Characterization of the yeast tRNA^{Ser}₂ gene family: genomic organization and DNA sequence

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ABSTRACT

Purified, isolated yeast tRNA^{Ser}₂ was used as a hybridization probe to estimate the number of tRNA^{Ser} genes in the yeast genome.

Molecular clones of several of the genes were obtained. Three examples were studied in detail with respect to their genomic organization, and DNA sequences were determined for them.

There appear to be eleven tRNA^{Ser} genes in the yeast genome. They are neither tandemly repeated, nor clustered with other tRNA genes. They contain no intervening sequences.

INTRODUCTION

The primary structures of the major serine tRNAs of yeast, tRNA^{Ser}₁ and tRNA^{Ser}₂ were among the first to be determined (Zachau, Dutting and Feldman, 1966). Minor serine isoacceptors have since been purified and characterized (Kruppa and Zachau, 1972) and sequenced, (Piper, 1978, Etcheverry, *et. al.*, 1979). The anticodon sequences for yeast tRNA^{Ser}₂ are: 5'-IGA-3' for ser₁ and ser₂ tRNAs (Zachau, Dutting and Feldman, 1966) and, for two minor serine tRNAs, U*GA and CGA (Etcheverry, *et. al.*, 1979). According to the wobble-pairing rules (Crick, 1966) the corresponding codons for these tRNAs should be UCU/C/A and UCG, respectively.

Each of the above-mentioned tRNA^{Ser} anticodons can, by a single base pair alteration in DNA, be changed to a suppressor anticodon. Therefore, we have chosen the genes coding for these tRNA^{Ser} molecules for structural study, anticipating that some of them will be nonsense suppressor loci amenable both to biochemical and genetic analysis. In this paper we report our initial studies on yeast tRNA tRNA^{Ser}₂ genes: the molecular cloning of genes coding for tRNA^{Ser}₂ and the DNA sequences of three of these genes.

MATERIALS AND METHODS

Growth of yeast strains

All yeast strains were cultured in yeast extract peptone broth (YEP) supplemented with adenine and uracil or on 1.4% agar plates in YEP unless otherwise specified.

Isolation and labelling of tRNA₂^{Ser}

A 2.5 x 22.5 cm column of BD-cellulose was equilibrated with 0.45M NaCl, 0.05M NaOAc pH 4.5, 0.01M MgCl₂. 15,000 A₂₆₀ units of total yeast tRNA were charged onto the column and eluted with a four liter linear gradient of NaCl from 0.45 to 1.8M. 10 ml fractions were collected and 5 µl of each fraction assayed for the ability to convert ³H-serine to acid-insoluble form when incubated with tRNA synthetase (Wimmer, Maxwell & Tener, 1968). Radiolabeling of tRNA₂^{Ser} was via iodination of the N⁶-isopentenyl adenosine residue (Olson, *et al.*, 1979a). The probe so prepared gave a single band on a one-dimensional denaturing acrylamide gel (data not shown) and did not cross-hybridize with known tRNA_{Minor}^{Ser} genes (Olson, *et al.*, manuscript submitted for publication). The unlabelled tRNA₂^{Ser} from this preparation was used as a competitor against total tRNA in some experiments presented below (e.g. *vis.* Fig. 5).

Acetylation of ³H-serine-tRNA₂^{Ser} with N-acetylsuccinimide

This procedure was used to stabilize the aminoacyl tRNA linkage during hybridization. N-acetylsuccinimide was prepared by mixing 1.2 ml glacial acetic acid with 2.3g N-hydroxysuccinimide in 40 ml dioxane containing 4.2g dicyclohexylcarbodiimide. After three hours at room temperature the reaction was transferred to -20° for 24 hours. Precipitated urea was removed by filtration and dioxane by evaporation. The resultant crystals were dissolved in 4 ml dioxane at 45° and allowed to cool slowly. The final crystals were collected by filtration and dried under a vacuum.

Serine transfer RNA was aminoacylated, the reaction was chilled and brought to 0.1 M sodium acetate pH 4.5 (total volume 150 µl). 200 µl of DMSO containing 22 mg/ml of N-acetylsuccinimide were added, and after 2 hours on ice a second 200 µl added. The solution was made 0.3 M in potassium acetate and the tRNA precipitated with ethanol.

Preparation of yeast DNA

Yeast DNA samples were prepared as described by Olson, *et al.* (1979b). DNA was customarily purified through the CsCl gradient step.

