Interplay among Processing and Degradative Enzymes and a Precursor Ribonucleic Acid in the Selective Maturation and Maintenance of Ribonucleic Acid Molecules†

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ABSTRACT: In order to understand why the first tRNA ($tRNA^{Gln}$) in the T4 tRNA gene cluster is not produced when T4 infects an RNase III⁻ mutant of *Escherichia coli*, RNA metabolism was analyzed in RNase III⁻ RNase P⁻ (*rnc*, *rnp*) cells infected with bacteriophage T4. After such an infection a new dimeric precursor RNA molecule of $tRNA^{Gln}$ and $tRNA^{Leu}$ has been identified and analyzed. This molecule is structurally very similar to K band RNA that accumulates in *rnc*⁺ *rnp* strains. It is four nucleotides shorter than K RNA at the 5′ end. This molecule like K RNA contains two RNase P processing sites at the 5′ ends of each tRNA. Both sites are accessible to RNase P. However, while in the K RNA the site at the 5′ end of $tRNA^{Leu}$ (the site in the middle of the substrate) is more efficiently cleaved than the other site, this differential is even increased in the Ks (K like) molecule. This difference is sufficiently large that in vivo in the RNase III⁻ strain the smaller precursor of $tRNA^{Gln}$ is degraded rather than being matured to $tRNA^{Gln}$ by RNase P. This information contributes to the elucidation of the key role of RNase III in the processing of T4 tRNA. It shows the dependence of RNase P activity at the 5′ end of $tRNA^{Gln}$ on a correct and specific cleavage by RNase III at a position six nucleotides proximal to the RNase P site, and it explains why in the absence of RNase III the first tRNA in the T4 tRNA cluster, $tRNA^{Gln}$, does not accumulate.

Most procaryotic RNA molecules are not transcribed in their final mature size (Altman & Smith, 1971; Pace, 1973). In order to become functional they often require a set of processing reactions (Pace, 1973; Perry, 1976; Smith, 1976; Altman, 1978; Abelson, 1979). In procaryotic cells there are extensive cleavages and trimming of the primary transcripts especially in the synthesis of rRNA and tRNA (Altman, 1975; Abelson, 1979; Mazzara et al., 1981; Gegenheimer & Apirion, 1981).

The bacteriophage T4 tRNA cluster, where the RNAs from 10 small genes are all cotranscribed from a single promoter, provides an excellent system for the analysis of RNA processing (McClain et al., 1972; Guthrie et al., 1975; Abelson et al., 1975; Abelson, 1979). When this system was used, it was found that in the absence of the host processing enzyme RNase[1] III the first tRNA in the cluster, $tRNA^{Gln}$, does not accumulate (McClain, 1979; Pragai et al., 1980). The same result, i.e., absence of $tRNA^{Gln}$, was obtained by using the deletion T4Δ27. This deletion simplifies the system considerably. This is an internal deletion missing 7 of the 10 genes in the cluster (Wilson et al., 1972; Abelson et al., 1975). In this deletion the first two genes ($tRNA^{Gln}$ and $tRNA^{Leu}$) and the last gene (species 1 RNA) remain intact. Infection of an *Escherichia coli* RNase III⁻ cell with T4Δ27 results in the transient accumulation of 10.5S RNA that contains sequences from all the three RNAs and which can be processed by extracts from an RNase III⁺ strain to the three final molecules (Pragai & Apirion, 1981); however, when extracts from an RNase III⁻ strain are used, like in vivo, the level of $tRNA^{Gln}$ is relatively very low. The 10.5S RNA is cleaved at the 5′ end by RNase III to give rise to 10.1S RNA which still contains all the three final molecules, and the cleavage is six nucleotides 5′ to $tRNA^{Gln}$ (Pragai & Apirion, 1981).

In the studies reported here we made an attempt to understand why $tRNA^{Gln}$ that exists in the precursor molecule fails to accumulate while its counterpart $tRNA^{Leu}$ does. We found that in the absence of RNase III a precursor of $tRNA^{Gln}$–$tRNA^{Leu}$ accumulates. This precursor contains only two extra nucleotides at the 5′ end, rather than six as is the case in the normal situation. This small difference in four nucleotides is sufficient to seal the fate of the immediate precursor of $tRNA^{Gln}$ which is apparently being degraded, rather than processed.

