BIOSYNTHESIS OF TRANSFER RNA*

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I. INTRODUCTION

The biosynthesis of transfer RNA (tRNA) is a multistep process which includes transcriptional and post-transcriptional events. The initial transcripts of tRNA genes are RNA molecules containing extra nucleotide sequences not present in tRNA. An important aspect of tRNA biosynthesis is therefore the conversion of the initial tRNA gene transcripts into mature functional molecules. This takes place via a series of enzymatic reactions termed RNA processing which include cleavage and trimming of tRNA precursors by endo- and exonucleases to tRNA size. The addition of the CCA sequence to the 3′ ends and the synthesis of modified nucleosides are also part of the precursor processing. Important advance in the understanding of tRNA gene organization and structure has been achieved in recent years with the help of the recombinant DNA[1] and the DNA sequencing techniques.[2,3] In prokaryotes, recent research has brought detailed information about the structure of several tRNA genes and their initial transcription products. It has also revealed the fact that some tRNA genes are associated with ribosomal RNA (rRNA) genes in a common transcription unit. In eukaryotes it was discovered that the phenomenon of noncolinearity of a gene with its product, generally observed in these systems,[4] applies also to some tRNA genes. It was found that several tRNA coding sequences are not continuous, containing intervening sequences of variable length. The removal of the intervening sequences was shown to take place at the tRNA precursor level and to involve a new type of RNA processing reaction called splicing, whose mechanism is not yet understood. The recent sequence analysis of several tRNA genes has helped in some cases to predict the structure of primary transcription products, which cannot always be detected in vivo, as well as the initial steps in the processing pathway. This, together with the information accumulated in intermediate precursor processing and the mode of action of several processing and nucleoside modifying enzymes, provides a better understanding of some aspects of tRNA precursor maturation.

Several excellent reviews on tRNA biosynthesis,[5,6] RNA processing,[7] and modified nucleosides in tRNA[8,9] have appeared in the last few years. In this article we shall describe general aspects of tRNA biosynthesis, concentrating on the recent advances in the study of gene organization and the structure of initial transcription products. Special attention will be given to the new information now available on eukaryotic tRNA biosynthesis.

* Abbreviations used: bp, base pair; kb, kilobase pair; ψ, pseudouridine; m^5U or T, 5′-methyluridine; D, dihydrouridine; Gm, 2′-0-methylguanosine; Cm, 2′-0-methylcytidine; m^1G, 1-methylguanosine; m^2A, 2-methyladenosine; m^7G, ^{7}methylguanosine; mam^5S^2U, 5-methylaminomethyl-2-thio-uridine; mo^5U, 5′-methoxyuridine; cmo^5U, uridine-5-oxyacetic acid; i^6A, N^6-isopentenyladenosine; t^6A, [N(9β-Dribofuranosylpurin-6-yl)carbamoyl)threonine].

II. ORGANIZATION OF tRNA GENES

Extensive knowledge has become recently available about the chromosomal location and organization of tRNA genes in prokoryotes, particularly in *Escherichia coli* and T4 phage. The location of some tRNA genes on the *E. coli* chromosome has been derived from genetic mapping of suppressor mutations, identified to occur in tRNA genes, which alter the anticodon of the tRNA and allow translation of nonsense and missense codons.[10-12] tRNA genes located near suppressor or ribosomal RNA genes can be mapped when carried together with these genes on the same transducing phage or plasmid. In this way genes coding for $tRNA_2^{Tyr}$ (*tyr*T) and $tRNA_3^{Thr}$ (*thr*T) were mapped near the missense suppressor suA36 derived from $tRNA_2^{Gly}$ when carried by bacteriophage λh80d*gly*Tsu_{36}^+.[13] Genes for $tRNA_1^{Ile}$, $tRNA_{1B}^{Ala}$, $tRNA_2^{Glu}$, $tRNA_1^{Asp}$, $tRNA^{Trp}$ have been located in rRNA operons (*rrn*) through the analysis of transducing phages and plasmids carrying rRNA genes.[14-18] Chromosomal locations of *E. coli* genes specifying 20 different tRNA species have been recently determined using the gene dosage effects caused by F′ factors and the amplified synthesis of tRNA after induction of lysogenes bearing λ integrated at different chromosomal sites.[19] The genetic mapping experiments show the tRNA genes to be distributed along the *E. coli* chromosome in a nonrandom fashion, some regions containing a higher density of tRNA genes.[19,20] Hybridization experiments have shown that *E. coli* DNA fragments, averaging 400 nucleotides, can accommodate two to three tRNA sequences.[21,22] This, together with biochemical analysis of multimeric tRNA precursor molecules isolated under special conditions in *E. coli* mutants,[23-25] has led to the conclusion that many *E. coli* tRNA genes are organized in clusters. Several tRNA gene clusters have been identified and shown to contain either different tRNA genes[13,26,27] or repeats of the same gene.[28,29]

The discovery that some tRNA genes are located within rRNA operons[14-18] has focused attention on the organization and expression of these genes. The (rRNA) genes in *E. coli* are organized in at least seven transcription units (rRNA operons) located at seven different chromosomal sites with the gene order 5′...16S-23S-5S...3′.[30] These rRNA operons were transposed either on plasmids or on λ phages and shown to contain genes for one or two tRNAs in the spacer region between the cistrons for 16S and 23S rRNAs. Out of the seven rRNA operons, four had $tRNA_2^{Glu}$ and the remaining three had both $tRNA_1^{Ile}$ and $tRNA_{1B}^{Ala}$ genes[14-16,31] (Table 1). These spacer tRNA genes are under the control of the rRNA promoter and are cotranscribed with the other rRNA genes.[17] The 30S rRNA precursor molecules, which accumulate in strains lacking functional RNase III and appear to be primary rRNA transcripts,[32-35] have been processed in vitro with *E. coli* extracts to produce $tRNA_1^{Ile}$, $tRNA_{1B}^{Ala}$ and $tRNA_2^{Glu}$.[36] Some rRNA operons have tRNA genes located at or near their distal end[18] in addition to the ones in the spacer region. For example, the *rrn*C operon has genes for $tRNA^{Trp}$ and $tRNA_1^{Asp}$ at its distal end (Figure 1); a gene for $tRNA_1^{Asp}$ is located at the distal end of group 1 rRNA operon and a gene coding probably for $tRNA^{Thr}$[18,36] is located at the end of the *rrn*D operon[18] (Table 1). There is evidence that the distal $tRNA^{Trp}$ $tRNA^{Asp}$ genes are in the same transcription unit as the other rRNA genes in the *rrn*C operon.[18] Different strains carrying hybrid plasmids with $tRNA^{Trp}$, $tRNA_1^{Asp}$ and parts of the *rrn*C operon overproduce the two tRNA species only when the promoter for *rrn*C is present. The rRNA operon carried by the transducing phage λ*ilv*5, which contains the genes for rRNAs, spacer tRNAs ($tRNA_1^{Ile}$ and $tRNA_{1B}^{Ala}$) and the two distal tRNAs ($tRNA_1^{Asp}$ and $tRNA^{Trp}$), is considered to be a hybrid rRNA operon probably formed by recombination between an unknown rRNA operon and the distal end of the *rrn*C operon.[31,16] Studies with deletion mutants in the promoter region of the rRNA operon from λ*ilv*5 or λ*ilv*5su7, the latter carrying an amber suppressor mutation in the $tRNA^{Trp}$

Table 1
tRNA GENES ASSOCIATED WITH rRNA OPERONS IN *E. COLI*[18,229]

Group	Locus	Map location (min)	Spacer tRNA gene	Distal tRNA gene
I		unmapped	Ile, Ala	Asp
II	*rrn*D	71	Ile, Ala	Thr
III	*rrn*A	85	Ile, Ala	
IV	*rrn*B	88	Glu	—*
V		unmapped	Glu	
VI	*rrnC*	83	Glu	Asp Trp
VII	*rrn*E	89	Glu	

* A four-tRNA gene cluster (Thr, Tyr, Gly, Thr)[26,27] located close to the distal end of *rrn*B operon was found to be transcribed independently.[27]

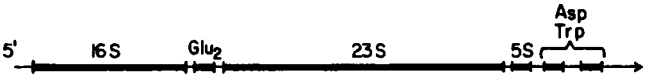

FIGURE 1. Organization of the rRNA operon *rrnC* from *E. coli*. The total length of the transcription unit shown is about 5500 bp.[18] The arrow indicates the direction of transcription.

gene, also show that the expression of the distal $tRNA^{Trp}$ gene is dependent on the promoter of the rRNA operon and that the $tRNA^{Trp}$ gene is cotranscribed with the rRNA and spacer tRNA genes of the operon.[37] The significance of the association of several tRNA genes with rRNA operons is not clear. There is no apparent correlation between the types of tRNA genes located in the spacer region and at the distal end of an rRNA operon (Table 1). Although all the rRNA operons so far analyzed have one or two types of spacer tRNA gene arrangements (either $tRNA_2^{Glu}$ or both $tRNA_1^{Ile}$ and $tRNA_{1B}^{Ala}$), not all rRNA operons seem to have tRNA genes at their distal ends. In addition, one of the two distal $tRNA_1^{Asp}$ genes is associated with an rRNA operon which contains $tRNA_1^{Ile}$ and $tRNA_{1B}^{Ala}$ as spacer while the other is associated with the *rrnC* operon which contains $tRNA_2^{Glu}$ gene as spacer.

Extensive studies have been carried out on the structure and organization of the genes coding for the two tyrosine-accepting tRNA species $tRNA_1^{Tyr}$ and $tRNA_2^{Tyr}$ in *E. coli*. The $tRNA_1^{Tyr}$ species differs in nucleotide sequence from $tRNA_2^{Tyr}$ by only two bases in the variable loop region.[38] There are two identical genes (*tyr*T) coding for $tRNA_1^{Tyr}$ and one gene (*tyr*U) coding for $tRNA_2^{Tyr}$. The *tyr* T locus is located at 27 min on the genetic map;[19,20,39,40] *tyr*U is located at 88 min, in close vicinity to gene *gly*T ($tRNA_2^{Gly}$) and *thr*T ($tRNA_3^{Thr}$).[13,19,20,41,42] The two $tRNA_1^{Tyr}$ genes, carried as a "doublet" on transducing phage $\phi 80psu_3^{+\prime -}$ have been studied by restriction enzyme analysis and DNA sequencing and were shown to consist of two 85 bp (base pair) tRNA sequences separated by a 200 bp intergenic spacer.[43-45] A "singlet" $tRNA_1^{Tyr}$ gene structure carried by $\phi 80psu_3^{+}$ was shown to arise from the $\phi 80u_3^{+\prime -}$ doublet by an unequal recombination event involving the two identical $tRNA^{Tyr}$ sequences through the loss of one of the two 85 bp $tRNA_1^{Tyr}$ sequences and the 200 bp intergenic sequence.[40,45,46] The results of in vivo and in vitro $tRNA_1^{Tyr}$ transcription studies suggest that the two $tRNA_1^{Tyr}$ genes are included in the same transcription unit.[47-49] The $tRNA_2^{Tyr}$ gene is tightly associated in the *E. coli* chromosome with three other tRNA

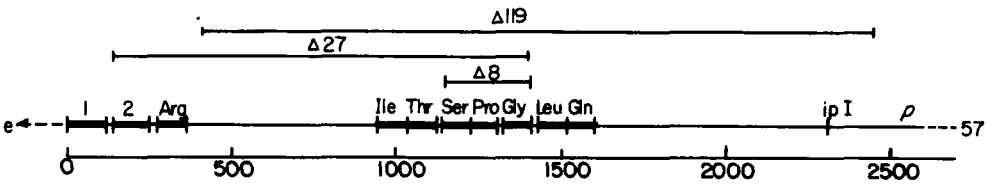

FIGURE 2. Organization of T4 tRNA genes. The arrow indicates the direction of transcription and the scale gives distances in base pairs. The sizes of the T4 tRNA deletions Δ119,Δ27, and Δ8 shown at the top of the figure are 2070 ± 190, 1330 ± 100 and 200 ± 30 bp, respectively.[60] A promoter for T4 tRNA transcription, located upstream from ip I gene at a distance of about 1000 bp from the large tRNA gene cluster,[71] is indicated as *p*.[69,70]

genes to form the cluster *Thr*U, *tyr*U, *gly*T, *thr*T coding for $tRNA_4^{Thr}$, $tRNA_2^{Tyr}$, $tRNA_2^{Gly}$ and $tRNA_3^{Thr}$, respectively.[26,27] Transducing bacteriophage λ*rif*18[50] carries the tRNA gene cluster in the same arrangement and orientation (relative to transcription) as in the *E. coli* chromosome:[26,45,46] 5'...$tRNA_4^{Thr}$-8bp-$tRNA_2^{Tyr}$-115bp-$tRNA_2^{Gly}$-6 bp-$tRNA_3^{Thr}$. Although located at the distal end of the *rrn*B ribosomal operon, the four tRNA genes are not cotranscribed with the rRNA genes and seem to have their own promoter.[27] Transducing bacteriophage λh80d*gly* Tsu36^{+13} carries a $tRNA_2^{Tyr}$, $tRNA_2^{Gly}$, $tRNA_3^{Thr}$ gene cluster. But for the absence of the $tRNA_4^{Thr}$ gene, this cluster is identical to that carried by λ*rif*18 phage.[26,46] Recent DNA sequence studies have demonstrated that the region upstream of $tRNA_2^{Tyr}$ on λh80d*gly*Tsu36$^+$ phage is identical to the region upstream of $tRNA_1^{Tyr}$ gene on ϕ80psu3$^+$ phage.[46] It appears that the λh80d*gly*Tsu36$^+$ transducing phage carries a hybrid structure composed of "upstream" sequences from the $tRNA_1^{Tyr}$ gene cluster and the "downstream" sequences from the $tRNA_2^{Tyr}$ gene cluster, an artifact probably generated during the isolation of this transducing phage.[26,45,46]

