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Structure of yeast phenylalanine-tRNA genes: An intervening DNA segment within the region coding for the tRNA

(gene regulation/DNA sequence/tRNA processing)

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ABSTRACT **Sixteen bacterial clones containing sequences complementary to yeast Phe-tRNA were isolated from a collection of hybrid plasmids containing *Bam*HI restriction endonuclease-generated yeast DNA fragments inserted in the plasmid vector pBR315. Ten of these clones contained hybrid plasmids with distinct *Bam*HI fragments. The sequence of the Phe-tRNA structural genes and adjacent regions of three of these clones is reported here. In the region flanking the tRNA gene, the sequence of two of the cloned DNAs is similar; the sequence of the third varies considerably. All three of the tRNA genes are bordered by A,T-rich regions. In particular, near the region coding for the 3′ end of the tRNA there is a long sequence of As in the coding strand. This is reminiscent of the region of termination of transcription of the yeast 5S rRNA gene. The sequences coding for the Phe-tRNA contain an additional segment of 18 or 19 base pairs (depending upon the clone) not predicted by the yeast Phe-tRNA sequence. These intervening segments are nearly identical in the three clones and are located within the structural gene, two base pairs from the nucleotides coding for the tRNA anticodon.**

It is axiomatic that knowledge of the organization and structure of eukaryotic genes will clarify some of the mysteries of their expression and regulation. In order to study the factors required for specific transcription of ribosomal genes, we have recently determined the nucleotide sequence of a substantial fraction of the 9000-base-pair yeast ribosomal DNA repeat which contains both the 35S ribosomal precursor (transcribed by RNA polymerase I) and the 5S rRNA genes (transcribed by RNA polymerase III) (1–3). Inspection of the flanking sequences of the 5S rRNA gene (which appears to be an unprocessed gene product) showed a striking A-rich sequence in the coding strand at the site of termination of transcription. On the other hand, the sequence of the putative 5S rRNA promoter region was not revealing (1, 4), nor was there sequence homology between this region and the analogous region of *Xenopus* 5S rRNA genes (5). These observations led us to seek further structural information about other genes transcribed by RNA polymerase III, such the yeast tRNA genes. A comparative sequence analysis of the regions of initiation and termination of transcription as well as processing could then be made in a single transcription system.

Previous studies show that there are about 360 tRNA genes per haploid genome in yeast (6); on the average, each yeast tRNA gene is reiterated 8–10 times (7). Evidence from gene cloning (8) as well as genetic (9) and UV mapping experiments (10) suggests that the yeast tRNA genes are dispersed; as yet there is no evidence for multimeric transcription units as has been found for certain tRNAs in *Escherichia coli* (11–13) and T4 phage (14, 15).

We selected the yeast Phe-tRNA genes for analysis because the sequence and three-dimensional structure are known (16, 17). We present here sequence of three Phe-tRNA genes cloned in *E. coli* from yeast DNA fragments. A number of interesting structural features of the Phe-tRNA genes are reported, the most striking of which is that the DNA sequence of the genes is not collinear with Phe-tRNA; an additional segment of 18 or 19 base pairs is present in the middle of the sequence encoding Phe-tRNA in each of the three cloned genes.

MATERIALS AND METHODS

Bacterial and Yeast Strains and Plasmid Vector. The bacterial host DG75 [an F^- derivative of DG73 (18)] is a derivative of *E. coli* K-12. The plasmid vector pBR315, which confers resistance to both ampicillin and tetracycline (19), was provided by H. Boyer. Yeast DNA was isolated from yeast strain 5178 IC^2X2B^2 provided by L. H. Hartwell. For bacterial growth, L broth containing 20 g of Tryptone (Difco), 5 g of yeast extract (Difco), and 5 g of NaCl per liter was used. Minimal medium (20) was supplemented with thiamine (10 μg/ml), 0.2% glucose, and 100 μg of required amino acids and vitamin-free amino acids (Difco). Drug selective medium contained 20–30 μg of either ampicillin (Bristol Laboratories) or tetracycline (Sigma) per ml.