Materials and Methods

Strains. The *rnc*⁺ *rnp*⁺ strain (wild type) was N2111 (Korn & Yanofsky, 1976), the *rnc* strain was N2328 (Apirion et al., 1978), the *rnp* strain was N2020 (Schedl & Primakoff, 1973; Apirion, 1980), and the *rnc rnp* strain was N2018 (Gegenheimer & Apirion, 1978). These strains were infected with the T4Δ27 deletion mutant (Wilson et al., 1972) at a multiplicity of 15 phage per bacterium.

Growth and Labeling of Cells. Cells were grown in low phosphate medium containing 0.6% peptone and 0.2% glucose (Gegenheimer & Apirion, 1980) at 30 °C. Cultures were shifted to 37 or 43 °C, as necessary, at an A_{560} of 0.3. Infection of cells was carried out 30 min after the shift, and 4 min later the cells were superinfected and labeled with [^{32}P]P_i. Labeling was terminated by adding 2 volumes of stop solution (80% ethanol, 0.1% diethyl pyrocarbonate, 0.3 mM aurintricarboxylic acid, and 1% dimethyl sulfoxide). Cells were harvested by centrifugation (5000*g*), and the cell pellet was suspended in lysis buffer (20 mM Tris-HCl, pH 7.4, 10 mM Na_2EDTA, 20% glycerol, 1% sodium dodecyl sulfate, 4 mM diethyl pyrocarbonate, and 0.01% bromphenol blue) and heated for 2 min at 100 °C.

Polyacrylamide Gel Electrophoresis. The electrophoresis was carried out as previously described (Laemmli, 1970) by using 5%/8% tandem thin (1.5 mm) polyacrylamide gels containing 0.2% $NaDodSO_4$ and 7 M urea. For purification

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[1] Abbreviations: RNase, ribonuclease; EDTA, ethylenediaminetetraacetic acid; p, precursor; $NaDodSO_4$, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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of RNA, the RNA bands were excised and rerun in polyacrylamide gels of different porosity. The elution of RNA from the gel and the precipitation with ethanol were done as previously described (Gurevitz et al., 1982).

Structural Analysis of RNA. Two-dimensional fingerprint analysis after digestion with RNase T1 was performed according to Volckaert et al. (1976). Subsequent oligonucleotide analysis using RNase A, RNase T2, and nuclease P1 was carried out as described by Volckaert & Fiers (1977) and Saneyoshi et al. (1969).

In Vitro Processing of K Band and Ks RNAs. Assays for RNase P activity were performed according to published procedures (Gardiner & Pace, 1980; Jain et al., 1982). Each assay in 20 μL contained 1 μg of yeast RNA and 2000–3000 cpm of the ^{32}P-labeled substrate. The reaction was terminated after 30 min by adding sample buffer (1% $NaDodSO_4$, 50 mM Na_2EDTA, 50% glycerol, and 0.02% bromphenol blue dye), and the tubes were heated at 90 °C for 2 min. Large-scale processing of K band and Ks RNAs was done in 250 μL volumes keeping the necessary ratio of RNase P to RNA as in the analytical scale.

Results

Accumulation of Small RNAs Specified by T4Δ27 in an rnc rnp E. coli Strain. In order to understand the events leading to the maturation of T4 tRNA in the absence of RNase III, we decided to use an *rnc rnp* (RNase III$^-$ RNase P$^-$) double mutant strain. Since RNase P introduces cleavages at the 5′ end of each of the two tRNAs produced from T4Δ27 (Guthrie et al., 1973, 1975; Guthrie, 1975; Abelson et al., 1975), we anticipated the accumulation in an *rnc rnp* double mutant of a dimeric tRNA^{Gln}–tRNA^{Leu} precursor that could be an intermediate in the processing of the tRNAs; indeed this expectation was fulfilled (see below). In order to make the analysis meaningful we compared the RNAs accumulated in the *rnc rnp* cells to RNAs that accumulate in the other three relevant strains: *rnc*$^+$ *rnp*$^+$, *rnc rnp*$^+$, and *rnc*$^+$ *rnp*.