Several *E. coli* bacteriophages, the T-even group (T_2, T_4, T_6, RB69) and T_5, carry tRNA genes that are expressed upon host infection.[51-59] Bacteriophage T4, which has been the most extensively studied, carries genes coding for eight tRNA and two other stable RNA species of unknown function.[60-62] The nucleotide sequences of all the T4 tRNAs and of species 1 RNA have been determined.[61,63-68] A set of T4 mutants, carrying deletions in the tRNA region, have allowed the mapping of the tRNA genes as a cluster located between genes *e* (lysozyme) and 57 on the T4 genome.[60,61] Recently, using endonuclease digestion and *in situ* hybridization of the DNA fragments with individual [(^{32}P)]-labeled T4 tRNAs, a restriction map of the T4 tRNA gene cluster and its control region has been constructed.[69] The map of the T4 tRNA region is shown in Figure 2. The tRNA genes are organized within a distance of 1600 bp in two clusters. A large cluster containing seven tRNA genes is located about 600 bp away from a second cluster which contains the genes for $tRNA^{Arg}$ and the two stable RNAs (species 1 and species 2). The T4 tRNA region was recently cloned[70,71] and the two tRNA gene clusters were sequenced.[72] The complete sequence of a DNA fragment of about 600 bp which includes the genes from $tRNA^{Gln}$ to $tRNA^{Ile}$ has shown that the T4 tRNA sequences are organized in a very compact structure.[74] Early evidence of clustering of the tRNA genes had suggested that the T4 tRNA region may constitute a single transcription unit.[60] While kinetics of in vitro synthesis of T4 tRNAs support this hypothesis,[73] attempts to isolate a large multimeric precursor from *E. coli* infected cells were not successful. It is probable that in vivo transcription is tightly coupled with processing and that cleavage takes place before transcription of the entire T4 tRNA region is completed. However, in a nuclease-free in vitro system, a polycistronic T4 tRNA precursor was recently isolated among T4 tRNA transcripts.[74] Further evidence that the T4 tRNA genes are included in a single transcription unit has been obtained with the

help of molecular cloning.[71] Recombinant λ-T4 phages containing a 5000 bp *Eco*R1 restriction fragment that encodes for the larger one of the two T4 tRNA gene clusters have been constructed. Difficulties encountered in the cloning of this T4 fragment have suggested that a small region close to the T4 tRNA genes, probably the ipl gene (internal protein I),[75] codes for a "lethal" function which has an inhibitory effect on the cell growth. Deletion of this "lethal" gene has allowed the location of a promoter, about 1000 bp upstream from the $tRNA^{Gln}$ gene, which directs the transcription of the T4 tRNA genes[71] (Figure 2). λ-T4 hybrid phages which had the intact T4 DNA fragment were shown to induce tRNA synthesis in infected cells, while little T4 tRNA synthesis was observed with λ-T4 phages which have the deletion. The presence of a promoter in the deleted region was also confirmed by RNA polymerase binding experiments.[71] Clustering of tRNA genes seems to be common in bacteriophages and evidence shows that in the genome of T5 phage about 20 tRNA genes are located in three regions within a 13,800-nucleotide-long DNA segment.[76]

In eukaryotes the number of tRNA genes is much larger than that observed in bacteria. There are about 600 genes coding for tRNA in *Drosophila melanogaster*[77] and 8000 in *Xenopus laevis*[78-80] compared to 60 tRNA genes in *E. coli*.[21,81,82] Since the number of tRNA species is not significantly larger than in bacteria, this implies that in eukaryotes the tRNA genes exist in multiple copies. From hybridization kinetics an average of 10 genes for each tRNA sequence was calculated in *Drosophila*[77] and about 200 in *Xenopus*.[80] The 300-fold reiterated genes for *Xenopus* $tRNA_i^{Met}$ were shown to be clustered[83] and organized in tandem repeats of 3.18kb.[84] Cloning and analysis by restriction endonuclease of such a repeat have shown that it contains two genes coding for $tRNA_i^{Met}$ and at least one other 4S RNA species separated by spacers of about 0.3 kb.[84] Presently it is not clear whether the clustering of identical tRNA genes found in *Xenopus* genome applies to other eukaryotes. *In situ* hybridization studies with purified tRNAs on the *Drosophila* polytene chromosomes suggest that some tRNA genes coding for the same tRNA species are distributed at different sites on the chromosome[85,86] while other tRNA genes seem to be clustered at a single chromosomal site as in *Xenopus*.[87,88] A cloned 9.3-kb *Drosophila* DNA fragment derived from a unique chromosomal site was shown to contain a repeat of three identical tRNA genes, coding for $tRNA_2^{Lys}$, separated by large spacers and two other tRNA genes coding for $tRNA^{Arg}$ and $tRNA^{Asn}$.[89,90] Evidence from gene cloning[91] and uv light mapping experiments[92] suggests that in the yeast *Schizosacharomyces cerevisae* genome the tRNA genes are not significantly clustered and possess a rather widely spaced distribution. The genes coding for $tRNA^{Tyr}$ have been located at eight different loci on several chromosomes[93] and digestion of yeast DNA with endonuclease $Eco R_1$ has produced eight distinct and separable fragments capable of hybridization with $tRNA^{Tyr}$.[94] That a limited clustering may exist in yeast is revealed by the analysis of a cloned DNA fragment in which the genes for $tRNA_3^{Arg}$ and $tRNA^{Asp}$ were found located in the same orientation and separated by a 9-bp spacer.[95] DNA sequencing of three cloned yeast $tRNA^{Tyr}$ genes has demonstrated that these genes all contain next to the 3′ side of the anticodon triplet a 17-bp intervening sequence which is not present in mature $tRNA^{Tyr}$[96] (Figure 3). In two of the $tRNA^{Tyr}$ genes the 14-bp sequence is identical, while in the third it differs by a single base pair. Similarly, short intervening sequences (18 or 19 bp) different from the $tRNA^{Tyr}$ inserts have been demonstrated next to the anticodon loop in three cloned $tRNA^{Phe}$ genes[97] (Figure 3). Several other tRNA genes from yeast, $tRNA^{Ser}_{UCG}$, $tRNA^{Trp}$, and $tRNA_3^{Leu}$ also contain intervening sequences.[98-100] The presence of intervening sequences in tRNA genes is an example of the phenomenon of noncolinearity between a gene and its product. While noncolinearity seems to be the general rule in eukaryotic genes coding for mRNA,[4] not all the eukaryotic tRNA genes so far examined contain intervening sequences.[84,95,98] In yeast the genes coding for $tRNA_3^{Arg}$,

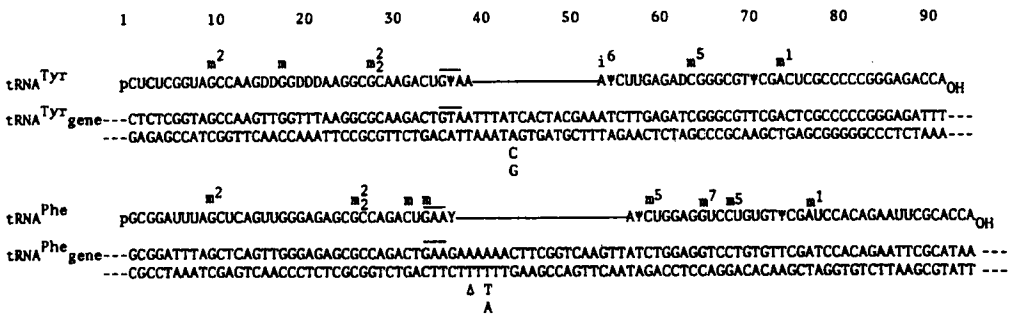

FIGURE 3. Nucleotide sequences of yeast tRNA^{Tyr} and tRNA^{Phe} genes and their corresponding tRNAs. The anticodon and its coding triplet are overlined in both the tRNA and the gene sequences. The position of the intervening sequence is indicated by the line interrupting the tRNA sequence. Note that the CCA residues present at the 3′ end of the mature tRNA molecules are not encoded for by the DNA.[96,97,240]

tRNA^{Asp}, tRNA$_2^{Ser}$, and tRNA$^{Ser}_{UAG}$ do not contain any inserts.[95,99] In eukaryotes, with the possible exception of yeast tRNA$_3^{Arg}$-tRNA^{Asp} genes,[100] there is no evidence as yet for multimeric tRNA transcription units. The 90 to 100 nucleotide tRNA-precursor molecules detected in yeast,[101,102] human KB cells,[103] or *Bombyx mori*[104,105] contain only one tRNA sequence, suggesting that eukaryotic tRNA genes are generally transcribed into monocistronic RNA molecules. This may reflect the different organization of eukaryotic tRNA genes, although it cannot be completely excluded that some of the tRNA precursors observed have already undergone partial processing or degradation during isolation.

Cell organelles such as mitochondria and chloroplasts contain specific tRNAs and tRNA genes.[106] Mitochondria from yeast contain about 20 tRNA genes, 17 of which are located in a narrow region representing less than 20% of the genome.[107] The mitochondrial genome of *Neurospora crassa* has been shown to contain about 25 genes for tRNA, clustered in two regions of the circular mtDNA molecule.[108] One of the clusters maps in a 4-kb spacer region between the 17S and 24S rRNA genes.[109] There are about 15 sites hybridizing with tRNA in mitochondrial DNA from *Xenopus* and about 19 in that from HeLa cells. Electron microscopic mapping of tRNA-DNA hybrids in HeLa cell and *Xenopus* mitochondrial DNA has shown that the tRNA cistrons are widely spaced, distributed uniformly around the genome, and transcribed from both strands.[110,111] Recently the location of tRNA hybridization sites along the chloroplast DNA has been determined in *Chlamydomonas*[112] and spinach.[113]

III. NUCLEOTIDE SEQUENCE OF tRNA PRECURSORS

A great deal of information concerning the organization of tRNA genes in transcription units and different aspects of tRNA biosynthesis such as precursor processing and base modifications has been obtained from an examination of nucleotide sequences of precursor molecules. Although precursors to tRNA molecules were first detected in mammalian cells,[114] most of our knowledge about their maturation into tRNAs has come from studies with microorganisms. In bacteria, where mutant isolation and highly radioactive labeling are possible, several tRNA precursors have been isolated and their nucleotide sequences determined. Recently, several yeast tRNA precursors were detected and analyzed with the help of a yeast temperature sensitive (ts) mutant.[102]

Transcription in vitro of tRNA genes by nuclease-free RNA polymerase affords a way of obtaining intact tRNA precursors.[48,74] These molecules, which cannot be detected in vivo because of their fast cleavage, are useful for the study of the enzymes involved in the first steps of tRNA precursor processing.

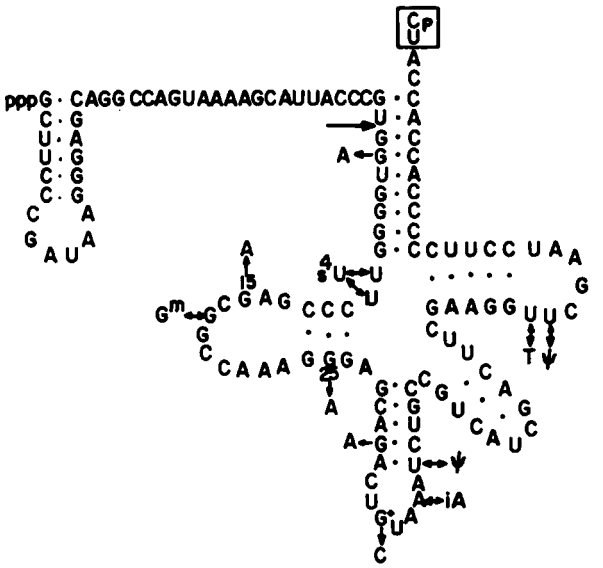

FIGURE 4. Nucleotide sequence of *E. coli* $tRNA_1^{Tyr}$ precursor. The site of RNase P cleavage is indicated by a large arrow and the 3′ extra nucleotides are boxed.[116] Small arrows indicate the anticodon su_3^+ (G→C) mutation and base-substitution mutations which decrease the tRNA yield.[151] Double head arrows show the positions of the modified nucleosides found in the mature tRNA molecule.[38]

A. *E. coli* tRNA Precursors

The extremely short half-life of tRNA precursors in normally growing bacteria makes their isolation and characterization very difficult. One way to increase the production of specific tRNA precursors takes advantage of transducing phage carrying tRNA genes.[115-117] Upon infection with these phages or induction of their prophage, the transcription of the transduced tRNA genes is highly amplified, due to the large number of tRNA genes in the cell resulting from phage DNA replication. In another approach, tRNA precursor molecules are caused to accumulate in *E. coli* as a result of mutations which block or slow down their processing by an alteration of the tRNA precursor structure or the processing enzymes. Thus, bacterial tRNA precursors were discovered in the study of a ϕ80 phage carrying a mutationally altered $tRNA_1^{Tyr}su_3^+$ gene, defective in suppressor activity.[115,116] Several $tRNA_1^{Tyr}$ mutants, selected for defective suppressor activity, were found to carry single base substitution in the $tRNA_1^{Tyr}$ gene and to transiently accumulate a $tRNA_1^{Tyr}$ precursor[115,116] (Figure 4). The mutations in the tRNA gene appear to alter the conformation of the tRNA precursor so that it cannot be normally processed. This example emphasizes the importance of precursor conformation for its recognition by the processing enzymes. Precursors to tRNAs may also accumulate as a result of mutations which alter the processing enzymes. Temperature sensitive (ts) *E. coli* mutants have been isolated which accumulate tRNA precursors at the nonpermissive temperature.[118-120] The best characterized mutants harbor a ts RNase P, an endonuclease that cleaves tRNA precursor at the 5′ end of the mature tRNA sequence.[121] When a culture of *E. coli* cells carrying a ts RNase P is incubated at the nonpermissive temperature in the presence of radioactive phosphate, a large number of [(^{32}P)]-labeled tRNA precursor species accumulate. These can be separated by electrophoresis in acrylamide gel and further characterized. The ts RNase P mutants accumulate precursors ranging in molecular sizes from slightly

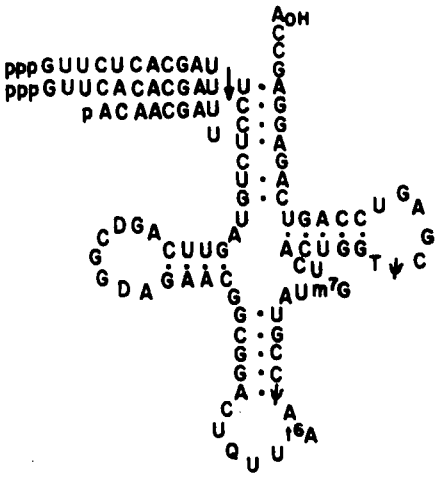

FIGURE 5. Nucleotide sequences of precursors to *E. coli* tRNA^{4m}. The four precursor species contain different 5′ leader sequences and mature 3′ ends. All the modified nucleosides of mature tRNA^{4m} are present in the precursors. The arrow indicates the site of RNase P cleavage (From Vogeli, G., Stewart, T. S., McCutchan, T., and Soll, D., *J. Biol. Chem.*, 252, 2311, 1977. With permission.)