Preparation and use of restriction endonucleases

The restriction endonuclease *EcoRI* was prepared as described by Hel-

ling, Goodman, and Boyer (1974) through the phosphocellulose step. PstI was prepared from Providentia stuartii following the method for preparation of HaeIII described by Roberts (1975). All other endonucleases were purchased from New England Biolabs, Bethesda Research Laboratories or Miles Biochemical. Agarose gel electrophoresis of DNA restriction fragments and hybridization analyses were done as described by Olson, et al. (1979a).

Hybridization to filter-bound DNA

The conditions used for all hybridizations were essentially those of Birnsteil (1972). The hybridization solutions contained 50% formamide, 0.5M NaCl, 0.15M Na_2HPO_4 pH 6.2, 0.005M EDTA. Iodinated tRNA probe was maintained at a concentration of $2-4 \times 10^6$ cpm/ml. When either ^{32}P - or ^3H -labeled tRNAs were used, probe concentrations were adjusted so as to have tRNA^{Ser} present at approximately 0.1 $\mu\text{g}/\text{ml}$. Hybridizations were typically carried out at 42° for 36 to 48 hours.

Preparation of phage lambda DNA

Preparations of DNA from the phage lambda vectors and from various lambda/yeast recombinant strains closely followed those detailed by J.R. Cameron (Ph.D. Thesis, Stanford University, 1977).

Construction of Recombinant Plasmids

Selected fragments of serine tRNA clones were transferred to the plasmid vector pBR322. The particular transfers are specified in Results. All ligations and transformations were done as described by Goodman, Olson, and Hall (1977).

RESULTS

I. Determination of the number of tRNA₂^{Ser} genes

The major species of seryl transfer RNA, tRNA₂^{Ser}, was purified from the "-phe" fraction of total yeast tRNA (Wimmer, et al., 1968), and was labeled for use as a hybridization probe by iodination of the N⁶-isopentenyladenosine residue located just 3' to the anticodon of the tRNA (Olson, et al., 1979a). The conditions used for labeling show specificity for this modified base, preventing radiochemical contamination of the hybridization probe by any non-i⁶A-containing tRNAs.

To obtain an estimate of the number of tRNA₂^{Ser} genes in the yeast genome, EcoRI restriction fragments of total yeast DNA from four different yeast strains were assayed by Southern hybridization with the labeled tRNA probe (Fig. 1). With the exception of Y4A, each of the strains displays a pattern of eleven bands.

Figure 1. Identification of $tRNA^{Ser}_2$ Restriction Fragments. DNA samples from yeast strains, (a) B596, (b) J12-9C, (c) 96C, and (d) Y4A were digested with EcoRI and the fragments separated on an agarose gel. A nitrocellulose filter replica was prepared from the gel and hybridized with ^{125}I - $tRNA^{Ser}_2$. Molecular weight estimates for the $tRNA^{Ser}_2$ restriction fragments were obtained by comparison with EcoRI fragments of phage λ . A schematic representation of the hybridization data is given at the right.

II. Construction and identification of the molecular clones of yeast $tRNA^{Ser}_2$ genes

A. Cloning in λ gt.10B

The vector λ gt.10B (Cameron, J.R., et al., 1975) was used to clone the EcoRI restriction fragments containing $tRNA^{Ser}_2$ gene sequences from strain B596 (vis. Fig. 1). A B596 hybrid phage pool was screened with iodinated $tRNA^{Ser}_2$ probe, and a total of approximately 20 clones isolated. Restriction analyses of DNA from four of the phage that gave positive hybridization responses are shown in Fig. 2. Each of the clones contains a DNA restriction fragment comparable in size to a yeast DNA fragment that

Figure 2. Cloning of $tRNA_{Ser}^2$ Genes. DNA from each of four recombinant phages that had been shown to carry $tRNA_{Ser}^2$ genes was restricted with EcoRI and run on an agarose gel along with similarly digested B596 DNA. A nitrocellulose filter replica was prepared from the gel and hybridized with iodinated $tRNA_{Ser}^2$. (A) Unfractionated DNA from strain B596; (B) DNA from clone λ gt. SerG; (C) DNA from clone λ gt. SerJ; (D) DNA from clone λ gt. SerB; (E) DNA from clone λ gt. SerF. The lighter intensity band observed in lane (B) arises from a partial digestion of the clone DNA.

hybridizes to that same probe. By the designation given in Fig. 1, the clones represent fragments SerB, SerF, SerG, and SerJ. SerA and SerE were also obtained (data not shown).