Upon infection of *rnc rnp* (RNase III$^-$ RNase P$^-$) *E. coli* cells with T4Δ27, few RNA molecules accumulate to a prominent level. 10.5S RNA (Figure 1, lane 4) which was previously reported and analyzed (Pragai & Apirion, 1981, 1982) can be found in all *rnc* strains infected with the Δ27 deletion mutant of bacteriophage T4. This molecule reaches its highest level in *rnc rnp* cells approximately 15 min after infection. Another RNA molecule which reaches its highest level of accumulation in these cells about 25 min after infection and has not been encountered before is Ks RNA (Figure 1, lane 4). This molecule migrates somewhat faster than the K band RNA (Figure 1, lane 3) and appears in relatively high amounts as compared to other RNAs in the gel. [The K band is the dimeric precursor for tNA^{Gln}–tRNA^{Leu} (Guthrie et al., 1975; Guthrie, 1975).] Below Ks RNA, another molecule designated as III′ is accumulated (lanes 2 and 4 in Figure 1). The level of this RNA is always elevated in *rnc* strains infected with T4Δ27 or other T4 strains but is irrelevant to the tRNA region of the T4 genome since it appears also in a deletion strain that is missing all the tRNA gene cluster (Pragai & Apirion, 1981).

The next prominent RNA in the gel is p1Sp1, a precursor molecule to species 1 RNA that contains additional nucleotides at its 5′ side as compared to species 1 RNA. [These nucleotides are the bridge between the 3′ end of tRNA^{Leu} and the 5′ end of species 1 RNA in the deleted T4Δ27 mutant (Pragai & Apirion, 1982).] This RNA was identified earlier in *rnp* mutants of *E. coli* infected with T4 or T4Δ27 (Abelson et al., 1975; Pragai & Apirion, 1982). This precursor, p1Sp1

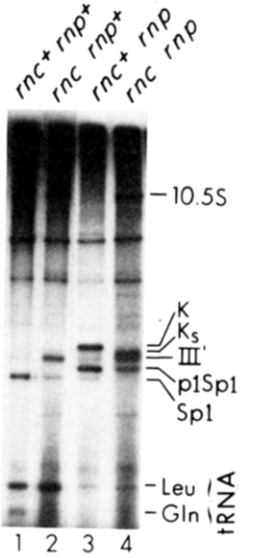

FIGURE 1: Display of T4Δ27 RNAs accumulated in various RNA processing mutants of *E. coli*. Cells were grown and infected as described under Materials and Methods; 250 μCi/mL [^{32}P]P_i was used for labeling at 37 °C. The *rnc*$^+$ *rnp*$^+$ and *rnc rnp*$^+$ strains were labeled for 30 min while the *rnc*$^+$ *rnp* and *rnc rnp* strains were labeled for 22 min. The cells were treated (see Materials and Methods) and their contents separated in a 5%/8% tandem thin polyacrylamide gel containing 0.2% sodium dodecyl sulfate and 7 M urea. The 5% portion of the gel was removed prior to autoradiography. About 300 000 cpm of the trichloroacetic acid precipitable material was loaded into each slot. (For further details see text.)

RNA, seems to appear in relatively large quantities, approximately in equal amounts to K and Ks RNAs.

Below p1Sp1 RNA, a small amount of species 1 RNA can be detected in the *rnc rnp* strain (lane 4) and in the *rnp* strain (lane 3) while in wild-type cells (lane 1) its level is much higher and is comparable to the level of the other mature tRNA^{Leu} and tRNA^{Gln}. In the *rnc* strain species 1 RNA appears also in a relatively low level. This is similar to what was found earlier (Pragai & Apirion, 1981). tRNA^{Leu} and tRNA^{Gln} that appear in high levels in the wild type cells (lane 1) are much reduced in the *rnp* and the *rnc rnp* strains (lanes 3 and 4); tRNA^{Leu} appears in the *rnc* strain (lane 2) and in the *rnc rnp* strain (lane 4), but tRNA^{Gln} is absent in both cases. Between 10.5S RNA and K band RNA there are two prominent RNA bands. These RNAs always appear after T4 infection and are not derived from the tRNA gene cluster (Pragai & Apirion, 1981). The experiment depicted in Figure 1 was performed at 37 °C. At this temperature *rnp* cells can grow, and they contain some RNase P activity. For this reason there is some maturation of tRNAs and species 1 in the *rnp* strains. This can be observed in lanes 3 and 4 of Figure 1.

We have decided to examine the Ks RNA molecule, which has not been detected previously, in order to get a better understanding of the set of reactions that convert 10.5S RNA to tRNA^{Leu} and species 1 RNA in cells depleted of RNase III and to try to shed some light on the problem of why a precursor that contains sequences for tRNA^{Gln}, tRNA^{Leu}, and species 1 RNA does give rise only to two of the three RNAs (tRNA^{Leu} and species 1 RNA but not tRNA^{Gln}).