larger than mature tRNA to about 500 nucleotides in length.[23,24,122-124] The fact that all tRNAs accumulate as precursors at the nonpermissive temperature in ts RNase P mutants indicates that RNase P plays an essential role in the biosynthesis of all tRNA in *E. coli*. Several types of precursors were observed to accumulate in ts RNase P mutants — small monomeric (4.0 to 4.5S), large monomeric (4.5 to 5.5S), dimeric, and multimeric precursors.[124] The small monomeric precursors which are the predominant type have only a few extra nucleotides at the 5′-termini of the corresponding tRNA molecules. Since the majority of these precursors have ribonucleoside monophosphates at the 5′ end, they appear to be derived from the internal or 3′-proximal regions of multimeric precursors or from the larger monomeric precursors as a result of processing reactions.[124] The larger monomeric precursors like tRNA$_1^{Tyr}$ precursor (Figure 4) have longer stretches of extra nucleotides and ribonucleoside triphosphates at their 5′ termini, and are probably transcripts of monomeric cistrons or derived from the 5′ proximal sites of multimeric precursors. Multimeric precursors containing as many as 5 to 7 tRNA sequences within a single molecule have been detected.[23,24,122-124] These multimeric precursors are evidently derived from clusters of tRNA genes, sometimes identical species repeated in tandem, which are transcribed as a unit. Their study provides us with some information on the organization of tRNA genes in *E. coli*. The small number of multimeric precursors so far identified probably represents only a fraction of the total multimeric precursors transcribed in *E. coli*. These molecules are metabolically unstable and are rapidly processed by endonucleolytic activities, probably RNase P2[122] or RNase O[125], to monomers and dimers. A complete nucleotide sequence has been determined only for a few *E. coli* tRNA precursors.[116,117,126,127] (Figure 5 and Figure 6). All *E. coli* tRNA precursors so far sequenced contain - CCA either as a mature 3′ end or followed by another sequence.[116,117,122,124,126,127] RNA precursors accumulating in ts RNase P mutant at the nonpermissive temperature contain some

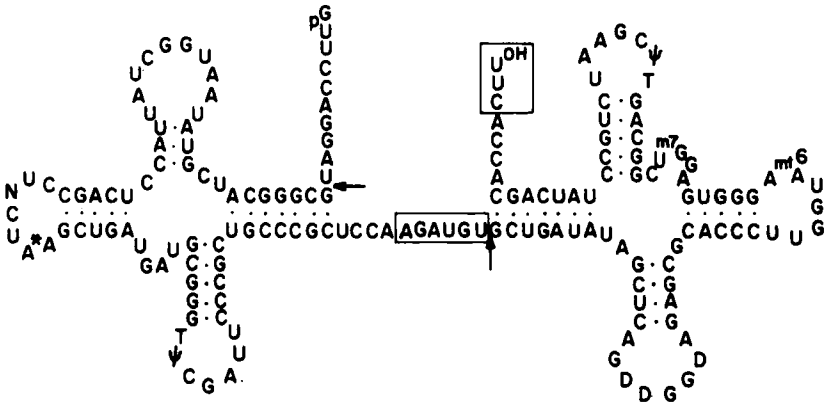

FIGURE 6. Nucleotide sequence of the dimeric $tRNA_2^{Gly}$ - $tRNA_3^{Thr}$ precursor of *E. coli*. Arrows indicate the RNase P cleavage sites and 3′ end nucleotides not found in the mature tRNA are boxed. (From Chang, S. and Carbon, J., *J. Biol. Chem.*, 250, 5542, 1975. With permission.)

modified nucleosides such as ψ,T(m⁵U), D, m²A and always lack the Gm modification.[124]

B. Bacteriophage T4 tRNA Precursors

Three dimeric T4 tRNA precursors have been isolated from *E. coli* cells infected with bacteriophage T4: $tRNA^{Gln}$-$tRNA^{Leu}$, $tRNA^{Pro}$-$tRNA^{Ser}$, and $tRNA^{Thr}$-$tRNA^{Ile}$. The sequences of all these precursors have been determined[65,128,129] (two of them are presented in Figure 7). The following features seem to characterize the structure of the dimeric precursors:

1. All have a nucleoside monophosphate at their 5′ end, indicating that they may be derived from a larger RNA transcript by nucleolytic cleavage.
2. They contain 5′ leader sequences of variable length which have no sequence homology and are composed of A and U residues only.
3. All three precursors have either no spacer or only one nucleotide between the two tRNA moieties and very short (0 to 3) 3′ extra nucleotides which are again composed of A and U residues only.
4. All modified nucleoside residues of the mature tRNAs are present in the precursors with the exception of the Gm modification in the 3′ distal tRNAs.

RNase P appears to be responsible for the 5′ end maturation of all the T4 tRNAs.[61,62,130] When ts RNase P mutants are infected with bacteriophage T4 at the nonpermissive temperature, no mature T4 tRNAs are produced. Instead, the dimeric precursors to $tRNA^{Gln}$-$tRNA^{Leu}$ and $tRNA^{Pro}$-$tRNA^{Ser}$ and the monomeric precursors to $tRNA^{Gly}$, $tRNA^{Thr}$, and $tRNA^{Arg}$ accumulate. No accumulation of the $tRNA^{Thr}$-$tRNA^{Ile}$ dimeric precursor is observed, probably due to an alternative pathway of processing.[129] RNase P participates also in the maturation of RNA species 1 at its 5′ end and precursors of this RNA also accumulate in RNase P mutants.[61]

The T4 tRNA precursors do not always contain the CCA sequence at the 3′ end. This implies that in some precursors the CCA must be added as part of their maturation process. A striking difference exists between the three dimeric T4 tRNA precursors concerning the presence of the CCA at the 3′ end of the tRNA sequences. In $tRNA^{Pro}$-$tRNA^{Ser}$ both tRNA sequences lack CCA ends[65]; in $tRNA^{Gln}$-$tRNA^{Leu}$ only $tRNA^{Leu}$ has a complete CCA sequence;[128] and in $tRNA^{Thr}$-$tRNA^{Ile}$ only the 5′ proximal $tRNA^{Thr}$

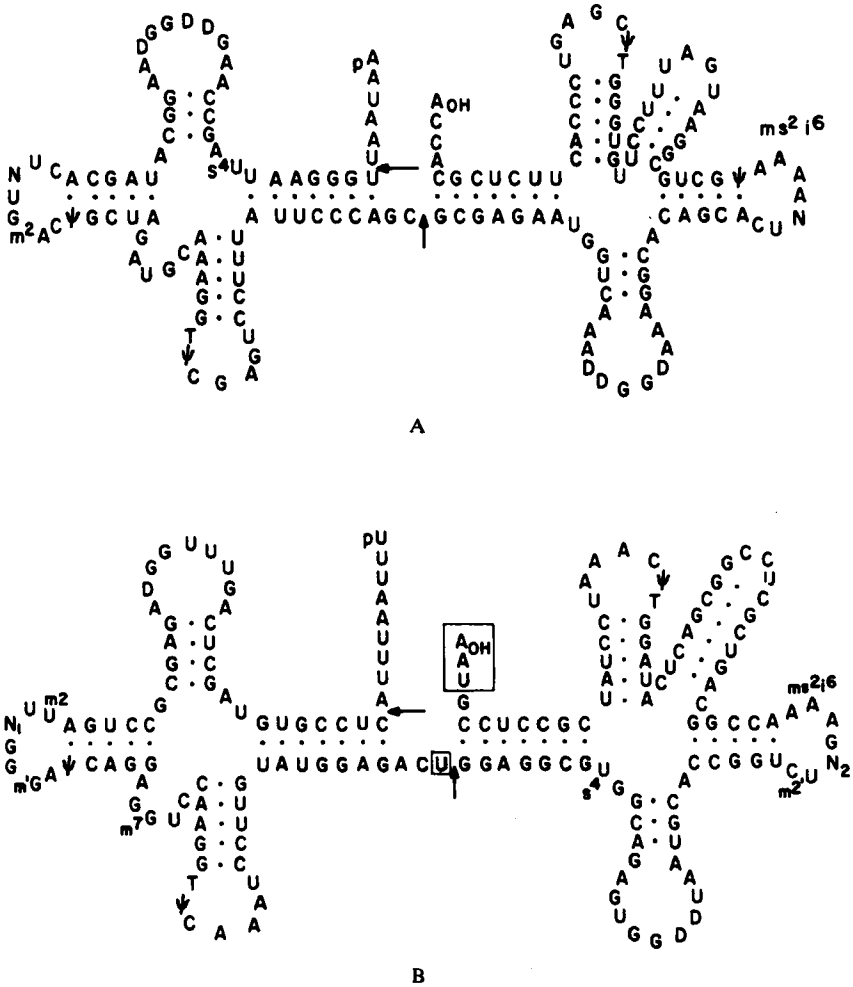

FIGURE 7. Nucleotide sequence of two dimeric T4 tRNA precursors. (A) Precursor to $tRNA^{Gln}$ - $tRNA^{Leu}$; (B) precursor to $tRNA^{Pro}$ - $tRNA^{Ser}$. Arrows indicate the RNase P cleavage sites and the 3′ end nucleotides not found in the mature tRNAs are boxed. (With permission from Guthrie, C., *J. Mol. Biol.*, 95, 529, 1975 and Seidman, J. C., Barrell, B. G., and McClain, W. H., *J. Mol. Biol.*, 99, 733, 1975. Copyright by Academic Press, Inc. (London), Ltd.)

contains the CCA end.[129] In view of the fact that the synthesis of CCA at the 3′-terminus of dimeric precursor is prerequisite for its cleavage by RNase P[131,132] the disposition of the CCA sequence in the dimer seems to determine the processing pathway of each of the precursors.[72,129] This aspect will be discussed in a following section.

C. Precursor tRNAs in Eukaryotes

Precursors to tRNAs have been detected by pulse-labeling experiments in various eukaryotic systems (for a review see Reference 114.) These are small RNA molecules about 100 nucleotides long, which contain a single tRNA sequence with extra nucleotides at both ends. Only recently individual RNA species with properties of tRNA precursors have been isolated from silkworms[133] and yeast[102] and purified to radiochemical purity. Some of the tRNA precursors derived from silk worms still have the transcription initiator purine nucleoside triphosphate at their 5′ termini and contain

modified and methylated bases typical of tRNA.[133] In yeast pulse-labeled with [(^{32}P)] orthophosphate, a large number of tRNA precursors of different sizes (4 to 5.8 S) have been identified. These can be converted into tRNA-size molecules by incubation with yeast cell-free extracts.[101] In yeast as in silkworms, it was suggested that the smaller size precursors were derived from the larger ones in the process of maturation.[101,134] A yeast mutant, ts 136, described initially as conditionally defective in transport of mRNA from the nucleus to the cytoplasm,[135] was recently shown to accumulate tRNA precursors at the nonpermissive temperature.[102] When ts 136 mutant was grown at 35.5°C in the presence of radioactive phosphate, about 12 tRNA precursors accumulated in a pattern of species similar to that obtained in the pulse-labeling experiments of wild-type yeast.[101] The [(^{32}P)]-labeled precursor tRNAs were separated by two dimensional polyacrylamide gel electrophoresis in a number of spots in the 4.5 to 5.8 S size range. The RNA from each spot was eluted and characterized by hybridization to a set of *E. coli* recombinant clones, each of which carrying one or more defined yeast tRNA genes.[91] In this way several tRNA precursors which accumulated in the yeast mutant ts 136 at the nonpermissive temperature were identified as precursors for $tRNA^{Phe}$, $tRNA^{Tyr}$, $tRNA_3^{Leu}$, $tRNA^{Trp}$, $tRNA^{Ser}_{UCG}$[98]. In addition, three other tRNA precursor spots were found to hybridize to a unique set of clones containing as yet unidentified tRNA genes.[98] It should be observed that in this mutant only a very restricted set of tRNA precursor species accumulate and it was nt possible to detect precursors for most of the yeast tRNAs. Sequence analysis of the precursors for $tRNA^{Phe}$ and $tRNA^{Tyr}$ was carried out by RNase T1 and pancreatic RNase digestion and fingerprinting. It was found that the precursors to both $tRNA^{Tyr}$ and $tRNA^{Phe}$ contain intervening RNA sequences, do not have extra nucleotides at their 5′ ends, and that their 3′ ends contain the uncoded CCA termini.[98,136] The precursors contain most of the modified nucleosides with the exception of Gm or Cm and the hypermodified bases Y and i^6A. Two intervening sequences differing by one base pair substitution have been observed in the three cloned $tRNA^{Tyr}$ genes[96] and both are present in $tRNA^{Tyr}$ precursors. Similarly, $tRNA^{Phe}$ precursors contain the nucleotide stretches derived from the two types of intervening sequences of the respective $tRNA^{Phe}$ genes.[97] The precursors to $tRNA^{Ser}_{UCG}$, $tRNA_3^{Leu}$ and $tRNA^{Trp}$, which also accumulate in the ts 136 mutant at the restrictive temperature, all contain intervening sequences.[98] Fingerprint analysis of the precursors to $tRNA^{Ser}_{UCG}$,[99] $tRNA^{Trp}$ [100], and $tRNA_3^{Leu}$ [95] shows that they also have mature 5′ and 3′ ends and an RNA intervening sequence located near the 3′ end of the anticodon loop. It may be concluded that the only extra sequences contained in the 4.5 S precursors which accumulate in ts 136 mutant are the intervening sequences. These precursors are not primary transcripts, since antecedent processing events have already produced mature 5′ and 3′ ends and introduced modified nucleosides. If these RNA molecules are to serve as precursors to tRNAs, the intervening RNA sequences must be removed by an enzymatic activity which would excise very specifically a segment of the polynucleotide chain and rejoin the ends. Evidence for the existence of such an excision-ligation activity in yeast was recently presented.[98,136] It should be observed that precursors for tRNAs whose DNA sequences do not contain intervening sequences, like $tRNA_3^{Arg}$, $tRNA^{Asp}$, $tRNA_2^{Ser}$, and $tRNA^{Ser}_{UCA}$ are not found to accumulate in the ts 136 mutant.[95,99]

Figure 8 shows the secondary structure proposed for the precursors to $tRNA^{Tyr}$ and $tRNA^{Phe}$.[98] The precursor structures obtained by maximization of base pairing retain the general features of the tRNA cloverleaf model. They diverge from it in the anticodon stem, which is extended by the additional base pairs formed with the precursor specific intervening sequences. A common feature shared by the two tRNA precursors and also by the precursors to $tRNA^{Ser}_{UCG}$[99] and $tRNA^{Trp}$,[100] is an intervening sequence rich in A and U which can form base pairs with the respective tRNA anticodon. The