The presence of a $tRNA_{Ser}^2$ gene in each of the clones was verified by hybridization to cloned DNA of 3H -seryl- $tRNA_{Ser}^2$. This probe was tested against DNA of the recombinant phages bound to nitrocellulose. The results are shown in Table I. 3H -seryl $tRNA_{Ser}^2$ does not hybridize to λ gt. λ B DNA, but does hybridize to DNA from each of the putative clones. As the label

Table I
Hybridization of (^3H)-seryl-tRNA^{Ser} to Clone DNA

Microliters probe (a)	added	λ gt- λ B DNA	λ gt-SerB DNA	λ gt-SerG	λ gt-SerJ DNA
20		26 ^(b)	315	454	329
40		27	378	454	329
60		26	416	625	470
80		30	420	708	387
100		31	458	663	480

(a) Probe was constructed as detailed in Materials and Methods.

(b) Numbers shown indicated in the column heading. Each filter contained 2 μg of the DNA indicated in the column heading. All hybridizations were done in a 500 μl final volume at 42 $^{\circ}$ for four hours.

in this probe can only be associated with seryl tRNA, each of the clones must contain a seryl transfer RNA gene.

B. Subcloning the tRNA^{Ser} genes into pBR322.

After restriction maps for each of the recombinant phages were determined (Fig. 3), a suitable restriction fragment was chosen from each phage

Figure 3. Restriction Maps of SerB, SerG, and SerJ Restriction Fragments. Each restriction map was determined by digestion with the specified restriction enzymes. Estimates of molecular weights of the products were made by comparison with EcoRI and HindIII restriction fragments of phage λ for agarose gels, and HaeIII fragments of pBR322 for acrylamide gels.

for transfer to the plasmid pBR322. SerB was transferred into pBR322 as a HindIII fragment of 3.4 kb, SerG as an EcoRI - BamHI fragment of 0.6 kb, and SerJ as an EcoRI- HindIII fragment of 0.4 kb. These recombinant plasmid products have been designated pGP.SerB, pGP.SerG, and pGP.SerJ.

III. Organization of the yeast tRNA₂^{Ser} genes

For present purposes the general question of the "organization" of the tRNA₂^{Ser} genes in the yeast genome will be broken down into two parts: (1) Do the tRNA₂^{Ser} loci ever occur as closely spaced tandem repeats of the tRNA₂^{Ser} sequence, as suggested by the variation in hybridization band intensity observed in Figure 1? (2) Are any of the tRNA₂^{Ser} genes linked closely to other tRNA genes?

Tandem Repeats of tRNA₂^{Ser} Genes

On the basis of preliminary restriction mapping of the SerB, SerG, and SerJ clones, we were able to limit all of the tRNA₂^{Ser} hybridizing DNA sequence to those fragments that had been subcloned into pBR322 (data not shown). However, this type of analysis does not distinguish tRNA-gene repeats in which the genes are located very close to one another.

Figure 4 describes an experiment used to test for the presence of closely spaced tandem repeats of tRNA₂^{Ser} sequences in the cloned DNA fragments. Each of the tRNA₂^{Ser} genes has a single site for the restriction endonuclease PstI (Kruppa and Zachau, 1972). If a clone contains tandemly repeated tRNA₂^{Ser} sequences, then cleavage of the clone DNA with PstI should generate, in addition to the plasmid fragment and the fragment derived from DNA surrounding the tRNA₂^{Ser} gene, a small fragment corresponding in size to the distance between two neighboring tRNA₂^{Ser} sequences, i.e. from the PstI site of one sequence to the PstI site of its neighbor.

DNA samples from clones SerB, SerG, and SerJ were digested with PstI and electrophoresed on a 5% acrylamide gel. The photograph in Figure 4 shows no fragment smaller than those predicted by the presence of a single PstI site (i.e., a single tRNA₂^{Ser} gene) in each of the clones. In none of the cases examined, then, is there a tandem repeat of the tRNA₂^{Ser} gene sequence. It should be noted that this experiment would not detect multiple tRNA₂^{Ser} genes if (a) they were inverse repeats with the PstI sites located less than 50 bp from one another or (b) if one of the genes did not contain the requisite PstI site (i.e. a sequence variant tRNA₂^{Ser}).