Structural Analysis of Ks RNA. Cells (1.5 mL) from *rnc rnp* and *rnc*$^+$ *rnp* strains were infected with bacteriophage T4Δ27 and labeled with 2 mCi/mL [^{32}P]P_i at 37 °C. After 22 min the labeling was stopped, the cells were lysed, and the RNA content of the cells was separated in 5%/8% $NaDodSO_4$–polyacrylamide gel containing 7 M urea. The K RNA (in *rnc*$^+$ *rnp* cells) and the Ks RNA (in *rnc rnp* cells) bands

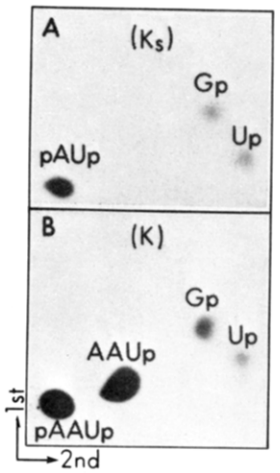

FIGURE 2: Composition analysis of the 5′ end of K and Ks RNAs. The T1 5′-end oligonucleotides from Ks RNA (A) and from K band RNA (B) were digested by ribonuclease A. The chromatography was performed according to Volckaert & Fiers (1977).

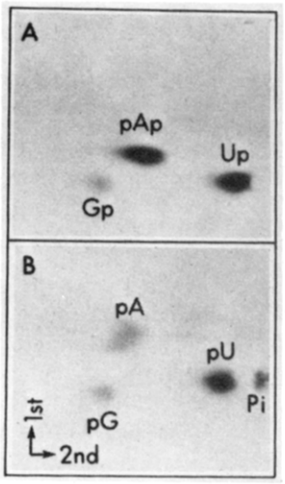

FIGURE 3: Determination of the 5′ terminus in Ks RNA. RNase T2 digestion (A) vs. nuclease P1 digestion (B) of the T1 5′-end oligonucleotide of Ks RNA.

were excised and purified. The isolated RNAs were digested by RNase T1 and fingerprinted followed a well-established procedure (Volckaert et al., 1976). The generated oligonucleotides were further digested with RNase A, and the end oligonucleotides were also incubated with RNase T2 and nuclease P1. The analysis of Ks RNA showed that it is very similar to K band RNA. The only difference found was in the 5′-end T1 oligonucleotide which is pAAUAAUUGp in K RNA and is four nucleotides shorter, pAUUGp, in Ks RNA. (The first tRNA, $tRNA^{Gln}$, starts with the last two nucleotides pUGp.) The digestion of the 5′-end T1 oligonucleotides from K or Ks RNAs by RNase A (pancreatic) is depicted in Figure 2. The 5′-end T1 oligonucleotides were also incubated with nuclease P1 and T2 RNase. In the first experiment the digestion revealed pA, pU, and pG in both cases, but the RNase T2 digestion released pAp, Ap, Up, and Gp in K band T1 5′-end oligonucleotide but only pAp, Up, and Gp in Ks RNA T1 5′-end oligonucleotide (Figure 3). (In Figure 3 only the analysis of the T1 5′-end oligonucleotide of the Ks RNA is presented.)

In Vitro Processing of Ks RNA vs. K RNA. In a previous report (Guthrie et al., 1975) it was shown that the two RNase P cleavage sites in K RNA are different in their accessibility to the enzyme. While the internal site between the 3′ end of $tRNA^{Gln}$ and the 5′ end of $tRNA^{Leu}$ is cleaved first, the other