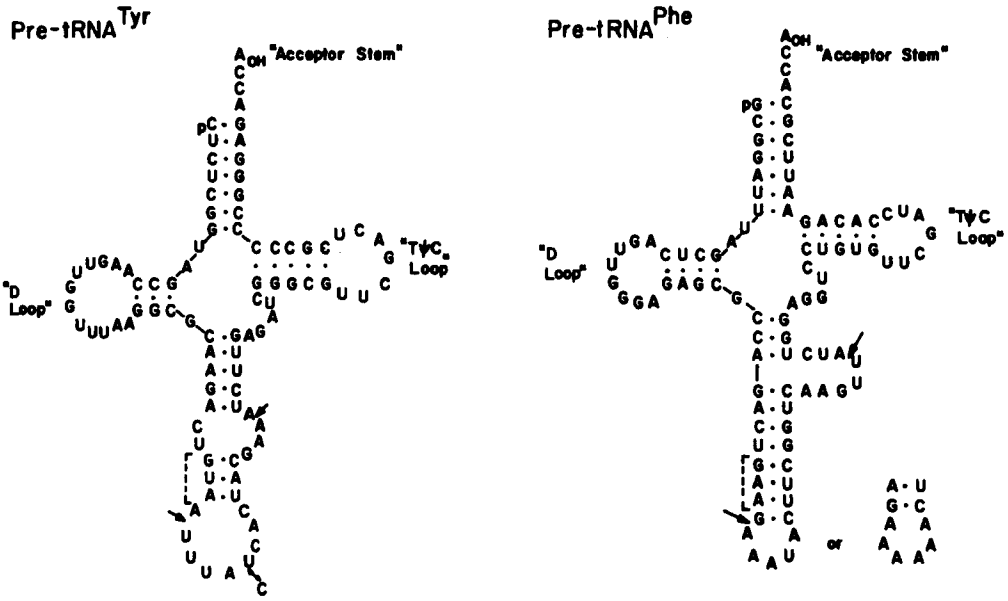

FIGURE 8. Possible secondary structures of precursors to yeast tRNA ^{Tyr} and tRNA ^{Phe}. Arrows indicate the splice points of the intervening sequence. (From Knapp, G., Beckmann, J. S., Johnson, P. F., Fuhrman, S. A., and Abelson, J., *Cell*, 14, 221, 1978. With permission. Copywright MIT. Published by The MIT Press, Cambridge.)

interaction of the anticodon with the intervening sequence allows the formation of two loops which contain the putative sites of enzymatic excision and ligation. Evidence for the existence of such precursor structures may be obtained from an analysis of precursor sensitivity to S1 nuclease[136] or limited RNAse T1 digestion.[99] It was shown that the anticodon of the precursors, unlike that of mature tRNAs, is not susceptible to enzymatic attack and that the cleavage takes place in the looped region of the intervening sequence.

D. In Vitro Synthesis of tRNA Precursors

Transcription of tRNA genes in vitro by purified RNA polymerase produces completely unmodified tRNA precursors and provides an opportunity to study in detail the maturation process of primary transcripts. Studied first in a system consisting of $\phi 80su_3^+$DNA, purified *E. coli* RNA polymerase, and ρ factor, the $tRNA_1^{Tyr}$ gene transcription product was analyzed by competitive hybridization or acrylamide gel electrophoresis and found to be larger than a 4S tRNA molecule.[137,138] Subsequently, the $tRNA_1^{Tyr}$ gene was transcribed from whole $\phi 80psu_3^+$DNA[48,139-142] or DNA restriction fragments[49,143] by several investigators who have characterized a transcription product of variable size (up to 350 nucleotides), but always larger than that of the $tRNA_1^{Tyr}$ precursor (129 nucleotide) isolated in vivo.[116] The size variability of the $tRNA_1^{Tyr}$ precursors synthesized in vitro was probably due to nuclease contaminations in the RNA polymerase preparations. With a nuclease-free RNA polymerase a 350 nucleotide precursor was transcribed from a single $tRNA_1^{Tyr}$ gene,[49,143] while a precursor of about 600 nucleotide was obtained from a $\phi 80su_3^+$-DNA which carried both $tRNA_1^{Tyr}$ genes.[48,144] Similarly, transcription of tRNA genes carried by the DNA of $\lambda 80dglyTsu_{36}^+$ ($tRNA_2^{Tyr}$, $tRNA_2^{Gly}su36^+$, $tRNA_3^{Thr}$) was shown to produce a large size (~8 S) polycistronic tRNA transcript.[48,145] The fidelity of the in vitro transcription was demonstrated by the processing of the synthesized tRNA precursors with crude *E. coli*

extracts into mature tRNAs.[48,49] These tRNA molecules were identified by the fingerprint patterns of their RNase T1 digests.[48,146] The primary transcripts of tRNA genes, isolated by electrophoresis on acrylamide gels, have been used as substrates for the study of enzymes involved in precursor maturation.[146] In a different approach, $tRNA_1^{Tyr}$ synthesis was studied in vitro, without isolation of the primary transcription product, by following the formation of a su_3^+ $tRNA_1^{Tyr}$ active in the suppression of amber mutations.[139,140] By separation and combination of fractions from crude cell extracts and isolation of intermediate precursors, several nuclease activities which participate in the processing of $tRNA_1^{Tyr}$ precursor have been identified.[140]

Transcription in vitro of the T4 tRNA genes followed by processing of the unfractionated product by crude cell extracts was shown to produce T4-specific tRNAs.[147] Although the T4 tRNA gene cluster was inferred to be transcribed in vitro as a polycistronic RNA product,[73] the size of this transcript and the presence of a multitude of other T4 DNA transcripts have been an obstacle to its isolation. Recently, transcription of DNA from wild type T4 and T4 mutants containing deletions in the tRNA region followed by separation of products by polyacrylamide gel electrophoresis allowed the identification of the T4 tRNA precursor among the high molecular weight RNA transcripts. Figure 9 shows that the T4 tRNA precursor is present only in the transcript from wild-type T4 DNA and is missing from the transcripts of the deletion mutants. Instead, new RNA species have appeared (indicated by small arrows) which were found to contain the remaining tRNA sequences of the tRNA gene cluster shortened by the deletions.[74] The T4 tRNA precursor, which is about 5000 nucleotides long, was isolated and processed into mature size tRNA molecules.[74] This precursor could be useful for the detection of the nucleolytic activities which generate the T4 tRNA dimeric and monomeric precursors observed in vivo.

IV. PROCESSING OF tRNA PRECURSORS

Much of the knowledge concerning the processing pathway of tRNA precursors from the initial transcript to mature tRNA molecules came from the study of *E. coli* $tRNA_1^{Tyr}$ and bacteriophage T4 tRNA biosynthetic systems. Partially processed tRNA precursors have been isolated and used as substrates for the detection and purification of several processing enzymes. These include endo- and exonucleases which bring the transcript to its mature size, tRNA-nucleotidyl transferase which adds the 3′ CCA sequence, and a multitude of nucleoside modifying enzymes. In eukaryotes, the processing of some tRNA precursors involves, in addition, an activity which removes intervening sequences by a cleavage-ligation process.

A. Endonucleases

1. RNase P

The first enzyme that has been shown to participate in the processing of tRNA precursors,[116-121] RNase P, was purified from *E. coli* as an endonuclease which cleaves tRNA precursors in vitro to generate the mature 5′ termini. The essential role of RNase P in tRNA biosynthesis was demonstrated by the isolation of ts lethal mutants of *E. coli* defective in this nuclease activity.[118-120] In the mutants at the restrictive temperature, the synthesis of all cellular as well as phage-encoded tRNA is arrested, resulting in an accumulation of precursors. Genetic studies have shown that there are two types of RNase P mutants which differ in their mutational site on the *E. coli* chromosome.[123] The mutational site of ts 709 mutant[120] was located at 67 min (*rnp*B), that of ts 241 and A 49 mutants[118,119] was located at 77 min (*rnp*A) on the genetic map of *E. coli*. Mutation in either one of these genes is equally effective in blocking the RNase P function, resulting in the accumulation of the same tRNA precursors.[123,124] These find-

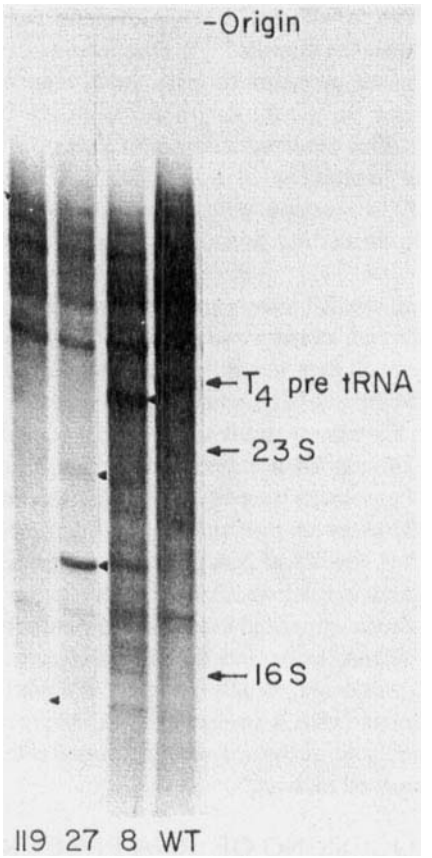

FIGURE 9. Autoradiogram of acrylamide gel separation of primary transcripts of T4 DNA with intact or deleted tRNA genes. DNA from wild type (WT) T4 and psu_b^- deletion mutants Δ119, Δ27 and Δ8 were transcribed by purified *E. coli* RNA polymerase, the RNA products extracted and separated by electrophoresis on acrylamide/agarose composite gel as described in Ref. 74. *E. coli* 23S and 16S [(^{32}P)] rRNA were run separately as reference markers. T4 pre-tRNA indicates the tRNA precursor band in WT T4 transcripts. The small arrows indicate the new tRNA precursor bands appearing in transcripts of the deletion mutants.

ings suggest that RNase P activity is the product of two separate genes. Recent studies with purified RNase P preparations have shown that this enzyme contains a discrete RNA species and has a buoyant density in CsCl characteristic of an RNA-protein complex composed of 80% RNA and 20% protein.[148] That the RNA is an essential component for enzyme function is indicated by the fact that the activity of RNase P can be abolished by treatment with micrococcal nuclease or pancreatic RNase A. The separation of RNase P into RNA and protein components, each devoid of any ribonuclease activity, has been obtained by column-chromatography in the presence of 7 *M* urea. Recently the reconstitution of RNase P activity from the separated components was reported.[149] Of several RNAs tried, only the RNA species found in purified RNase P was active in the reconstitution experiments. The exact role of the RNA component

is not clear; it may stabilize the protein moiety in an active configuration, or it may participate actively in RNase P-precursor tRNA recognition by a mechanism involving RNA-RNA interactions.[148] The site specificity of RNase P is not known. An examination of the nucleotide sequences surrounding the RNase P cleavage site on a number of different tRNA precursors reveals no similarity among them (see Figures 4 to 7). In addition, isolated fragments of $tRNA^{Pro}$-$tRNA^{Ser}$ precursor containing the nucleotide sequence at the cleavage site for RNase P were not attacked by the enzyme.[62] These observations indicate that the specificity of the enzyme cannot be determined exclusively by the primary sequence of the substrate. Additional information concerning RNase P processing specificity has been obtained from the study of mutations which inactivate suppressor tRNAs from *E. coli* and T4 phage.[150-155] In some of these tRNA mutants single nucleotide substitutions have affected tRNA biosynthesis causing an accumulation of precursors. A characterization of the mutant precursor tRNAs for their sequence alteration and their defects in enzymatic processing suggested that the mutations which disrupt the secondary and/or tertiary structure of the molecule result in a reduced rate of cleavage by RNase P. Figure 4 shows the location of several mutations which affect RNase P processing of $tRNA_1^{Tyr}$ precursor. The importance of conformation of the tRNA sequence for efficient processing by RNase P is supported by the following observations: mutations in the anticodon loop which do not affect secondary and/or tertiary structure of the tRNA do not alter RNase P recognition of precursors;[150,151] the inhibitory effects of nucleotide substitutions in the hydrogen-bonded stems can be reversed by second site mutations that change the nucleotide opposite in the stem so that an A:U or G:C base pair can be reformed.[150,155] Thus RNase P seems to recognize some structural features common to all tRNA precursor molecules, probably their tRNA moiety, rather than nucleotide sequences at the cleavage site. How exactly this recognition takes place is a question still open to speculation.[148,155] A study of tRNA precursor cleavage by the single-stranded specific nuclease S1 shows that the predominant structural feature of the precursors is their close resemblance to mature tRNAs.[156] The S1 digestion products of T4 $tRNA^{Gln}$-$tRNA^{Leu}$ dimeric precursor were compared with those of the mature cognate tRNAs. The location and accessibility of S1 cleavage sites were observed to be identical in both precursor and mature tRNAs. On the basis of these findings it was argued that the dimeric precursor is composed of two domains in which the secondary and tertiary conformations are very close to those found in mature tRNAs.[156] The two sites of T4 $tRNA^{Gln\text{-}tRNA^{Leu}}$ precursor cleavage by RNase P (Figure 7A) were found to be inaccessible to S1 nuclease digestion, which may suggest a participation of their nucleotides in secondary or tertiary interactions.[156] It is not clear yet to what extent the 5′ extra sequences proximal to the RNase P cleavage site play a role in enzyme attack. In vitro, a $tRNA_1^{Tyr}$ precursor containing only three extra nucleotides at the 5′ end was reported to be processed.[151] The 3′ terminal region of the precursor affects cleavage by RNase P,[151,157,158] a fact which may be explained by the three-dimensional structure of the tRNA sequence in the precursor which brings the RNase P cleavage site at the 5′ end very close to the 3′ terminus of the molecule. In the case of T4 $tRNA^{Pro}$-$tRNA^{Ser}$ precursor which has an immature 3′ end, it was shown that the removal of the 3′ extra nucleotides and subsequent synthesis of the CCA sequence must take place before cleavage by RNase P.[157] In contrast, the T4 $tRNA^{Gln}$-$tRNA^{Leu}$ precursor which contains a mature 3′ CCA end is rapidly processed by RNase P. From this it may be concluded that the presence in a precursor molecule of a mature 3′ end which includes the CCA sequence is a prerequisite for its efficient cleavage by RNase P. It has been suggested that RNase P must bind to the CCA sequence of the precursor for the cleavage to occur.[155] The effect of an intact CCA sequence or an untrimmed 3′ terminus upon RNase P cleavage seems, however, to vary with the precursor substrate. *E. coli* tRNA

precursors which accumulate in ts RNase P mutants contain nucleotide residues beyond the terminal CCA, yet in vitro they are excellent substrates for cleavage by RNase P.[116,117,124] It is observed that for many *E. coli* precursors the maturation of the 5′ end by RNase P cleavage takes place before the trimming of the 3′ extra sequences.[25,151] Bearing in mind that all *E. coli* tRNA precursors so far examined contain the CCA sequence, it may be argued that its presence is required for efficient cleavage by RNase P. On the other hand, the cleavage by RNase P which generates the 5′ terminus of T4 $tRNA^{Pro}$ was reported to occur in exonuclease-deficient cells in the absence of 3′ end trimming and CCA synthesis.[155] Thus, the synthesis of a mature CCA 3′ end before 5′ end maturation of precursor by RNase P seems to be an absolute requirement only in the case of the T4 tRNA dimeric precursors $tRNA^{Pro}$-$tRNA^{Ser}$ and $tRNA^{Thr}$-$tRNA^{Ile}$. An additional aspect of the role played by the 3′ terminal region of a precursor in the interaction with RNase P is exemplified by the way in which the processing of dimeric precursor takes place. A number of dimeric precursors from both *E. coli* and T4 phage are cleaved by RNase P at two sites to generate the 5′ ends of the tRNA moieties. There is evidence for a preferential order of cleavage, both in vivo and in vitro, such that the 5′ end of the 3′ proximal tRNA is generated first.[124,128,159] In vitro RNase P was also shown to recognize and cleave some RNA molecules which are not tRNA precursors, such as an *E. coli* 4.5 S RNA and a ϕ80-induced M3 RNA.[160]

