Escherichia coli.* Biochemistry **4**:1319–1330.
158. **Smith, J. D.** 1975. Mutants which allow accumulation of $tRNA^{Tyr}$ precursor molecules. Brookhaven Symp. Biol. **26**:1–11.
159. **Smith, J. D.** 1976. Transcription and processing of transfer RNA precursors. Prog. Nucleic Acid Res. Mol. Biol. **16**:25–73.
160. **Sogin, M. L., B. Pace, and N. R. Pace.** 1977. Partial purification and properties of a ribosomal RNA maturation endonuclease from *Bacillus subtilis.* J. Biol. Chem. **252**:1350–1357.
161. **Sogin, M. L., N. R. Pace, M. Rosenberg, and S. M. Weissman.** 1976. Nucleotide sequence of a 5S ribosomal RNA precursor from *Bacillus subtilis.* J. Biol. Chem. **251**:3480–3488.
162. **Sprague, K. U., and J. A. Steitz.** 1975. The 3′ terminal oligonucleotide of *E. coli* 16S ribosomal RNA: the sequence in both wild-type and RNase $III^-$ cells is complementary to the polypurine tracts common to mRNA initiator regions. Nucleic Acids Res. **2**:787–798.
163. **Stahl, D. A., B. Meyhack, and N. R. Pace.** 1980. Recognition of local nucleotide conformation in contrast to sequence by a rRNA processing endonuclease. Proc. Natl. Acad. Sci.

U.S.A. 77:5644-5648.

164. **Stahl, D. A., T. A. Walker, B. Meyhack, and N. R. Pace.** 1979. Precursor-specific nucleotides can govern RNA folding. Cell **18**:1133-1143.

165. **Stark, B. P., R. Kole, E. J. Bowman, and S. Altman.** 1978. Ribonuclease P: an enzyme with an essential RNA component. Proc. Natl. Acad. Sci. U.S.A. **75**:3717-3721.

166. **Stiekema, W. J., H. A. Raué, and R. J. Planta.** 1980. Sequence analysis and *in vitro* maturation of five precursor 5S RNAs from Bacillus Q. Nucleic Acids Res. **8**:2193-2211.

167. **Studier, F. W.** 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol. **79**:237-248.

168. **Studier, F. W.** 1975. Genetic mapping of a mutation that causes ribonuclease III deficiency in *Escherichia coli*. J. Bacteriol. **124**:307-316.

169. **Studier, F. W., J. J. Dunn, and E. Buzash-Pollert.** 1979. Processing of bacteriophage T7 RNAs by RNase III, p. 261-269. *In* T. R. Russell, K. Brew, H. Faber, and T. Schultz (ed.), From gene to protein: information transfer in normal and abnormal cells. Academic Press, Inc., New York.

170. **Szybalski, W., K. Bøvre, M. Fiandt, S. Hayes, Z. Hradecna, S. Kumar, H. A. Lozeron, H. J. J. Nijkamp, and W. F. Stevens.** 1970. Transcriptional units and their controls in *Escherichia coli* phage λ: operons and scriptons. Cold Spring Harbor Symp. Quant. Biol. **35**:341-353.

171. **Talkad, V., D. Achord, and D. Kennell.** 1978. Altered RNA metabolism in ribonuclease III-deficient strains of *Escherichia coli*. J. Bacteriol. **135**:528-541.

172. **Turco, E., F. Altruda, A. Ponzetto, and G. Mangiarotti.** 1974. Ribosome biosynthesis in *Escherichia coli*. Concerning the limiting step. Biochemistry **13**:4752-4757.

173. **Vogeli, G., H. Grosjean, and D. Söll.** 1975. A method for the isolation of specific tRNA precursors. Proc. Natl. Acad. Sci. U.S.A. **72**:4790-4794.

173a. **Watson, N., and D. Apirion.** 1981. Ribonuclease F, a putative processing endoribonuclease from *Escherichia coli*. Biochem. Biophys. Res. Commun., in press.

174. **Wilder, D. A., and H. A. Lozeron.** 1979. Differential modes of processing and decay for the major N-dependent RNA transcript of coliphage λ. Virology **99**:241-256.

175. **Wireman, J. W., and P. S. Sypherd.** 1974. Properties of 30S ribosomal particles reconstituted from precursor 16S ribonucleic acid. Biochemistry **13**:1215-1221.

176. **Wu, M., and N. Davidson.** 1975. Use of gene 32 protein staining of single-strand polynucleotides for gene mapping by electron microscopy: application to the $\phi 80$ $d_3$ *ilv* $su^+7$ system. Proc. Natl. Acad. Sci. U.S.A. **72**:4506-4510.

177. **Young, R. A.** 1979. Transcription termination in the *Escherichia coli* ribosomal RNA operon *rrnC*. J. Biol. Chem. **254**:12725-12731.

178. **Young, R. A., R. Macklis, and J. A. Steitz.** 1979. Sequence of the 16S-23S spacer region in two ribosomal RNA operons of *E. coli*. J. Biol. Chem. **254**:3264-3271.

179. **Young, R. A., and J. A. Steitz.** 1978. Complementary sequences 1700 nucleotides apart form a ribonuclease III cleavage site in *Escherichia coli* ribosomal precursor RNA. Proc. Natl. Acad. Sci. U.S.A. **75**:3593-3597.