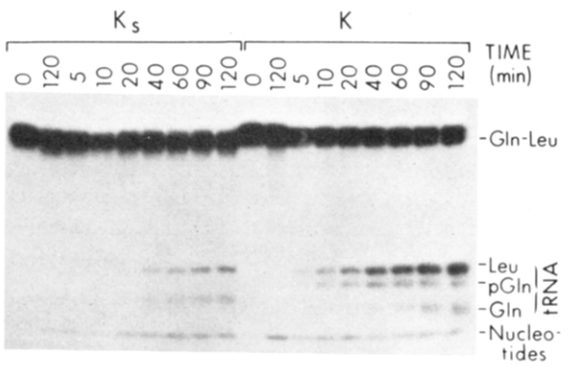

FIGURE 4: Limited processing of K and Ks RNAs by RNase P. The reactions were carried out at 30 °C for 30 min by using RNase P assay conditions. Amount of enzyme used was 1 arbitrary unit per assay. (This amount cleaves K band RNA under the above conditions in the presence of 1 μg of yeast RNA to about 30% in 30 min.) The first two lanes on the left hand for each substrate were controls without the enzyme, incubated in ice (0) or at 30 °C for 2 h in the presence of the assay mix (120). The products originating from cleavages of K RNA are designated. The dimeric Gln–Leu tRNA precursor and $tRNA^{Gln}$ were not produced when Ks was the substrate. The molecule that appears below $tRNA^{Leu}$, when Ks RNA is the substrate, is $ptRNA_s^{Gln}$ which is four nucleotides shorter as compared to $ptRNA^{Gln}$ derived from K RNA and is migrating faster than $ptRNA^{Gln}$ in the gel. The reaction products were separated in 5%/10% tandem thin polyacrylamide gel containing 0.2% sodium dodecyl sulfate without urea. About 2000 cpm of substrate was subjected to electrophoresis in each slot, and the autoradiography was carried out for 15 h at −80 °C with an intensifying screen (Swanstrom & Shank, 1978).

site at the 5′ end of $tRNA^{Gln}$ is cleaved later. This finding implied that the internal cleavage site is probably recognized more efficiently by RNase P than the other site. However, under conditions of enzyme excess both sites can be cleaved to yield an equimolar ratio of the products, $tRNA^{Gln}$ to $tRNA^{Leu}$. We anticipated the cleavage by RNase P at the 5′ site of Ks RNA to be further decreased due to the shorter 5′ end as compared to that of K RNA.

The two main questions that we have tried to answer were the following: can RNase P recognize and cleave both sites in Ks RNA, and which one of them is cleaved preferentially. We therefore digested Ks RNA by relatively small amounts of RNase P, and the kinetics of the accumulation of the products was examined and compared to a similar kinetics experiment conducted with K band RNA as a substrate (Figure 4). Identification of the cleaved products was carried out by using the same assay conditions on a large scale (in 250 μL volumes). The different RNA products were purified as described before and fingerprinted after being digested with RNase T1. The final determination of the T1-generated oligonucleotides was confirmed by using RNase A and RNase T2 for 5′-end analyses. As can be seen in Figure 4, K band RNA can be digested independently in both sites to create four different products. (1) The first product is a $tRNA^{Gln}$–$tRNA^{Leu}$ dimer without the 5′ end of the K band. This cleavage takes place at the 5′ end of $tRNA^{Gln}$. This dimer is six nucleotides shorter than the K RNA. (2) The second product is $tRNA^{Leu}$ which is produced when the cleavage takes place in the internal RNase P site at the 5′ end of $tRNA^{Leu}$ either in K band RNA or in the dimer if already created. (3) The third product is $ptRNA^{Gln}$ which comprises $tRNA^{Gln}$ plus six nucleotides from the 5′-end sequence of the K RNA band and is produced directly from K RNA at the same time when $tRNA^{Leu}$ is produced during the reaction. (4) The fourth product is $tRNA^{Gln}$ that is derived from $ptRNA^{Gln}$. $tRNA^{Gln}$ does not appear as a sharp band, probably due to its immature

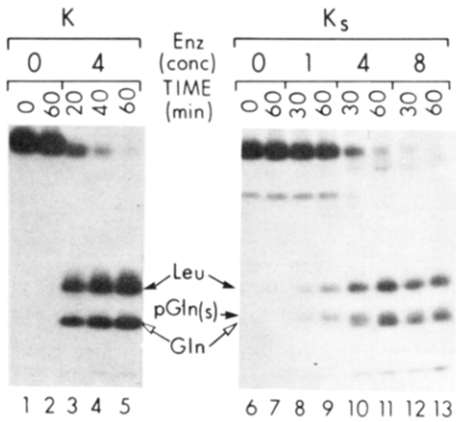

FIGURE 5: Processing of K and Ks RNA with excess RNase P. The reactions were performed as described in Figure 4. The amount of enzyme used per assay varied from 1 unit (as defined in the legend to Figure 4) up to 8 units per 1 μg of carrier RNA. Lanes 1, 2, 6, and 7 are the controls as described before (Figure 4). In lanes 3–5 there is equimolar production of $tRNA^{Leu}$ and $tRNA^{Gln}$ when RNase P is in excess and with no accumulation of the $tRNA^{Gln-Leu}$ dimer or pre-$tRNA^{Gln}$ molecules. Similar cleavage conditions seem to be achieved with Ks RNA when 8 units of enzyme was applied to the same amount of substrate (lanes 12 and 13). Lanes 8 and 9 depict the production of $tRNA^{Leu}$ and $ptRNA_s^{Gln}$ which can also be detected in lanes 10 and 11 concomitantly with increasing amounts of $tRNA^{Gln}$ itself. For further details see Figure 4. The Ks substrate used was slightly contaminated with a faster migrating molecule (lanes 6–9) that is degraded probably by the contaminating nuclease(s) in the RNase P preparation.