2. RNase P2 and RNase O

An endonucleolytic activity which cleaves in the spacer regions of multimeric tRNA precursors was detected in heat-treated cell extracts from *E. coli* mutants with a ts RNase P. Such an activity originally observed in extracts from *E. coli* A49 mutant was designated RNase P2[122] and a similar enzyme from ts 241 mutant was designated RNase O.[125] RNase P2 and RNase O are functionally very similar and it remains to be clarified whether they represent the same enzymatic activity. Mutants affecting these enzymes have not yet been isolated. RNase P2 and RNase O have been partially purified[23,161] and their specificities studied in vitro using as substrate tRNA precursors which accumulate at the restrictive temperature in ts RNase P mutants. The partially purified enzymes cleave specifically at the intercistronic region of multimeric tRNA precursors, producing smaller precursor molecules that contain one or two tRNA sequences. These cleavage products, which are sometimes identical with the tRNA precursors that accumulate stably in RNase P mutants, can be subsequently processed by RNase P to yield tRNA-sized molecules. In a multimeric tRNA precursor which contains several cleavage sites for the intercistronic endonuclease (RNase P2 or O) and for RNase P the question arises about the order in which the two enzymes process their substrate. In a study of the in vitro processing of a trimeric precursor for the $tRNA_m^{Met}$-$tRNA^X$-$tRNA_1^{Gln}$ it was found that cleavage reactions catalyzed by RNase O precede those of RNase P[124]. The action of RNase O was highly ordered, the enzyme having preference for the site proximal to the 5′ terminus.[124,25] On the other hand, purified RNase P was shown to attack the trimeric precursor at a much slower rate than purified RNase O and to cleave only at the site proximal to the 3′ terminus, producing a dimeric $tRNA_m^{Met}$-$tRNA^X$ and a 5′ mature $tRNA_1^{Gln}$ precursor.[124] Shimura and Sakano[25] have proposed a model for the sequential processing of tRNA precursors, according to which RNase O cleaves first the multimeric precursors providing substrates which are more susceptible to attack by RNase P (Figure 10A). This model, however, does not explain why multimeric precursors do accumulate in the ts RNase P mutants despite the fact that they are substrates for RNase O. From in vitro studies of multimeric precursor processing by RNase P2 and RNase P, Schedl et al.[23,122] have shown that complete spliting to monomer precursors is achieved only by a combination of the two endonucleases. According to their model for sequential processing, cleavage

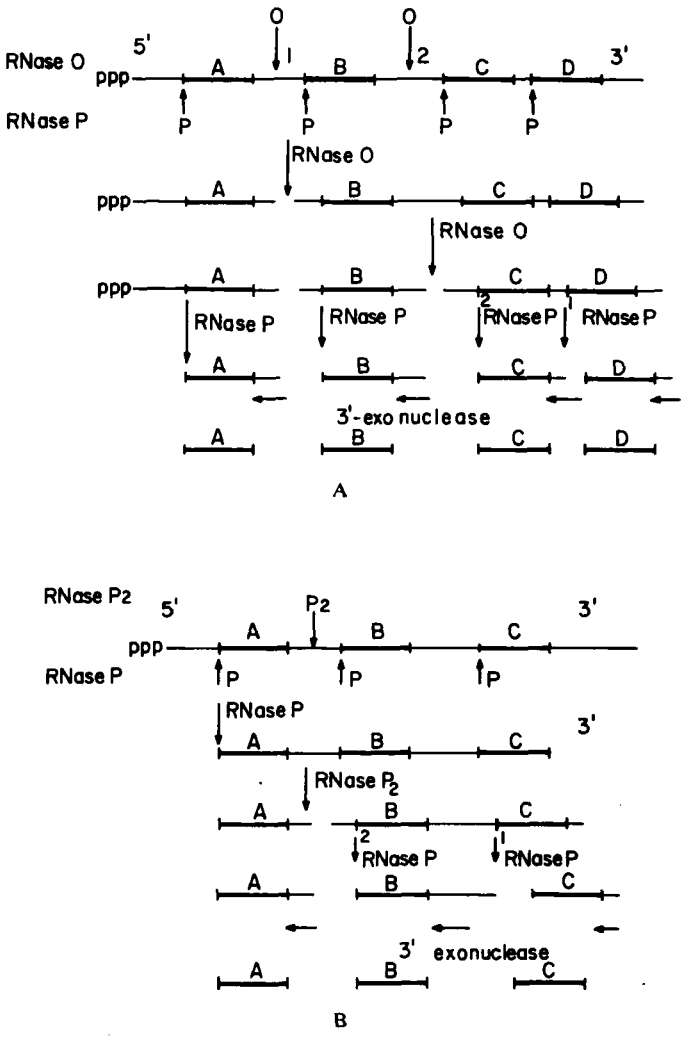

FIGURE 10. Sequential processing models for multimeric *E. coli* tRNA precursors. (A) Scheme proposed in Reference 25. (B) Scheme proposed in Reference 23. In some cases where the spacer region does not contain the RNase O or RNase P2 cleavage site, a dimeric precursor is generated. The dimeric precursor is cleaved by RNase P at two sites following the order indicated. The extra 3′ nucleotides of the monomers are subsequently removed by exonuclease.

by one endonuclease exposes a site for the second enzyme as shown in Figure 10B. Thus RNase P2 and RNase P cleave the multimeric precursor in an alternating fashion, starting with the removal of the 5′-leader sequence by an RNase P cleavage. This model may explain the accumulation of multimeric precursors in the RNase P mutants. It does not account, however, for the experiments described by Sakano and Shimura,[124] according to which multimeric precursors are cleaved into monomeric ones by RNase O alone. One should however consider these conclusions derived from in vitro studies with caution and keep in mind that the proposed schemes for processing of large precursors may not have their counterpart in vivo. It is probable that in vivo processing of a transcript takes place before its entire length is transcribed and that large precursors such as those observed in RNase P mutants are not normally formed.

At present we do not know what are the structures recognized by RNase P2 and O. On the one hand, RNase O was reported to have some resemblance with another *E. coli* endonuclease, RNase III,[162] and to cleave more efficiently double stranded RNA structures such as poly(A), poly(U).[161] The relationship between the two enzymes is not clear. On the other hand, there is evidence that RNase P2 is different from RNase III.[23] The cleavage products obtained by processing an in vitro synthesized T4 tRNA multimeric precursor with purified RNase P2 were completely different from the cleavage products obtained with purified RNase III.[163] In fact, RNase P2 was observed to cleave the in vitro synthesized multimeric T4 tRNA precursor, specifically yielding several intermediate tRNA precursors which could be processed by cell extracts to mature size tRNAs.[163] While these experiments may indicate a recognition of spacers in the multimeric T4 tRNA precursor by RNase P2, it is not clear yet whether this enzyme is responsible in T4 infected cells for the cleavage of the primary T4 tRNA transcript.

3. RNase III

The role of RNase III in tRNA precursor processing is presently unclear. The synthesis of T4 $tRNA^{Gln}$ was found to be severely depressed in an RNase III deficient strain, which suggests a participation of this enzyme in the processing of a precursor RNA containing $tRNA^{Gln}$.[164] This hypothesis needs additional evidence since the accumulation of such a precursor in the RNase III mutant has not been observed.

RNase III is responsible for the generation of smaller rRNA precursor molecules by cleavage of the large 30 S polycistronic rRNA precursor[32,33] and as such it may be expected to play a role in the processing of the cotranscribed tRNA precursors. In vitro, 30 S rRNA precursor molecules were cleaved by RNase III to produce intermediate precursors of spacer tRNAs containing $tRNA_2^{Glu}$ or the $tRNA_1^{Ile}$-$tRNA_{1B}^{Ala}$ dimer. These precursors were then cleaved by purified RNase P to produce the 5′ ends of $tRNA_2^{Glu}$ and $tRNA_{1B}^{Ala}$.[165] The 5′ end of $tRNA_1^{Ile}$ is not produced in vitro by RNase P, although it must be assumed that the enzyme performs such a cleavage in vivo. The secondary structure at the 5′ end of the $tRNA_1^{Ile}$ precursor was invoked to be responsible in vitro for the inhibition of the RNase P action.[165] In vitro, RNase P can cleave directly the intact 30 S rRNA precursors to generate the mature 5′-termini of $tRNA_2^{Glu}$ and $tRNA_{1B}^{Ala}$ which still contain 3′ immature ends.[165] Results demonstrating conclusively the lack of involvement of RNase III in spacer tRNA processing have been obtained from the study of an RNase III, RNase P double mutant of *E. coli*.[166] In this mutant, under restrictive conditions a 19S RNA precursor containing both 16S rRNA and $tRNA_2^{Glu}$ accumulates. The $tRNA_2^{Glu}$ can be released from the 19S RNA by RNase P. These results imply the existence of an unidentified endonuclease which is responsible for a cleavage distal to tRNA sequences in the rRNA precursor and which together with RNase P is responsible for the excision of spacer tRNA. In accord with these observations are the data obtained from the study of a ribosomal operon (*rrn*B) carried by λ*rif*^{d}18.[27] In this operon a deletion of the promoter and a major part of 16S rRNA gene did not prevent transcription of spacer $tRNA_2^{Glu}$ and 5S RNA genes from a λ promoter and normal processing to yield mature RNAs. Since the site for RNase III cleavage was deleted (this site is formed by the interaction between sequences preceeding the 16S rRNA and those following it[167]), it may be concluded that RNase III is not required for the formation of mature $tRNA_2^{Glu}$ or 5S RNA[27].

4. Other Endonucleases

An endonucleolytic cleavage, which may be of general significance in the processing of polycistronic transcripts, was observed to occur in the 3′ region of an in vitro synthesized $tRNA_1^{Tyr}$ precursor.[140] This precursor, which contained about 100 nucleotides beyond the 3′ terminus of the $tRNA_1^{Tyr}$ sequence, was shown to require both an endon-

uclease and an exonuclease for the processing of the 3′ end. The occurrence of the endonuclease cleavage site seven nucleotides downstream from the CCA end was demonstrated using a synthetic $tRNA_1^{Tyr}$ gene containing only 16 bp distal to the 3′ end of the structural sequence.[168] The transcript derived from the 16 bp is expected to form a hairpin of 5 bp. Since the synthetic gene was transcribed and its product correctly processed in a crude in vitro system, it was concluded that an endonuclease recognizes and cleaves the hairpin loop and that the remaining seven nucleotides at the 3′ end of the tRNA precursor are then removed by an exonuclease. Sequencing of the gene for $tRNA_1^{Ile}$ and the surrounding region in a ribosomal RNA operon of *E. coli* has revealed the existence of a similar hairpin structure adjacent to the 3′ end of $tRNA_1^{Ile}$, which may be the site for an endonucleolytic cleavage.[169] Other still uncharacterized endonucleolytic activities have been found to be involved in the cleavage at the 3′ end of spacer tRNAs in ribosomal operons[165,166] and in the processing of the primary transcript of T4 tRNA genes.[72]

B. Exonucleases

The tRNA precursors so far identified seem to fall into two types according to their 3′ extra sequences. In one type, generally observed in *E. coli*,[23,116,117] the CCA sequence is present, followed by a variable number of nucleotide residues. In the second type of precursor, which is observed in several T4 tRNAs[65,129] and probably in eukaryotes,[96,97,100] part or all of the CCA sequence is missing and its place occupied by other nucleotides. The generation of mature 3′ termini must therefore involve a stepwise exonucleolytic trimming of extranucleotides up to the CCA sequence, or to that sequence which could have all or part of the CCA added to it by tRNA-nucleotidyl transferase. From the specificity requirements of these reactions we may expect to find that different nucleases participate in the processing of the two types of tRNA precursors. Enzymatic activities which remove in vitro the 3′ extra nucleotides of tRNA precursors have been observed in crude *E. coli* extracts.[48,116,139,140] Several exonucleases have been partially purified but the identification of individual enzymes responsible for 3′ trimming of tRNA precursors is still confused.

Schedl et al.[23] have isolated a 3′ processing activity which copurified with the 3′ to 5′ exonuclease RNase II.[170] In addition, partially purified RNase II preparations were observed to remove 3′ extra nucleotides from an in vitro synthesized $tRNA_1^{Tyr}$ precursor,[140,141] making this enzyme a possible candidate for 3′ end processing of tRNA. An additional exonuclease RNase P III (distinct from RNase II) which removes 3′ extranucleotides from $tRNA_1^{Tyr}$ precursors after an endonucleolytic digestion has been described.[140] A similar exonuclease, RNase Q, was recently purifed and shown to process the 3′ extra nucleotides from monomeric *E. coli* tRNA precursors only after RNase P has removed the 5′ extra sequences.[125,161] Snce many tRNA precursors which accumulate in ts RNase P mutants have additional residues at their 3′ termini it might be concluded that trimming the 3′ end occurs in vivo after maturation of the 5′ end. It appears, therefore, that in vitro RNase P III and RNase Q both follow the same order of processing as observed in vivo. Another 3′ exonuclease activity, RNase Y,[161] can digest monomeric precursors into acid-soluble form. However the removal of the 5′ terminal extra nucleotides by the action of RNase P makes the precursor molecule less sensitive to RNase Y. It is not known however whether this enzyme generates correct 3′ ends. The observation that RNase II also becomes less active on a precursor which has had its 5′ extra sequences removed by RNase P leads to the assumption that RNase II is the same activity as RNase Y.[171] Thus the involvement of RNase II in tRNA precursor processing is not definitely established.