Association of tRNA₂^{Ser} Genes With Other tRNA Genes

A test to determine whether any of the tRNA₂^{Ser} genes is closely linked to another tRNA gene was carried out by a competition hybridization in

Figure 4. Tandem Repeats of $\text{tRNA}_{\text{Ser}}^{\text{Ser}}$ Genes. DNA samples from plasmids pGP.SerB, pGP.SerG, and pGP.SerJ were each digested with *Pst*I and the fragments separated on a 5% acrylamide gel. The amount of DNA in each lane was chosen so as to assure visualization of any fragment 50 base pairs or greater in length. The upper portion of the gel is overloaded. The arrow indicates the size of a fragment that would be generated by *Pst*I digestion of tandemly repeated $\text{tRNA}_{\text{Ser}}^{\text{Ser}}$ gene. Molecular weight markers are *Hae*III fragments of pBR322. The arrow (at ~ 85 bp) indicates the minimum size of a tandem repeat.

which total ^{32}P -labeled $\text{tRNA}_{\text{Ser}}^{\text{Ser}}$ was hybridized to cloned sequences with or without purified $\text{tRNA}_{\text{Ser}}^{\text{Ser}}$ as a competitor.

Yeast cells were uniformly labeled with $^{32}\text{PO}_4$, and tRNA was prepared from them. This tRNA was hybridized to EcoRI-restricted DNA from four representative tRNA₂^{Ser} clones. Each of the DNA samples was prepared in duplicate; one sample was hybridized in the presence of cold tRNA₂^{Ser} competitor and one in its absence. The level of ^{32}P -tRNA was adjusted so that any species present in a 1% representation would be at a final concentration of 0.1 $\mu\text{g}/\text{ml}$ in the hybridization solution; the level of competitor was adjusted to be in a 10 to 20-fold excess over the tRNA₂^{Ser} in the ^{32}P -labeled population.

The results of the hybridization are shown in Fig. 5. The ^{32}P -tRNA that hybridizes to DNA from clones SerB, SerF, and SerJ is efficiently com-

Figure 5. Linkage of tRNA₂^{Ser} Genes to Other tRNA Genes. Duplicate DNA samples from the clones of SerB, SerF, SerG, and SerJ were digested with EcoRI, electrophoresed on an agarose gel and transferred to nitrocellulose as separate lanes. The filters were hybridized to unfractionated ^{32}P -tRNA; one of each pair of filters was hybridized in the presence of cold tRNA₂^{Ser} competitor (+) and one in its absence (-).

peted by the cold $\text{tRNA}_2^{\text{Ser}}$. On these DNA fragments there are no tRNA genes other than for $\text{tRNA}_2^{\text{Ser}}$. In contrast, the cold $\text{tRNA}_2^{\text{Ser}}$ does not compete all the hybridization to DNA from clone SerG. A densitometer scan of the autoradiogram showed the SerG hybridization response in the presence of competitor to be approximately 50% that in its absence, suggesting that in addition to the $\text{tRNA}_2^{\text{Ser}}$ gene, there is only one other tRNA gene on the fragment. The data presented in Table 1 suggest that the other tRNA gene on the SerG fragment may be a tRNA^{Ser} . The SerG fragment does not hybridize particularly strongly, however (Figs. 1,2). As such it is unlikely that there is another $\text{tRNA}_2^{\text{Ser}}$ gene. It is also unlikely that there is a tRNA^{Ser} since none of the EcoRI fragments that hybridizes to purified $\text{tRNA}_{\text{min}}^{\text{Ser}}$ corresponds in size to the SerG fragment (Olson, *et al.*, 1981). Thus, the identity of the second tRNA gene on the SerG fragment must be left indeterminate.

IV. DNA sequence determination of three $\text{tRNA}_2^{\text{Ser}}$ genes

The tRNA^{Ser} sequence determined by Zachau *et al.*, (1966) predicts the presence of an endonuclease HaeIII restriction site 10 base pairs (bp) from the DNA sequence corresponding to the 5' end of the mature tRNA and a PstI restriction site 10 bp from that DNA sequence corresponding to the 3' end. Each of these sites provides a point from which DNA sequence may be obtained by the method of Maxam and Gilbert (1977). The positions of these restriction sites within the tRNA gene allow determination of sequences for both strands of the coding region (that portion of the DNA complementary to the mature tRNA molecule). DNA sequences of each of genes SerB, SerG, and SerJ were determined from their HaeIII and PstI restriction sites.