3′ end, which could be trimmed by exonucleases. [In T4, the terminal CCA of $tRNA^{Gln}$ is not coded (Guthrie et al., 1975; Fukada & Abelson, 1980).] On the other side of Figure 4, Ks RNA was digested with the same amount of enzyme, but no dimer of $tRNA^{Gln}$–$tRNA^{Leu}$ was detected; also, no $tRNA^{Gln}$ was accumulated while $tRNA^{Leu}$ was produced together with $ptRNA_s^{Gln}$ which comprises $tRNA^{Gln}$ plus the 5′ fragment that exists in Ks RNA. This experiment clearly indicates that the site at the 5′ end of $tRNA^{Gln}$ in Ks RNA is very inefficient as a cleavage site for RNase P as compared to the same site in K band RNA. However, the 5′ site of $tRNA^{Leu}$ in Ks RNA is accessible to the enzyme.

In order to find out whether the 5′ site of $tRNA^{Gln}$ is accessible at all to RNase P in the Ks RNA, we have performed the next experiment which is depicted in Figure 5. After the level of the enzyme vs. substrate was increased, K RNA was cleaved totally to what seems to be equal amounts of $tRNA^{Leu}$ and $tRNA^{Gln}$ (left side of Figure 5). When Ks RNA was incubated with increasing levels of enzyme, some $tRNA^{Gln}$ was formed, but at a much lower level in comparison to $tRNA^{Leu}$. This experiment implies that the 5′-cleavage site of $tRNA^{Gln}$ can be recognized by RNase P but only at relatively high enzyme levels.

Discussion

In the experiments presented here we tried to explain why from a precursor that contains sequences for a tRNA molecule, in the absence of RNase III this tRNA ($tRNA^{Gln}$) does not mature but is rather degraded.

The experiments described here suggest that in the absence of RNase III a shorter intermediate is produced which is an improper substrate for the final endonucleolytic RNA processing enzyme RNase P and this leads to degradation rather than to processing of the precursor of $tRNA^{Gln}$. Thus, the experiments discussed here clearly show that the level of an RNA molecule is determined by an interplay between a precursor and RNA processing and degradation enzymes.

One possibility to explain these results is to assume that $tRNA^{Gln}$ is produced but is unstable in the absence of RNase III. This was investigated previously and found not to be the case; i.e., the stability of existing $tRNA^{Gln}$ is not affected by the absence of RNase III (Pragai & Apirion, 1981; B. Pragai and D. Apirion, unpublished experiments). Therefore, it was clear that a different kind of explanation is necessary, where a precursor rather than the final product is subjected to degradation.

Using the *rnc rnp* double mutant, we could idenitfy a precursor for $tRNA^{Gln}$–$tRNA^{Leu}$ that was different from the one identified in an *rnp* rnc^+ strain. In the later, one can find the K RNA (Guthrie et al., 1975; Guthrie, 1975) which is derived by a cleavage of 10.1S RNA (see Figure 6), probably by RNase F. The 5′ end of this molecule (10.1S RNA) is created by RNase III which cleaves six nucleotides from the 5′ end of $tRNA^{Gln}$ (Pragai & Apirion, 1981, 1982). The two RNase P sites in this molecule (10.1S RNA) are not accessible to RNase P (B. Pragai and D. Apirion, unpublished observations), and in order to become accessible the 10.1S RNA molecule has to be first separated to K and p3Sp1 RNA (see Figure 6). In the 10.5S RNA this cleavage cannot take place; the 5′ region of the molecule has to be removed first. RNase III is normally responsible for this event; however, in its absence a nonspecific nucleases(s) can digest the 10.5S RNA molecule (Gegenheimer et al., 1977). This could be a combination of endonucleolytic cleavage(s) and exonucleolytic trimming. The digestion of the molecule stops at a specific site two nucleotides proximal to the 5′ end of $tRNA^{Gln}$ (Ks RNA) probably because of the tertiary structure of the tRNA precursor that endows it with a certain level of resistance to nonspecific nucleases. After the removal of the 5′-end nucleotides from 10.5S RNA, the separation of the RNA to Ks and p3Sp1 becomes feasible. At this point the Ks RNA becomes a substrate for RNase P. It contains two sites, but the one in front of $tRNA^{Leu}$ is accessible while the other is not (see Figure 4–6). As a result of this cleavage a mature $tRNA^{Leu}$ and an unusual precursor of $tRNA^{Gln}$, designated $ptRNA_s^{Gln}$, are produced. This precursor is a very poor substrate for RNase P (Figure 5), and in most cases it is degraded by nonspecific nucleases before having a chance to be processed by RNase P.