A major difficulty in the purification and characterization of nucleases involved in the processing of tRNA precursors is the scarce amount of substrates available. To

overcome this difficulty, large amounts of tRNA precursors specifically labeled at their 3′ end were prepared with the help of tRNA nucleotidyl transferase.[171-173] Since there are two types of tRNA precursors, with or without CCA, the synthetic precursors prepared to represent them were: t-RNA-CCAC*C* and tRNA-CU* (where the stars indicate radioactive label). Using these substrates, an enzyme specific for the removal of extra residues following the CCA sequence, RNase D, was purified from *E. coli*.[171,174] This enzyme does not remove residues from the CCA sequence and in fact regenerates by its action the amino acid acceptor activity of tRNA-CCAC*C*. The resistance of the CCA sequence to enzyme attack after extranucleotide removal suggests that the 3′ processing enzyme recognizes the tRNA structure as well as the CCA sequence and senses conformational changes which may occur in the precursor during its maturation. It cannot be excluded however that the CCA is stabilized in vivo by aminoacylation. The other synthetic tRNA precursor, tRNA-CU*, which lacks part of the CCA sequence, was resistant to RNase D, supporting the assumption that different nucleases are involved in the 3′ processing of the two types of tRNA precursors.[131] Since trimming by RNase D of natural tRNA precursors like the precursor to $tRNA_1^{Tyr}$ requires the prior maturation of the 5′ end by RNase P, it is probable that RNase D represents the same activity as RNase P III[140] and RNase Q.[161]

The biosynthesis of T4 $tRNA^{Pro}$, $tRNA^{Ser}$, and $tRNA^{Ile}$ requires an exonuclease activity which is absent in *E. coli* strain BN.[175] This enzyme termed RNase BN, removes the 3′ extra sequences of T4 tRNA precursors before the synthesis of the CCA terminus.[131] Using as substrate the synthetic tRNA-CU* precursor, RNase BN was purified and shown to cleave efficiently a pU residue from the 3′ terminus of immature $tRNA^{Pro}$.[173] For unclear reasons, however, it was less efficient in the removal of 3′ extranucleotides from immature $tRNA^{Ile}$ and from the $tRNA^{Pro}$-$tRNA^{Ser}$ and $tRNA^{Thr}$-$tRNA^{Ile}$ dimeric precursors.

C. tRNA Nucleotidyl Transferase — Synthesis of the CCA

All tRNAs contain the 3′-terminal CCA sequence which is the site of attachment of the aminoacid. This sequence can be synthesized by tRNA nucleotidyl transferase, an enzyme of wide distribution in both prokaryotes and eukaryotes, which incorporates AMP and CMP residues into tRNA molecules lacking all or part of the CCA terminus.[176] The question raised in the biosynthesis of different tRNAs is whether the 3′ terminal CCA is transcribed as part of the precursor RNA or is added post-transcriptionally by tRNA nucleotidyl transferase. The involvement of tRNA nucleotidyl transferase in tRNA biosynthesis was studied with the help of *E. coli cca* mutants defective in this enzyme.[177] The tRNA-nucleotidyl transferase activity does not appear to be required for the biosynthesis or function of *E. coli* tRNAs; the majority, if not all, of *E. coli* tRNA genes encode the CCA sequence in their DNA. In addition, the suppressor function of *E. coli* suppressor tRNAs is expressed at normal levels in the *cca* mutant strain.[178] A participation of tRNA nucleotidyl transferase in the metabolism of 3′ CCA terminus of tRNA in *E. coli* is indicated by the finding of a small fraction of tRNA molecules which lack a complete CCA sequence in the *cca* strain.[179,180] Among these, $tRNA^{Cys}$ appears to be the most severely affected by the *cca* mutation (79% of the molecules lacked a complete CCA sequence).[180] Since the 3′ CCA sequence of $tRNA^{Cys}$ was shown to be coded by the gene, the defective $tRNA^{Cys}$ molecules in *cca* cells were assumed to be the result of a high turnover at the terminus. In this respect tRNA nucleotidyl transferase plays the role of a repair enzyme. tRNA nucleotidyl transferase is required for the biosynthesis of several T4 tRNAs. As expected from the nucleotide sequences of their precursors, $tRNA^{Gln}$, $tRNA^{Ile}$, $tRNA^{Pro}$, and $tRNA^{Ser}$ acquire their CCA terminus post-transcriptionally.[65,128,129] *E. coli cca* mutants do not support the production of mature T4 tRNA species which require CCA addition and

were shown to accumulate tRNAs as well as dimeric tRNA precursors with immature 3′ end.[181] The accumulation of T4 $tRNA^{Pro}$-$tRNA^{Ser}$ precursor in *cca* mutants indicates that the 3′-CCA sequence of $tRNA^{Ser}$ is normally synthesized by nucleotidyl transferase before the precursor is cleaved by RNase P.[157,182] In order to establish the origin of the CCA sequence, whether gene encoded or post-transcriptionally added, McClain et al.[183] have comparatively studied the tRNA species induced by T-even bacteriophages T2, T4, T6, and RB69 in a *cca* mutant strain. In general, the biosynthetic origin of the CCA sequence of a given tRNA was found to be the same in the different bacteriophages. In two cases, however, ($tRNA^{Ser}$ and $tRNA^{He}$) the 3′ CCA residues were formed by tRNA nucleotidyl transferase in one phage but by transcription in another phage. Assuming that the different modes of CCA synthesis reflect tRNA gene evolution, McClain et al.[183] speculated that primitive tRNAs did not require 3′ CCA residues to function and that this requirement has evolved with the refinement of the protein-synthesizing machinery. Initially the requirement for CCA was fulfilled by tRNA nucleotidyl transferase; subsequently, for a more rapid tRNA biosynthesis the tRNA genes mutated to forms that encoded the CCA residues. It should be observed that in prokaryotes the majority of tRNA genes encode 3′ CCA residues in their DNA.

D. Nucleoside Modifications

One of the characteristics of tRNA is the presence of modified nucleosides in addition to the four nucleosides (adenosine, cytidine, uridine, and guanosine) normally found in RNA. At least 50 different modified nucleosides have been isolated and characterized.[8,184] The distribution of the modified nucleosides in the cloverleaf model of the tRNA is not random and shows site-specific regularities. Of special interest are the hypermodified nucleosides such as i^6A, t^6A, Q, and Y, which are located either in the first position or next to the 3′ end of the anticodon, and which seem to play a role in the recognition of codon sequences.[8] Some other modified bases like m^5U(T), ψ and D are present at specific locations in the tRNAs of most organisms. Since the tRNA gene transcripts are made by RNA polymerase from the four unmodified nucleotides, the synthesis of all modified bases must occur at the polynucleotide level as part of the post-transcriptional maturation process. This fact is clearly demonstrated by experiments in which tRNA genes carried by the DNA of transducing phages have been transcribed in vitro by purified *E. coli* RNA polymerase to yield completely unmodified tRNA precursors. Upon subsequent incubation with crude cellular extracts mature size tRNAs were formed which contained several modified nucleosides.[49,185] In some cases the yield of these modifications, which included the formation of hypermodified bases, was very high and the tRNA synthesized and processed in vitro could be aminoacylated.[185] The formation of almost all the modified bases, with the exception of ψ,[186] requires the presence of donor molecules (*S*-adenosylmethionine, threonine, isopentenylpyrophosphate, etc). In the absence of these, tRNA precursors synthesized enzymatically in vitro have been processed by *E. coli* extracts to mature size tRNA molecules which did not contain modified bases.[48,49,185] Similarly, precursors isolated from *E. coli* whose nucleoside modification was only partial have been processed efficiently in vitro.[124] This shows that nucleoside modifications are not prerequisite for the size maturation of tRNA gene transcripts.

The biosynthesis of the modified nucleosides in tRNA is still poorly understood. Most of our knowledge derives from in vitro studies with purified tRNA modifying enzymes and tRNA precursors[124,128,187,188] or undermodified tRNAs[186,189,190] isolated from bacterial mutants. In general it was observed that all tRNA precursors isolated from cells contain a certain number of modified nucleosides, the type of modification varying with the stage of precursor size maturation.[98,124,187] The analysis of several different *E. coli* tRNA precursors shows that nucleoside modifications, such as T, ψ,

D, and m^2A, occur at early stages of tRNA maturation while others, such as m^1G and Gm, occur at later stages. In fact the Gm modification is absent even from the monocistronic tRNA precursors which accumulate in ts241 mutant and appear to occur only after precursor processing by RNase P.[124,187] The ψ in the anticodon region was reported to be synthesized as a last step in the maturation of -$tRNA_1^{Tyr}$ from an *E. coli* $tRNA_1^{Tyr}$ mutant.[188] Recently, however, using in vitro synthesized $tRNA_1^{Tyr}$ precursor it was shown that a purified pseudouridylate synthetase 1 can synthesize the anticodon ψ directly on the initial transcript before any size maturation has taken place.[191] In yeast ts136 mutant tRNA precursors which accumulate contain some modification such as ψ, D, T, and m^7G but lack Gm or Cm.[98] It appears that some modifying enzymes may possess an absolute specificity for mature-size tRNA while others may recognize particular sequences on a tRNA structure which is part of larger precursor molecule. The study of nucleoside modifications in T4 tRNA precursors $tRNA^{Pro}$-$tRNA^{Ser}$ and $tRNA^{Gln}$-$tRNA^{Leu}$ from T4 tRNA mutants have demonstrated the importance of the tRNA conformation for recognition by modifying enzymes.[154] Nucleotide substitutions that occurred in one of the tRNA sequences of the dimeric precursors (e.g., in the $tRNA^{Ser}$ or $tRNA^{Gln}$ moiety) reduced the extent of nucleoside modification only in that tRNA sequence which contained the mutant nucleotide. The second tRNA moiety of the dimeric precursor was found to be completely modified, probably because it retained the conformation recognized by the modifying enzymes.[154,155]

In view of the importance of substrate RNA conformation for nucleoside modification, it is not surprising to find that introduction of the same modification at different sites involves different enzymes. Thus the biosynthesis of ψ in the bacterial tRNA is carried out by two different enzymes: (1) the pseudouridylate synthetase I coded by the *his* T gene is responsible for the synthesis of ψ in the anticodon region of several tRNA species; and (2) the pseudouridylate synthetase II responsible for the synthesis of ψ in the TψCG loop of every tRNA molecule.[186,192] A well-studied class of tRNA modifying enzymes are the tRNA methylases. These include a large number of different enzymes, each responsible for the synthesis of a specifically methylated nucleoside. The methyl donor for tRNA methyl-transferase reactions is in most cases *S*-adenosyl-L-methionine, although sometimes the methyl group is derived from formyl-tetrahydrofolate.[193] The isolation of *E. coli* mutants with defects in RNA methylation[189] has enabled the identification of the *trm*A locus with the gene of the methyl transferase responsible for the production of m^5U modification in tRNA.[194] Other *E. coli* mutants were found to be defective in the biosynthesis of m^7G (*trm*B) or mam^5s^2U (*trm*C).[195] Recently a general method for the isolation of *E. coli* mutants defective in nucleoside modifications, based on the accumulation of undermodified RNA under nonpermissive growth conditions and detection of mutants by remodification either in vivo at permissive conditions or in vitro, has enabled the isolation of two ribosomal and two tRNA methylation defective mutants.[196] In one of the mutants the tRNA was shown to lack mam^5s^2U due to a permanent nonfunctional methyltransferase (*trm*C), while in another mutant a defect in the synthesis of m^1G was caused by a ts transferase (*trm*D).[197] From the map locations of mutations affecting tRNA methylation and pseudouridylation it appears that the genes involved in tRNA modification are scattered all over the *E. coli* chromosome. The level of tRNA (m^5U) methyl transferase was shown to be under the same stringent control as stable RNA and ribosomal proteins.[198] However, there is no coordinated regulation of the different tRNA methyltransferases since the enzymes synthesizing mam^5s^2U and m^1G were not found to be stringently regulated.[199]

The biosynthesis of several hypermodified nucleosides such as ms^2i^6A, t^6A, and Y appears to be a multistep process which involves several enzymes and donor substrates, and has been recently reviewed.[9] The synthesis of mo^5U and cmo^5U, which occupy the

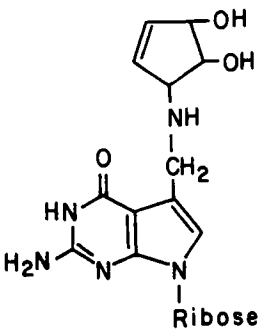

FIGURE 11. Nucleoside Q (queuosine).

first position of anticodons in certain tRNA species, was shown to occur via a common 5-hydroxyuridine precursor which is subsequently methylated or acetylated.[200] Recent studies of the biosynthesis of nucleoside Q (queuosine) (Figure 11) have revealed a novel type of post-transcriptional modification in tRNA: insertion of a modified base into the polynucleotide chain by cleavage of the N-C glycoside bond without breakage of the phosphodiester bond.[201] An enzyme with a tRNA transglycosylase activity was initially discovered in the lysate of rabbit reticulocytes.[202] This enzyme was shown to carry out a specific exchange of guanine with queuine (Q base) or guanine located in the first position of the anticodon of $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$ or $tRNA^{Asp}$.[203] A similar guanine insertion enzyme was found in *E. coli* and was recently purified to homogeneity.[204] It was shown that the actual substrates of the *E. coli* tRNA transglycosylase are the Q precursors rather than guanine, and queuine (Q base) itself was not incorporated into tRNA nor released by the transglycosylase reaction.[201] In fact, one of the Q precursors, 7-(amino-methyl)-7deazaguanine was found in the acid-soluble fraction of *E. coli* cells[205] and in vitro was irreversibly exchanged by the purified tRNA transglycosylase with the guanine located in the first position of the anticodon of undermodified $tRNA^{Asn}$ and $tRNA^{Tyr}$.[201] It seems therefore that a Q precursor, probably 7-(aminomethyl)-7-deazaguanine, is incorporated into tRNA by transglycosidase reaction and then it is successively modified to form a complete Q molecule.[201] The *E. coli* transglycosylase shows a very high specificity toward the base and its location in the polynucleotide chain. The exchange of base by cleavage of N-C glycoside bond takes place without requirement of an enzyme source.