Cloning and Screening Methods. DNA ligation and bacterial transformation were essentially as described (21). Individual plasmid-bearing clones were screened for the presence of DNA sequence complementary to yeast Phe-tRNA by the colony hybridization technique (22). The Phe-tRNA was obtained from Boehringer Mannheim. These experiments were conducted in a P2 facility with the precautions outlined in the "National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules."

Restriction Endonuclease Analysis. Restriction endonuclease *Eco*RI (*E. coli* RY13) was purified and assayed according to Bingham *et al.* (23). Other restriction endonucleases were obtained and assayed as described (2, 3).

***In Vitro* Labeling and Sequence of Nucleic Acids.** DNA was labeled with [γ-^{32}P]ATP and polynucleotide kinase as described by Maxam and Gilbert (24). tRNA was labeled with $Na^{125}I$ according to the procedure of Tereba and McCarthy (25). DNA was sequenced by the chemical method of Maxam and Gilbert (24).

Resolution of DNA Fragments and Hybridization. Analytical and preparative gel electrophoresis, in either acrylamide or agarose, was carried out as described (2, 3). After electrophoresis, the DNA fragments were transferred to a nitrocellu-

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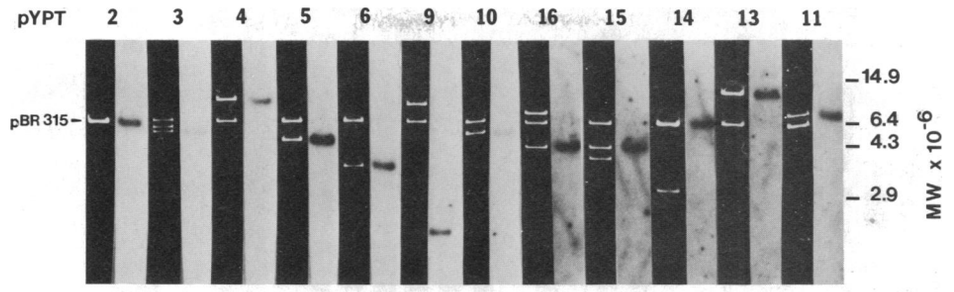

FIG. 1. Agarose gel electrophoresis of yeast Phe-tRNA containing plasmids digested with *Bam*HI and localization of sequences complementary to yeast ^{125}I-labeled Phe-tRNA. After digestion with *Bam*HI, the DNA fragments were resolved by gel electrophoresis in 1% agarose. Alternate columns show the ethidium bromide staining pattern. The right side of each staining pattern is an autoradiogram of hybridization with yeast ^{125}I-labeled Phe-tRNA after transfer from the gel to a nitrocellulose filter (26). MW, molecular weights of fragments obtained by digestion of λ DNA with *Hin*dIII, used as standards.

lose filter (Millipore) as described by Southern (26). The filters were incubated with ^{125}I-labeled Phe-tRNA and processed as described (2).

RESULTS

Yeast gene bank

To obtain a collection of hybrid plasmids containing a large proportion of the yeast genome, DNA digested with the restriction endonuclease *Bam*HI was ligated to *Bam*HI-digested pBR315 [pBR315 has a single *Bam*HI site (19)]. The introduction of a heterologous DNA fragment into the *Bam*HI site of pBR315 eliminates the plasmid's ability to confer tetracycline resistance to its bacterial host (19). Therefore, after the ligation mixture was used to transform the *E. coli* K-12 strain DG75, the transformants were selected for ampicillin resistance. Approximately 20% of the transformants that harbored hybrid plasmids were distinguished by their tetracycline sensitivity. For use as a resource from which the Phe-tRNA genes as well as others could be obtained 3550 clones containing hybrid plasmids were isolated. All of the clones analyzed harbored plasmids that contained at least one *Bam*HI yeast DNA fragment of average molecular weight 6,500,000. This yeast DNA bank contained no ribosomal DNA sequences (5–10% of yeast DNA) because there are no *Bam*HI endonuclease sites within the yeast ribosomal repeat (2, 3). Therefore, assuming that each clone is independently derived, there is a probability of at least 88% that any unique yeast DNA sequence is represented in this collection (27).