This mechanism explains why $tRNA^{Gln}$ is almost completely missing in an RNase III^- strain. Apparently, the four nucleotides make enough of a difference to RNase P. While previously changes in the precursor of $tRNA^{Tyr}$ that affected RNase P activity were confined mainly to the mature domain (Altman, 1975, 1978; Smith, 1975, 1976), in this case, missing the first four precursor nucleotides affects the precursor sufficiently to render it rather inaccessible to RNase P.

There are a number of points raised by the studies reported here that deserve some comment. First, these studies show that an RNA precursor of $tRNA^{Gln}$ can be cleaved at its 5′ end by RNase P even though its 3′ end is missing CCA (Figure 5). This is not too surprising and could have been anticipated, since a $tRNA^{Gln}$ with a mature 5′ end and an immature 3′ end was found in a *cca* host mutant (McClain & Seidman, 1978). These findings do not, however, contradict the previous conclusion that RNase P prefers to cleave tRNA precursors that already contain CCA (Schmidt et al., 1976).

Second, it is of interest that in a particular bacteriophage T2(H) strain that contains a mutation in the anticodon stem of $tRNA^{Gln}$, there is a decreased accumulation of $tRNA^{Gln}$, but again a dimeric precursor of $tRNA^{Gln}$–$tRNA^{Leu}$ can be observed in *rnp* cells (Likover-Moen et al., 1978). We would