E. Processing of T4 tRNA Precursors

Genetic and biochemical data have established that T4 tRNA genes are transcribed as a polycistronic RNA molecule. However, in vivo the initial transcript has not been detected and there are no experimental data describing the first processing steps leading to the generation of the intermediate monomeric and dimeric precursors observed in T4 infected cells. The recent DNA sequence analysis of the T4 tRNA gene cluster, together with the complete sequence of all T4 tRNA precursors, make it possible to retrace the way these precursors are generated[72] (Figure 12). It appears that the T4 tRNA precursors must have been joined together in a primary transcript and generated by endonucleolytic cleavages. The first endonucleolytic cleavage generating the 5′ end of the $tRNA^{Gln}$-$tRNA^{Leu}$ precursor is made by an enzyme of unknown specificity. It is not clear whether RNase III is responsible for this cleavage, although the synthesis of T4 $tRNA^{Gln}$ was reported to be severely depressed in RNase III deficient cells.[164] Other sites of cleavage, which occur in regions containing exclusively A and U, imply an endonuclease activity that recognizes a tRNA structure.[7,72] This nuclease cleaves the

FIGURE 12. Schematic representation of the transcript of the T4 tRNA gene cluster. The tRNA structures are represented by boxes. The arrows mark the endonucleolytic cleavages that must occur in order to generate the tRNA precursors detected in vivo.[72]

transcript next to a CCA or CCAA sequence generating the 3′ ends tRNA of tRNA^{Gln}-tRNA^{Leu} and tRNA^{Gly} precursors. The 3′ ends of tRNA^{Pro}-tRNA^{Ser} and tRNA^{Thr}-tRNA^{Ile} precursors may be similarly produced (Figure 12). Here in the absence of an encoded 3′ CCA sequence, the cleavage takes place in such a way that both dimeric precursors have three nucleotides, UAA in tRNA^{Ser} and UAU in tRNA^{Ile}, which have to be removed before addition of the CCA. The tRNA^{Thr}-tRNA^{Ile} precursor, due to the presence of the CCA at the interstitial sequence, is probably cleaved by the same endonuclease to produce a tRNA^{Thr} CCA precursor.[72,129] Such a cleavage may explain why this dimeric precursor is found in low yields and does not accumulate in ts RNase P mutants.[129] The DNA sequence analysis of the smaller T4 tRNA gene cluster indicates that tRNA^{Arg} species 2 and species 1RNA are probably cleaved from the T4 tRNA primary transcript by the same endonucleolytic activity, generating the 3′ ends of tRNA^{Arg}-CCAA, species 2 RNA-CCA and species 1 RNA-ACA.[72] The endonuclease responsible for these cleavages is probably a host enzyme not yet identified.

The detailed information now available about the processing of intermediate T4 tRNA precursors tRNA^{Pro}-tRNA^{Ser}, tRNA^{Gln}-tRNA^{Leu}, and tRNA^{Thr}-tRNA^{Ile} into mature tRNAs sheds light on the diversity of this process. It was shown to involve several enzymes and a different pathway of maturation for each tRNA.[72,129] The maturation of tRNA^{Pro} and tRNA^{Ser} from their dimeric precursor requires the participation of RNase P, 3′-exonuclease BN and tRNA nucleotidyl transferase in a sequential set of reactions established by McClain and collaborators[132,155] and summarized in Figure 13. The important feature of the processing of tRNA^{Pro}-tRNA^{Ser} precursor is the absolute requirement for 3′ end maturation (removal of 3′ extra nucleotides and addition of CCA) before cleavage by RNase P. The transient accumulation of T4 tRNA precursors in T4 infection of wild-type *E. coli* as compared to mutant cells with a ts RNase P seems to reflect this requirement. While the dimeric precursors tRNA^{Pro}-tRNA^{Ser} and tRNA^{Thr}-tRNA^{Ile} show transient accumulation in wild-type infections, the monomeric tRNA^{Gly} and dimeric tRNA^{Gln}-tRNA^{Leu} precursors can be detected only in infections of a mutant with ts RNase P. The reason for this behavior resides in the fact that tRNA^{Gly} precursor and the tRNA^{Leu} moiety of the dimeric tRNA^{Gln} tRNA^{Leu} precursor have the CCA sequences encoded and are thus rapidly processed in cells containing a wild type RNase P. Figure 14 shows the scheme of this processing. In the processing of precursors with immature 3′ ends like tRNA^{Pro}-tRNA^{Ser} and tRNA^{Thr}-tRNA^{Ile} the rate-limiting step must be their trimming at the 3′ end prior to RNase P cleavage. The processing pathway of the tRNA^{Thr}-tRNA^{Ile} was recently described.[72,129] This precursor can be processed in an analogous fashion to tRNA^{Pro}-tRNA^{Ser} (Figure 15). However, the maturation of tRNA^{Thr}-tRNA^{Ile} was observed to be assymetric in wild-type cell infections and to yield tRNA^{Ile} in 30% the amount of tRNA^{Thr}.[129] An additional pathway of processing was proposed which takes into account the unique disposition of a mature 3′ terminal CCA sequence in this dimer (Figure 15). According to this scheme. the previously described endonucleolytic activity which recognizes a tRNA-CCA moiety and cleaves the large T4 tRNA primary transcript, would cleave the tRNA^{Thr}-tRNA^{Ile} next to the tRNA^{Thr} CCA sequence. The cleavage is assumed to occur at several sites, including the 5′ end sequences of tRNA^{Ile} which, as a result, would be labile and

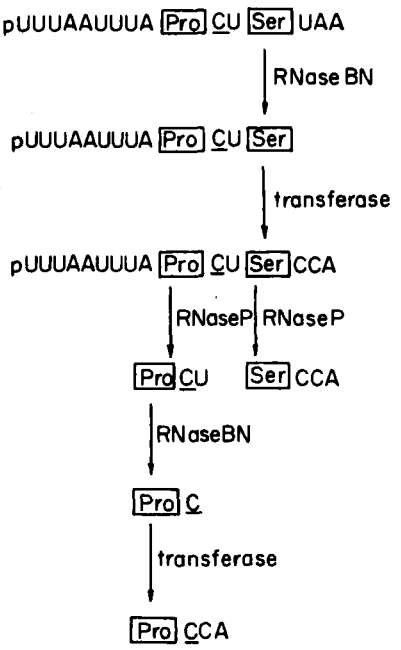

FIGURE 13. The processing scheme of T4 $tRNA^{Pro}$ -$tRNA^{Ser}$ precursor. The tRNAstructures are represented by boxes and the encoded residue corresponding to part of the mature 3' CCA terminus is underlined. The precursor is processed by exonuclease RNase BN[131,173], tRNA nucleotidyl transferase and RNase P in the sequential order established in Reference 132.

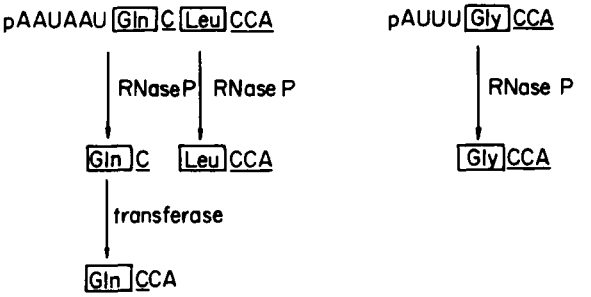

FIGURE 14. The processing scheme of T4 $tRNA^{Gln}$ -$tRNA^{Leu}$ and $tRNA^{Gly}$ precursors. The encoded residues corresponding to all or part of the mature 3' CCA terminus are underlined. $tRNA^{Leu}$ and $tRNA^{Gly}$ are generated by single RNase P cleavages. tRNA nucleotidyl transferase is required for the completion of the CCA sequence of $tRNA^{Gln}$[72,129]

degraded.[129] Thus it appears that although the T4 tRNA region is transcribed as a single molecule, the relative amounts of individual mature T4 tRNAs vary as a result of the maturation process. The organization of the T4 tRNA genes and the surrounding sequences seem to be designed in such a way as to allow a control of the final level

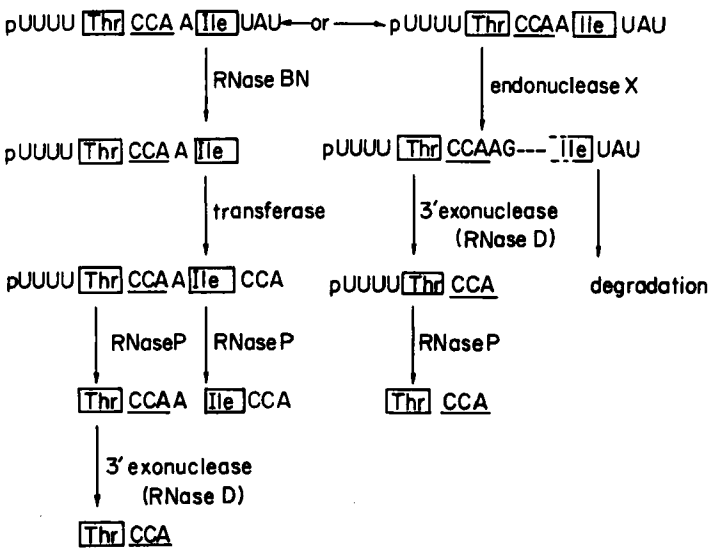

FIGURE 15. Two processing pathways for the $tRNA^{Thr}$ -$tRNA^{Ile}$ precursor. (Left) Maturation is initiated by exonuclease RNase BN[131,173] followed by a sequential processing reaction involving tRNA nucleotidyl transferase, RNase P and another exonuclease, tentatively indicated as RNase D.[171] An alternative pathway (right) implies an endonucleolytic cleavage by a still unidentified enzyme, which generates a $tRNA^{Thr}$ precursor with a mature 3' CCA end or, as shown in the figure, a 3' CCA end containing several extranucleotides. The extranucleotides are then removed by an exonuclease. When endonucleolytic cleavage occurs into the nucleotides from the 5' end of $tRNA^{Ile}$ the latter becomes unstable and is degraded.[72,129]

of each tRNA by modulating the efficiency of RNA precursor processing. The enzymes involved in T4 tRNA processing seem to be all host enzymes and mutations in *E. coli* RNase P, exonuclease BN, and tRNA nucleotidyl transferase restrict T4 tRNA synthesis. The complete maturation of $tRNA^{Leu}$, $tRNA^{Ser}$, $tRNA^{Ile}$, and $tRNA^{Gly}$ requires as a final step the synthesis of the Gm modification in the D loop.

A T4 phage function, *mb*, coded for by a gene located at some distance from the tRNA region, was reported to be required for the synthesis or stability of the $tRNA^{Pro}$ $tRNA^{Ser}$, $tRNA^{Ile}$ and species 2 RNA.[57,206,207] Conclusive evidence was presented that T4 *mb* function is not an endonuclease.[72]

F. Processing of Eukaryotic tRNA Gene Transcripts

It has been known for some time that cytoplasmic extracts of mammalian cells, yeast, and silkworms[101,208,209] contain activities which can reduce the tRNA precursors to the size of tRNAs. However, the fact that until recently no radiochemically pure precursors to a single eukaryotic tRNA species were avilable has hampered the attempts to identify and purify specific processing enzymes. The lack of a well-defined eukaryotic substrate has led research workers to the use of the much studied *E. coli* $tRNA_1^{Tyr}$ precursor. It was argued that since the secondary/tertiary structure of the tRNAs was conserved during evolution, the processing enzymes from eukaryotes may be similar to the bacterial enzymes in recognizing the tRNA conformation of the *E. coli* tRNA precursor. Such an approach has revealed in KB cells on endoribonuclease activity with RNase P-like specificity that generates the 5' end of the mature tRNAs.[103] The cleavage products generated from *E. coli* $tRNA_1^{Tyr}$ as well as from other precursors

by the KB cell activity were identical in size to those produced by the *E. coli* RNase P. With the isolation and identification of several tRNA precursors from silkworms and yeast it became possible to study the action of eukaryotic processing enzymes on their natural substrates. In the silk gland of *Bombyx mori* two enzymatic activities analogous to those from *E. coli* have been detected: an RNase P-like activity and a 3′ to 5′ exonuclease.[210] In vitro, the maturation of the tRNA precursors from *B. mori* was found to be a two-step process which involves first the removal of the 5′ end extranucleotides and then an exonucleolytic trimming of the 3′ end. From these studies it appears that the enzymes participating in the processing of the 5′ end 3′ ends of tRNA precursors are similar in eukaryotes and prokaryotes. Data from sequence analysis of several cloned $tRNA^{Tyr}$ and $tRNA^{Phe}$ yeast genes[96,97] indicate that the regions upstream from the 5′ end of mature tRNAs are dissimilar, and imply a variability in the 5′ leader sequences of the precursors. It may be assumed therefore that the 5′ end processing enzymes from eukaryotes, like their counterpart in prokaryotes, must recognize precursor structure rather than nucleotide sequence. Since the yeast tRNA genes so far sequenced do not code for the CCA sequence,[96,97] the 3′ end maturation of the tRNA precursors must include removal of extra nucleotides and CCA addition. The intervening sequences observed in several yeast tRNA genes[98-100,136] are transcribed and are found in the precursors accumulating in ts 136 mutant. Eukaryotic tRNA maturation must therefore involve not only nucleolytic cleavage, base modification, and CCA addition, but also excision of the intervening sequences and religation — a process referred to as "splicing". Using as substrates the tRNA precursors accumulating in ts 136 mutant, an excision-ligase activity was detected in yeast extracts which will process the precursors to mature tRNAs.[98,100,136] So far no definite cellular location was determined for this activity. It requires Mg^{++}, ATP, and 0.05 to 0.15 *M* salt and is inhibited by tRNA. It should be observed that only a limited number of tRNA precursor species accumulate in the ts 136 mutant and that all the accumulating precursors so far analyzed contain intervening sequences. This suggests a connection between the mutant in the *rna* 1 gene[135] which causes RNA accumulation in the nucleus, and the lack of removal of intervening sequences. The simplest explanation would be to assume that the lesion in the ts 136 mutant lies in the RNA splicing enzyme. There is no indication, however, of an increased temperature sensitivity of the enzyme from the mutant as compared to that from wild-type cells. At the same time we do not know the number of enzymes involved in the splicing of different precursors containing intervening sequences and more experimental data are required for the understanding of this tRNA maturation pathway particularly to eukaryotes.