Selection of clones with DNA sequences complementary to yeast Phe-tRNA

Each of the tetracycline-sensitive clones was tested for the presence of DNA sequences complementary to yeast Phe-tRNA by the colony hybridization technique of Grunstein and Hogness (22), by using iodinated yeast Phe-tRNA as a probe. Of the 3550 clones screened, only 16 gave positive signals. All of these clones contained DNA fragments that hybridized to yeast Phe-tRNA. Twelve of the 16 clones contained distinct hybrid plasmids as judged by the number and size of the *Bam*HI fragments. These 12 clones were further characterized by *Bam*HI endonuclease digestion and hybridization of ^{125}I-labeled Phe-tRNA to the restriction endonuclease generated fragments (26). Fig. 1 shows the fragment patterns obtained by agarose gel electrophoresis of plasmid DNAs digested with endonuclease *Bam*HI and the autoradiograms of the *Bam*HI fragments hybridized with ^{125}I-labeled Phe-tRNA. These data, as well as the pattern of combined *Bam*HI/*Eco*RI digestions (data not shown), indicate that there are at least 10 different *Bam*HI DNA fragments containing sequences complementary to Phe-tRNA, ranging in size from 2.2–13 megadaltons.

DNA sequence of the cloned Phe-tRNA genes

The strategy for DNA sequencing was based on the existence of an *Eco*RI cleavage site within the Phe-tRNA structural gene, near the region that codes for the 3′ end of the tRNA (16). *Eco*RI digestion of the *Bam*HI fragment containing a Phe-tRNA gene should produce at least two DNA fragments. The *Eco*RI fragment containing the major portion of the tRNA gene can be identified by hybridization (Fig. 2), whereas the *Eco*RI fragment containing the remaining sequence (eight base pairs) cannot be detected by this technique. Therefore, in order to analyze the region coding for the 3′ end of the tRNA it was necessary to sequence all the ends generated by digestion of the inserted yeast DNA by *Eco*RI.

Yeast Phe-tRNA Gene from pYPT2. This plasmid contains a *Bam*HI yeast insert of a size similar to the vector pBR315 (6.1 megadaltons) (Fig. 1). When pYPT2 was digested by *Bam*HI and *Eco*RI, four yeast DNA fragments of about 2, 1.75, 1.4, and 1.3 megadaltons were obtained (Fig. 2). The 1.4-megadalton fragment gave a positive signal by hybridization to ^{125}I-labeled Phe-tRNA. For DNA sequencing, about 1–2 mg of plasmid DNA was digested to completion with *Eco*RI. The resulting fragments were end-labeled with [γ-^{32}P]ATP, digested with *Bam*HI, and separated by gel electrophoresis. The 1.4-megadalton fragment was digested with *Hae*III and the two labeled fragments (0.56 and 0.12 megadalton) were isolated and sequenced from their *Eco*RI sites. The smaller fragment was found to contain the major portion of the Phe-tRNA gene (Fig. 3). The 1.75-megadalton fragment was digested with *Hin*dII, and two labeled fragments (0.34 and 0.1 megadalton) were isolated and sequenced from their *Eco*RI sites. The larger fragment contained sequences coding for the 3′ end of the