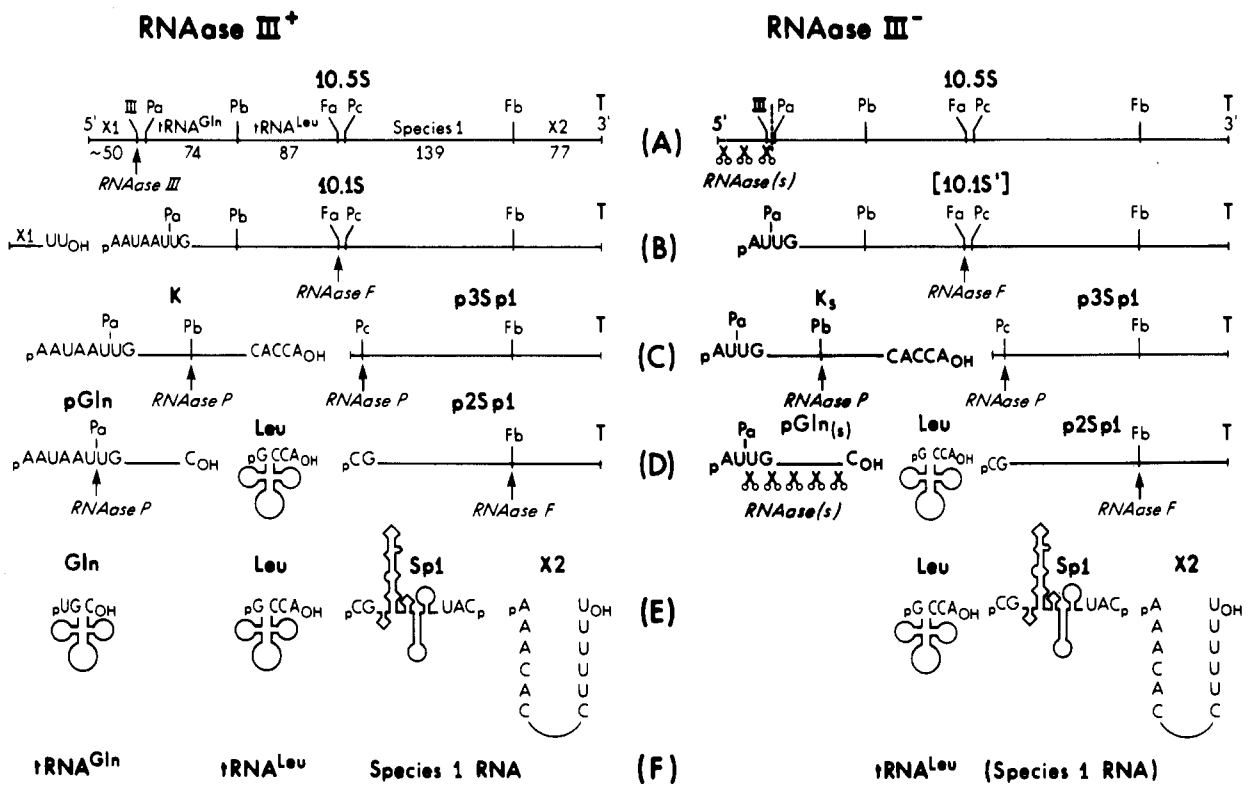

FIGURE 6: Schematic presentation of the processing events of T4 tRNA in RNase III$^+$ and RNase III$^-$ strains. This scheme is based on the studies reported here as well as on previous studies (see the text). The various processing steps are depicted successively on the various lines of this figure (A–E). On the left and right the 10.5S RNA molecule is presented, with the various positions of the enzymatic cleavages (III, RNase III; Pa, Pb, and Pc, RNase P; Fa and Fb, RNase F). T stands for termination. Below the line is shown the size of the segment in nucleotides. X1 and X2 are RNA fragments that can be found in the cell. On the right-hand side (RNase III$^-$) the vertical broken line represents the point where the degradation of the 5′ end of the 10.5S RNA is stopped in the absence of RNase III. This point represents the 5′ end of Ks RNA. In the fourth line (D) the degradation of ptRNA$_s^{Gln}$ by nonspecific nucleases is represented by the scissor action. Similarly, in the first line (A, RNase III$^-$) the scissor action at the 5′ end symbolizes the degradation of RNA at the 5′ end of 10.5S RNA in the absence of RNase III.

like to suggest that in this case like in the situation analyzed here the cleavage by RNase P in front of tRNA^{Leu} took place as usual but RNase P cleavage at the 5′ end of tRNA^{Gln} became less efficient and the altered ptRNA^{Gln}, this time because of a change in the anticodon, was degraded.

It should be mentioned that even in an *rnc*$^+$ strain the ratio of tRNA^{Gln} to tRNA^{Leu} is less than 1 to 1 (Pragai & Apirion, 1981). Thus, even in a wild-type strain the degradative enzyme(s) could probably attack some of the ptRNA^{Gln} molecules, prior to them having a chance to be processed by RNase P.

The situation with the dimeric precursor of tRNA^{Gln}–tRNA^{Leu} seems to be very similar to that of the dimeric precursor for tRNA^{Pro}–tRNA^{Ser} (Seidman et al., 1975; Schmidt & McClain, 1978). In this case these authors showed very clearly that in vivo the RNase P site at the 5′ end of tRNA^{Ser} is cleaved first and the other site is cleaved only after the first one, even though in vitro both sites are independently accessible but with a varying degree of efficiency; the middle site is more efficiently cleaved (Schmidt & McClain, 1978).

The results presented here also point to a very important feature of RNA molecules. They show that the stability of one part of an RNA molecule could be substantially affected by other parts of the molecule and that parts of a molecule could be more stable when they are removed from the mother molecule. This phenomenon of different stabilities of the same RNA sequence in different size RNAs is rather common and plays apparently an important role in maturation of RNAs. For instance, in the case of 5S rRNA in the precursor form, 9S RNA, it contains about 80 nucleotides at its 5′ end and a termination stem and loop at its 3′ end (Singh & Apirion, 1982). These regions of the 9S RNA precursor are relatively stable in the precursor form but are very unstable, especially the termination stem, after they are separated from the 5S rRNA (Misra & Apirion, 1979; Roy et al., 1983).

Comparing the T4 tRNA system to the rRNA system of *E. coli* is rather interesting since in both cases RNase III is not absolutely necessary for most of the processing to occur, and in both cases it seems that in the absence of RNase III this cleavage can be circumvented by a combination of degradation and/or trimming by nonspecific nucleases. The most interesting point is that even though the degradation and trimming are carried out by apparently nonspecific nucleases, the products in both cases are new kinds of RNA molecules, not found in the wild-type strain, but contrary to the common expectation they contain very specific ends rather than ragged ends (here the Ks RNA and, in the case of rRNA, the larger p16 and p23 rRNA precursors, p16b and p23b; Gegenheimer & Apirion, 1980). As pointed out above, this is very likely caused by the secondary–tertiary structure of the RNA that permits the nonspecific nucleases to cleave and trim only to a particular point.

In summary, the studies reported here show that the level of an RNA molecule could be determined by an interplay between precursor molecules and RNA processing and degradative enzymes.

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