The frog oocyte provides a useful system for the study of eukaryotic tRNA gene transcription as well as precursor processing. DNA injected into the nucleus of *Xenopus* oocyte is transcribed in vivo.[211,212] In this fashion the *Xenopus* oocyte has been proved to be especially efficient in expressing those genes which are transcribed by RNA polymerase III, the 5S ribosomal RNA, and the tRNA genes. Shown first to transcribe faithfully cloned genes from *Xenopus* coding for 5S RNA[213] and $tRNA_1^{Met}$,[214] the oocyte is also able to express cloned tRNA genes from heterologous systems like nematode[215] and yeast.[216] A cell-free system recently derived from the *Xenopus* oocyte is capable of accurate in vitro transcription of *Drosophila, Schizosacharomyces pombe*[90] *and S. cerevisiae*[100] tRNA genes.

From these studies it was observed that the transcription of the tRNA genes in the *Xenopus* system produces intermediate precursor molecules which are slowly processed into mature tRNAs.[90,100,215,216] The expression of several cloned yeast $tRNA^{Tyr}$ genes and a yeast $tRNA^{Trp}$ gene, which all contain intervening sequences, was studied in the *Xenopus* oocyte in vivo or in cell-free extracts.[100,216] The RNA synthesis was conducted in the presence of [(α-^{32}P)]-labeled nucleoside triphosphates, and the products were

separated by acrylamide gels and analyzed. These experiments have enabled for the first time the isolation of a eukaryotic tRNA gene primary transcript having an extra fragment at the 5′ end and an intervening RNA sequence, but lacking the 3′CCA end as well as modified bases.[216] A 5′ leader sequence containing the initiator nucleoside triphosphate was observed to be present in the primary transcripts derived from four different yeast $tRNA^{Tyr}$ genes examined. In all these transcripts the 5′ leader sequence presents considerable variability in length and nucleotide sequence.[216] The *Xenopus* RNA polymerase III seems to recognize the yeast tRNA transcription unit and initiates in close proximity to the $tRNA^{Tyr}$ genes. Sequence analysis of the major RNA species and the time course of their accumulation in the oocyte indicate that removal of the 5′ extra sequences, 3′ end trimming, and addition of the CCA take place before the splicing. Several modifications occur probably at the stage of termini processing and are for the first time detectable in a precursor which has mature ends but still contains the intervening sequence. The splicing activity of the *Xenopus* system converts both $tRNA^{Tyr}$ and $tRNA^{Trp}$ precursors into covalently linked mature size tRNA.[100,216] Although the splicing of yeast tRNAs is reported to be less efficient in *Xenopus* oocytes than in wild-type yeast,[216] the fact that it takes place at all and with apparent fidelity in such a heterologous systems is remarkable. The presence of a splicing activity in *Xenopus* implies the presence of intervening sequences within tRNA genes in this organism, a fact which has to be demonstrated. It may also suggest a common evolutionary origin of intervening sequences and splicing activities in eukaryotes. The specificity of these enzymes seems to be directed toward a common feature of their substrates, probably the secondary and tertiary structure of the tRNA precursors. A comparison of the known intervening sequences of four different yeast tRNAs[98-100,136] shows no sequence homology; nevertheless, it is possible to fit them all in similar secondary structure models as shown in Figure 8. In all the tRNA precursors so far examined the 5′-end splice site is always adjacent to the position of the modified base, next to the 3′ end of the tRNA anticodon, and the 3′ end of the splice is located next to a similar mature tRNA sequence, AUC.

V. tRNA GENE EXPRESSION

A. Transcription of tRNA Genes

Transcription is governed by two events — initiation and termination of RNA synthesis which occur at different specific sites along the template. The sequence and structural features of these DNA sites, promoters, and terminators serve as recognition signals for RNA polymerase. In *E. coli* the correct initiation of RNA synthesis has an absolute requirement for an RNA polymerase containing the σ factor.[217] The termination of transcription in vitro requires in some systems the presence of the termination factor ρ,[218] which seems to have an essential function in vivo.[219] Information on the transcription of a tRNA gene has become available from in vitro studies on the *E. coli* $tRNA_1^{Tyr}$ system, using the DNA of transducing ϕ80 phages carrying one or both $tRNA_1^{Tyr}$ genes.[48,49,143,220,221] The complete sequencing of the $tRNA_1^{Tyr}$ region together with in vitro transcription studies has established a promoter and a terminator which define the $tRNA_1^{Tyr}$ transcription unit.[44,143,222] The DNA sequence following the 3′ end of the structural gene was found to have an unusual structure. It consists of a 178-bp sequence that is repeated 3.14 times[44] and contains an in vitro ρ-dependent transcription termination site.[143] The first repeat unit starts 19 bp before the end of the sequence encoding the mature tRNA. In vitro transcription studies with purified restriction fragments derived from $\phi 80psu_3^+$DNA indicate that the transcript of the single $tRNA_1^{Tyr}$ gene contains approximately 350 nucleotides. RNA synthesis is initiated 41 nucleotides upstream from the 5′ end of the mature tRNA and continues 224 to 226 nucleotides

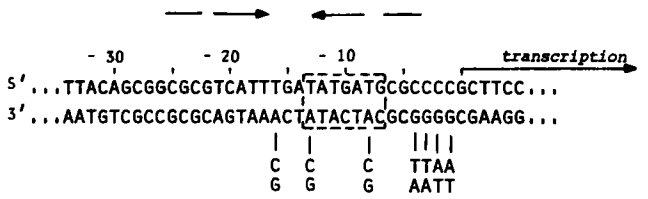

FIGURE 16. Nucletide sequences in the promoter region of $tRNA_1^{Tyr}$ gene. Part of the nucleotide sequence of the $tRNA_1^{Tyr}$ promoter region [222] is presented indicating the promoter heptamer sequence, "Pribnow box,"[238,239], the regions of inverted repeats (→←) and the initiation of transcription site.[49] Two types of bp alterations have been introduced in the $tRNA_1^{Tyr}$ gene promoter region: (a) Four G:C have been substituted by four A:T into a synthetic $tRNA_1^{Tyr}$ gene, resulting in an increased expression of the gene.[225] (b) Mutants containing single base pair A:T to G:C transitions in the A:T rich region of the promoter show a considerable reduction in the expression of $tRNA_1^{Tyr}$ suppressor activity.[46]

beyond the CCA 3′ end of the tRNA, terminating at ρ-dependent site.[143] A promoter for $tRNA_1^{Tyr}$ gene was located within 50 nucleotides upstream from the initiation of transcription site.[222] The complete chemical synthesis of $tRNA_1^{Tyr}su_3^+$ gene[223] and its in vitro transcription[168] and in vivo expression[224] help establish the functional structure of this gene. The transcription of the synthetic gene, which contained 51 bp upstream from the initiation site and only 16 bp following the CCA end, was promoter controlled and initiated with pppG at the correct site. The primary transcript was then correctly processed in vitro by cell extracts.[224] The synthetic $tRNA_1^{Tyr}su_3^+$ gene was cloned into plasmid and phage vectors and found to be functional in suppressing amber mutations.[224,225] A sequence alteration was introduced into the synthetic su_3^+ gene by substituting for the four G:C base pairs immediately precedingthe point of transcription initiation four A:T base pairs (Figure 16). It was assumed that a decrease in G:C content of the promoter will allow a higher rate of transcription by lowering the Tm of the region and facilitating stand separation by RNA polymerase. The modified synthetic gene was cloned and indeed found to be transcribed to a greater extent than the unmodified gene. The enhancement is however less than twofold, which suggests that the four changed nucleotides are not of critical importance for the expression of the gene.[225] It should be observed that the synthetic $tRNA_1^{Tyr}$ gene lacked the transcription termination signal and the 178-bp repeat sequence observed in the naturally occurring gene.[44] Apparently a $tRNA_1^{Tyr}$ gene containing only 16 bp beyond the tRNA 3′ CCA end can be functional due to the existence of a processing signal in this region.[168] An endonucleolytic cleavage will process the tRNA precursor close to the CCA end. In contrast with the in vitro transcription studies of $\phi 80psu_3^+$ (Cambridge)[46,49,143] phage DNA, transcription of the $tRNA_1^{Tyr}$ genes carried as a doublet by the $\phi 80psu_3^{+,-}$ (Kyoto)[226] phage DNA did not require ρ factor for termination.[144,221] It seems that the distal region of the $tRNA_1^{Tyr}$ gene carried by this phage does not contain the 178-bp repeat sequences and the ρ-dependent termination site.[46]

Information about the properties of tRNA gene promoters was obtained from in vitro transcription studies. Early experiments have shown that tRNA genes can be efficiently transcribed using purified DNA and RNA polymerase (holoenzyme) without the requirement of any additional factors.[137,138,145] The formation of RNA polymerase-promoter complex is characterized by a binding constant specific for each promoter which is determined by its structure and the conditions affecting the state of DNA template such as salt concentration, temperature, and nonionic solvents.[227] The

$tRNA_1^{Tyr}$ promoter, when studied by transcription from DNA restriction fragments, appeared to be exceptionally salt sensitive, to require glycerol for function, and to be unable to form a rifampicin resistant promoter-polymerase complex.[49] However, all these properties which may indicate a weak promoter-polymerase interaction were not observed when the same tRNA gene was transcribed from a whole $\phi 80psu_3^+,^-$ phage DNA template.[144,220,221] In fact the $tRNA_1^{Tyr}$ gene appears to have a rather strong promoter which is preferentially selected from $\phi 80psu_3^+,^-$ DNA in transcription reactions containing limiting amounts of RNA polymerase.[48,220,221] These observations suggest that the size of the template may affect the efficiency of promoter function. In vitro studies with high molecular weight *E. coli* DNA templates show also a selective and efficient transcription of tRNA genes by RNA polymerase which takes place in the presence of relatively high salt concentrations (up to 0.2 *M*).[228]

B. Regulation of tRNA Synthesis

There are two ways in which tRNA synthesis can be regulated: at the transcriptional level through the control of the amounts of transcripts produced, and at the maturation level through the control of the amount of mature tRNA formed. The way tRNA transcription is regulated is poorly understood. In *E. coli* the rate of transcription of tRNA and rRNA genes depends on the growth rate of the cell and is under stringent control.[229] During amino acid starvation, stringent (rel^+) bacteria but not relaxed (rel^-) mutants stop stable RNA synthesis. Concomitantly, the starved cells accumulate ppGpp which has been proposed to be the mediator of the stringent response in vivo. It has been suggested that ppGpp acts as an effector of negative control of transcription initiation by interacting directly with RNA polymerase and reducing its affinity for rRNA and tRNA promoter.[230] Although in vivo the synthesis of rRNA seems to be inversely correlated with the concentration of ppGpp, the question whether this compound has a direct effect on rRNA synthesis has not been settled. Selective inhibition by ppGpp of in vitro rRNA synthesis was observed in a number of laboratories, while negative results were obtained by others (for a review see Reference 30). Recently it was shown that there are two transcription initiation sites for each of the *rrn*A and *rrn*E *E. coli* ribosomal operons, and ppGpp was found to inhibit in vitro transcription from both sites in each rRNA operon.[231] The effect of ppGpp on in vitro tRNA gene transcription is still uncertain. In one case the transcription of *E. coli* $tRNA_1^{Tyr}$ gene was reported to be selectively inhibited by ppGpp,[232] while in others the synthesis of $tRNA^{Tyr}$ and bulk *E. coli* tRNA (directed by *E. coli* DNA) were found to be unaffected.[228,233] The involvement of a positive factor such as protein elongation factors TuTs in rRNA and tRNA synthesis is also controversial.[30,220] Thus in vitro studies have not provided consistent information on the regulation of stable RNA transcription. The recently described association of several tRNA genes with rRNA operons[14-18] defines perhaps a special class of tRNA genes. These genes, which code for major tRNA species in *E. coli*, are transcribed from a rRNA promoter and are coregulated with the rRNA genes. This may explain some of the common features observed in the regulation of both rRNA and bulk tRNA synthesis. The transcription of the tRNA genes which are not associated with rRNA operons may be regulated by a different mechanism. This may account for the observations that under the same growth conditions there are some variations in the rate of tRNA synthesis as compared to that of rRNA.[234,235] The synthesis of one *E. coli* tRNA species, $tRNA_1^{Leu}$ was found to be controlled independently.[236] The relative amounts of the two tyrosine-accepting tRNA species were also observed to vary during cell growth. Although $tRNA_2^{Tyr}$ is coded for by a single gene, the amount of this tRNA species is equivalent to that of $tRNA_1^{Tyr}$ (coded for by two genes) early in the *E. coli* growth cycle and becomes twice that amount in the late

cell cycle.[237] There is no explanation yet for the way the amounts of these tRNAs are regulated.

Control of tRNA synthesis at the maturation level may be exerted through any of the enzymatic steps involved in processing. The T4 tRNA system previously described furnishes an example where processing controls the level of the tRNAs. Here although the eight tRNA species are transcribed as a single RNA molecule, the final amounts of mature tRNAs can vary several folds due to the build-in requirements of the precursors for a different processing pathway.[72,129]

VI. CONCLUDING REMARKS

The organization of tRNA genes is a field of research which promises to advance rapidly in the near future. With the molecular cloning and DNA sequencing techniques now available one may anticipate that the nucleotide sequences of more tRNA genes will soon become known. Much effort is expected to be spent on the study of the structure and organization of eukaryotic tRNA genes, especially from higher organisms.

The isolation and characterization of tRNA precursors has led to a knowledge of many of the steps and enzymes involved in the processing of tRNA gene transcripts. Basically, the process of precursor maturation does not seem to differ going from simple organisms to the eukaryotic cell. Whether derived from prokaryotes or eukaryotes, the processing enzymes have similar requirements for recognition of tRNA-like structures and perform similar reaction. Thus, together with the conservation of tRNA structures, the principles governing tRNA precursor processing seem to have been preserved during evolution. In eukaryotes, tRNA precursor processing involves in addition "RNA splicing", which appears to be a general way of RNA processing in these systems.

Due to its complexity, the control of tRNA gene expression is still poorly understood. One may hope that information about the structure of controlling sites in tRNA genes, which can be obtained from DNA sequencing, will help unravel some aspects of the regulation of tRNA synthesis. The role of precursor processing as a control mechanism for the modulation of tRNA gene expression has only started to emerge from recent studies. Due to the presence of intervening sequences, this mode of control may prove of major importance in the expression of genes in higher organisms.

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