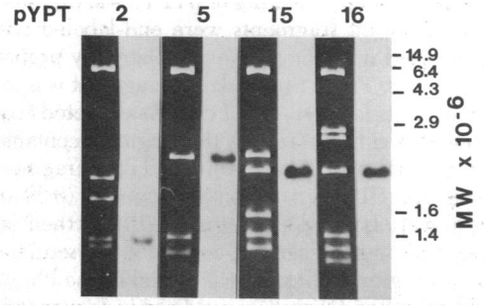

FIG. 2. Agarose gel electrophoresis of plasmids pYPT2, pYPT5, and pYPT15 digested with *Bam*HI and *Eco*RI and localization of sequences complementary to yeast ^{125}I-labeled Phe-tRNA. Fragments were separated by gel electrophoresis in 1.5% agarose. Other details as in Fig. 1. MW, molecular weight.

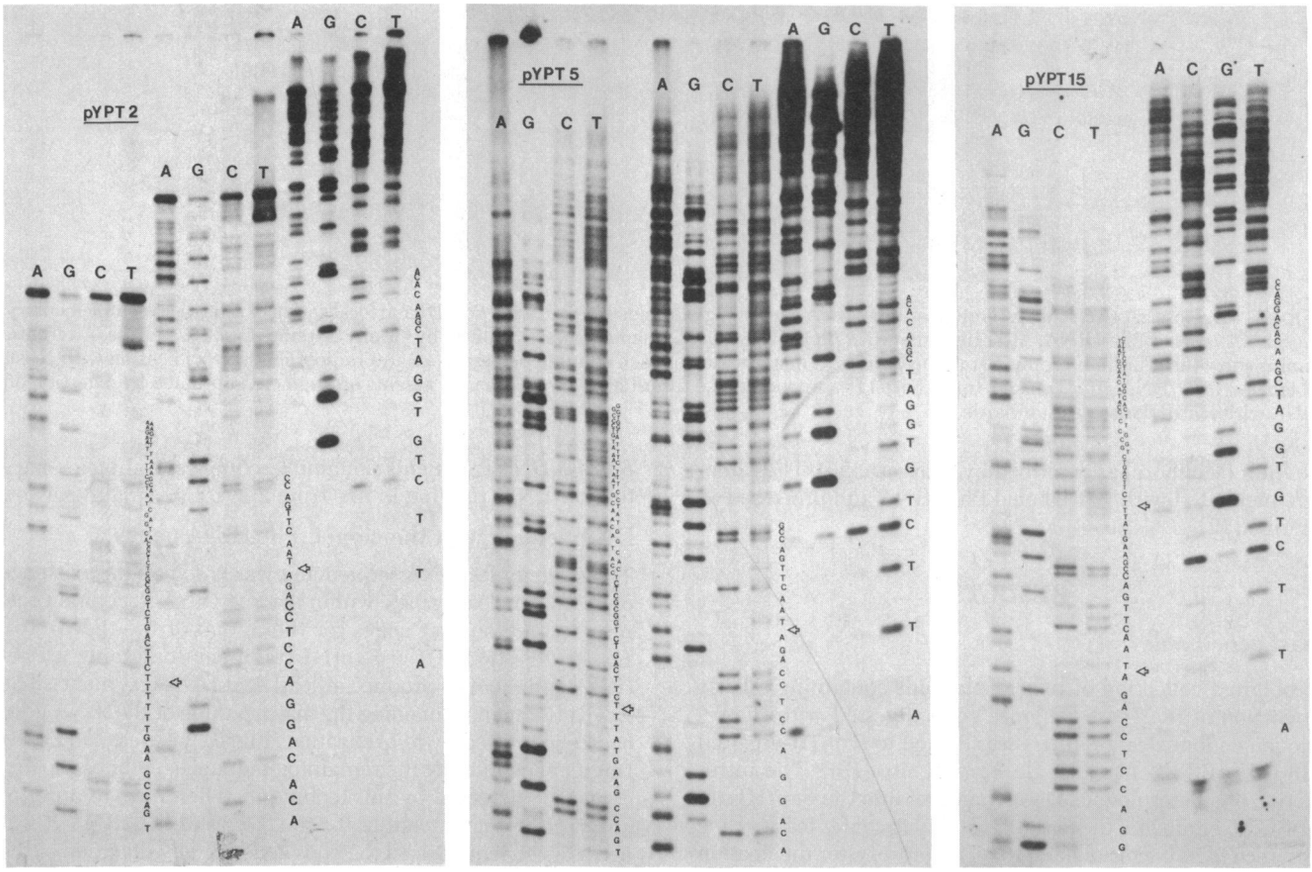

FIG. 3. Electrophoretic separation of ^{32}P-labeled partial digests obtained by specific base cleavage of fragments derived from pYPT2, pYPT5, and pYPT15 containing part of the sequence of yeast Phe-tRNA gene. The columns A, G, C, and T indicate wells loaded with products from reactions partially specific for adenine, guanine, cytosine, and thymine, respectively (24). Electrophoresis was in 20% acrylamide as described by Maxam and Gilbert (24). The arrows indicate the beginning and end of a DNA segment that interrupts sequence collinearity with the known yeast Phe-tRNA.

Phe-tRNA gene. The 2.2- and 1.3-megadalton fragments were sequenced directly from their *Eco*RI ends and found to lack sequences related to the Phe-tRNA gene. Therefore, we conclude that the Phe-tRNA gene is present at the *Eco*RI site encompassed by the 1.4- and 1.75-megadalton fragments. From these results the complete DNA sequence of the Phe-tRNA gene coding and flanking regions from pYPT2 was derived as shown in Fig. 4.

Yeast Phe-tRNA Gene from pYPT5. This plasmid contains a *Bam*HI DNA insert of about 5 megadaltons (Fig. 1). When pYPT5 was digested with *Bam*HI plus *Eco*RI, three fragments containing yeast DNA of 2.4, 1.40, and 1.25 megadaltons were obtained; the first one gave a positive hybridization signal (Fig. 2). For DNA sequencing, 1–2 mg of pYPT5 was digested with *Eco*RI. The resulting fragments were end-labeled with [γ-^{32}P]ATP, digested with *Bam*HI, and isolated by preparative gel electrophoresis. The 2.4-megadalton fragment was directly sequenced from its labeled *Eco*RI end. As expected from the hybridization experiments (Fig. 2), this fragment contained the Phe-tRNA gene (Fig. 3). The 1.40-megadalton fragment was digested with *Hae*III, and two labeled fragments (0.39 and 0.1 megadalton) were isolated and sequenced from their labeled *Eco*RI sites. The larger fragment contained the sequence expected for the region coding for the 3′ end of the Phe-tRNA. The 1.25-megadalton fragment was directly sequenced from its *Eco*RI site. The sequences bore no relationship to the Phe-tRNA gene. We conclude that the Phe-tRNA gene resides at the junction of the 2.4- and 1.4-megadalton fragments. The complete nucleotide sequence of the Phe-tRNA gene coding and flanking regions from pYPT5 is shown in Fig. 4.

Yeast Phe-tRNA Gene from pYPT15. This plasmid contains two *Bam*HI yeast DNA inserts of about 4.1 and 3.8 megadaltons (Fig. 1); only the first gave a positive hybridization signal to yeast Phe-tRNA (Fig. 1). When pYTP15 was digested with a combination of *Bam*HI and *Eco*RI, five fragments (2.4, 2.2, 1.6, 1.4, and 1.3 megadaltons) containing yeast DNA were obtained (Fig. 2). From a comparison of the pattern of *Bam*HI plus *Eco*RI digested pYPT16, which contains the same 4-megadalton *Bam*HI insert, it was concluded that only the fragments of 2.2, 1.4, and 1.3 megadaltons were derived from the 4-megadalton yeast insert in pYPT15. Of these, the 2.2-megadalton fragment gave a positive hybridization signal to Phe-tRNA (Fig. 2). For DNA sequencing, 1–2 mg of pYPT15 was digested with *Eco*RI, end-labeled with ^{32}P, digested with *Bam*HI, and isolated by preparative gel electrophoresis. The 2.2-megadalton fragment was directly sequenced from its *Eco*RI site. As expected, it contained the main part of the yeast Phe-tRNA (Fig. 3). The 1.4-megadalton fragment was digested with *Hpa* II, and two end-labeled fragments (0.35 and 0.09 megadalton) were isolated and sequenced from their *Eco*RI site. The larger fragment contained the sequence coding for the 3′ end of the tRNA and the smaller fragment contained no tRNA gene sequences. The 1.3-megadalton fragment was sequenced from its *Eco*RI site and found to contain no Phe-tRNA gene sequences. Therefore, we conclude that the tRNA gene resides at the junction of the 2.2- and 1.4-megadalton fragments. The complete DNA sequence of the Phe-tRNA gene coding and flanking regions from pYPT15 is presented in Fig. 4.

FIG. 4. Nucleotide sequence of three cloned yeast Phe-tRNA genes and adjacent regions. The results are depicted in three blocks of the sequences coding for the region flanking the 5′ end, the structural gene, and the 3′ end of the tRNA, respectively. The sequence of the corresponding yeast Phe-tRNA is shown in italics. The anticodon is underlined.

DISCUSSION

The hybridization experiments reported in this paper suggest that there are at least 10 Phe-tRNA genes in this tetraploid yeast strain. The clones containing the Phe-tRNA-specific sequences are derived from independent DNA segments because they have different size and restriction endonuclease cleavage patterns. The sequence of the Phe-tRNA genes from three of these clones is reported here.

The sequences of the regions flanking the Phe-tRNA structural gene are A,T-rich. Similarly, the ends of the intervening DNA segments are A,T-rich. Such A,T-rich regions are also present in the transcribed spacer regions adjacent to the 5.8S rRNA gene in the yeast ribosomal DNA (2). Thus, it seems plausible that they represent processing regions. In addition, there is a distinctive A,T-rich region near the end of the region coding for the 3′ end of the tRNA: all three genes have a sustained sequence of As in the coding strand. This is analogous to the putative termination region of the yeast 5S rRNA gene (1, 4) as well as the termination region of several prokaryotes (28). We presume that this is the termination signal for transcription. In pYPT15 and pYPT5, this sequence is close to the end of the structural gene, implying little if any trimming at the 3′ end of the tRNA, whereas in pYPT2 the A-rich sequence is approximately 27 nucleotides downstream, suggesting a longer primary transcript.

No specific structural features can be ascribed to the promoter region because the length of the Phe-tRNA primary transcript is still unknown and the DNA sequencing of this area is not yet extensive. However, there is no homology with the sequence of the yeast 5S rRNA promoter region (1, 4). Nevertheless, in all three cloned DNAs analyzed, there is a region of about 20 nucleotides that is A,T-rich and includes the sequence

5′ . . A-A-T-A-T-A . . 3′ .
. . T-T-A-T-A-T . .

This sequence may represent a signal for processing at the 5′ end.

Another characteristic of the yeast Phe-tRNA gene is the absence of sequences coding for C-C-A, which is found at the 3′ end of mature tRNAs. This indicates that these bases are added after transcription at some stage in the maturation process. This contrasts with the situation found in *E. coli* where some of the tRNA precursors contain the C-C-A trinucleotide (12, 29).

The most remarkable feature of the sequence of the cloned yeast DNA is the segment of 18 or 19 base pairs (depending on the clone) within the yeast Phe-tRNA gene sequences that is not predicted from the sequence of the tRNA (16). This intervening sequence is located one or two bases from the nucleotides complementary to the anticodon. Because the intervening sequence has the residue A at both ends and the insertion site is next to an A, there is some ambiguity as to the exact position of the intervening sequence. The nature of the intervening DNA and its position near the anticodon are intriguing. The sequence of this segment is essentially identical in all three cloned tRNA genes. This contrasts with the considerable dissimilarity in the sequences at the 3′- and 5′-flanking regions of two of the clones (pYPT2 versus pYPT5 and pYPT15) (Fig. 4). As yet, we have no direct evidence that these genes are functional as Phe-tRNA genes. Analysis of the remaining members of this set of Phe-tRNA genes may be necessary to prove this point. The presence of this intervening DNA segment does not appear to be the result of cloning because the DNA segment is small and the sequence of the putative insertion site does not have features (inverted repeats, palindromes) found in other systems in which insertions have been detected (30, 31). In addition, the cloned Tyr-tRNA genes including a suppressor Tyr-tRNA gene (32) also contain intervening DNA segments, but of a sequence different from that reported here for the Phe-tRNA gene. Thus, the nucleotide sequence of this segment appears specific for a set of genes of a given tRNA as if the intervening sequence

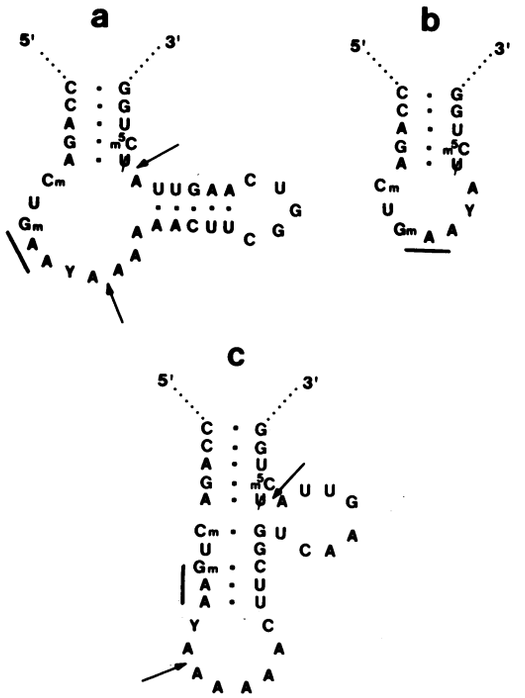

FIG. 5. Two hypothetical secondary structures of the anticodon loop region of putative yeast Phe-tRNA precursors. (*a* and *c*) Alternative Phe-tRNA precursor structures based on the sequence from pYPT2. (*b*) Mature Phe-tRNA. The arrows indicate the beginning and end of the extra 19 bases. The position of the anticodon is indicated by underlining.

played some functional role. Finally, additional DNA sequences have recently been discovered within other eukaryotic genes such as ribosomal RNA (33), globin (34), and immunoglobulin (S. Tonegawa and W. Gilbert, personal communication). In adenovirus, it has become clear that the several mRNAs apparently produced from a single transcription unit (35) are derived from the ligation of RNA fragments coded by noncontiguous regions in the DNA (36, 37).

It may be significant that the intervening segment in the Phe-tRNA gene is adjacent to the anticodon. In the tRNA, the additional nucleotides can form at least two alternative secondary structures. One involves the formation of an additional stem and loop (Fig. 5*a*). The other structure involves the rearrangement of the anticodon loop so as to alter the position of the anticodon from a loop to a stem (Fig. 5*b*). This feature may eliminate anticodon activity of the putative pre-Phe-tRNA without substantially changing the conformation of the rest of the molecule (17).

The findings reported here suggest that maturation of the tRNA transcripts involves not only endonuclease cleavage and base modification (38) but also ligation and C-C-A addition. The complexity of this process suggests a role in gene expression. This strengthens the emerging views that regulation can be effectively controlled not only at the level of transcription but also at the level of processing. With such tandem controls acting in concert, the degree of regulation can be more stringent and dependable.

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