The results of the determinations are shown in Fig. 6. In several respects the $\text{tRNA}_2^{\text{Ser}}$ genes are similar to other yeast tRNA genes. The CCA-OH found at the 3' end of the $\text{tRNA}_2^{\text{Ser}}$ molecule is not encoded in the DNA. The sequences 3' to the coding region of the tRNA gene are A + T rich, although the long strings of T residues in the "+" strand 3' to both tRNA^{Tyr} and tRNA^{Phe} genes do not seem to be present. The regions 5' to the three $\text{tRNA}_2^{\text{Ser}}$ genes bear no apparent homologies to one another, nor to any of the comparable regions adjacent to other yeast tRNA genes. In none of the three genes sequenced here was an intervening sequence found.

DISCUSSION

The experiments described here constitute an initial characterization of the yeast $\text{tRNA}_2^{\text{Ser}}$ family. We have made an estimate of the number of

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      50          40          30          20          10
Ser B T T T C C C T G C C A G T G G A C A G T G T T G T C C T T A C A A A C T C A A G A G A T T A A A
Ser G      A G G A A T C A C A A A G A A A C A G A A T A C A C T A A T C A A G T T A G T A T
Ser J      C C T A G G T T T G G G A T A T A T C T T A A G T C A A T A C A T C A T T A G T A A

      10          20          30          40          50
Ser B G G C A A C T T G G C C G A G T G G T T A A G G C G A A A G A T T A G A A A T C T T T T G G G C T T T G
Ser G G G C A A C T T G G C C G A G T G G T T A A G G C G A A A G A T T A G A A A T C T T T T G G G C T T T G
Ser J G G C A A C T T G G C C G A G T G G T T A A G G C G A A A G A T T A G A A A T C T T T T G G G C T T T G

      60          70          80          90          100
Ser B C C C G C G C A G G T T C G A G T C C T G C A G T T G T C G
Ser G C C C G C G C A G G T T C G A G T C C T G C A G T T G T C G T T A T G T G T G C C T
Ser J C C C G C G C A G G T T C G A G T C C T G C A G T T G T C G T C A T T T T A T A T T C C A A T

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Figure 6. DNA Sequences of Three tRNA_{Ser} Genes. DNA sequences of SerB, SerG, SerJ. Sequences were determined from the HaeIII site (GGCC) as position 10, and the PstI site (CTGCAG), at position 71. The sequence in italics represents the mature tRNA_{Ser} . The first row shows bases 5' to the tRNA coding sequences, the second and third rows, bases of the tRNA-coding sequences and in the cases of SerG and SerJ some bases 3' to the tRNA sequence. The actual extent of sequence determinations for the three genes was as follows: SerB, Hae III > 5', 60 bases (2 determinations); Hae III > 3', 72 bases (1 determination); PstI > 5', 73 bases (1 determination). SerG, Hae III > 5', 52 bases (2 determinations); Hae III > 3', 75 bases (1 determination); PstI > 5', 64 bases (1 determination); PstI > 3', 19 bases (1 determination); SerJ, Hae III > 5', 52 bases (2 determinations); Hae III > 3', 55 bases; PstI > 5', 63 bases (1 determination); PstI > 3', 25 bases (1 determination). All sequence presented from a single determination was wholly unambiguous in interpretation.

tRNA_{Ser} genes, examined the organization of these genes, and determined the DNA sequences of three examples.

Among the serine transfer RNA's tRNA_{Ser} is the predominant species. The codons translated by it - UCA, UCC, and UCU, - are the most frequently used serine codons in yeast mRNA (J. Bennetzen, unpublished information). Concurrently, the RNA/DNA hybridization data presented here show the tRNA_{Ser} genes to be the greatest in number among the several classes of tRNA_{Ser} genes (cf. Olson, *et al.*, 1981). These observations suggest the hypothesis that the availability a tRNA species to the yeast translation system may be determined by the number of genes which encode that species. While this may be part of a general mechanism for regulating the levels of tRNA, it is likely not to be the only one. An example of an apparently more complex regulatory system is seen within the class of yeast tRNA^{Tyr} genes, which show variations in level of expression among themselves (Rothstein, 1977). Another example of apparent variation in transcription efficiency within a class of tRNA genes has recently been presented by DeFranco *et al.* (1984), who support the hypothesis that 5'-flanking sequences play

a role in modulation of rate of transcription. It must be noted, however, that the conclusions of DeFranco, *et.al.*, are based on experiments done with *Drosophila* tRNA genes in a heterologous *in vitro* system.

The eleven tRNA₂^{Ser} sequences identified in Fig. 1 appear to be distributed more or less randomly throughout the yeast genome. The examination of their distribution presented here revealed no systematic patterns of linkage of the tRNA₂^{Ser} genes, either to one another or to other tRNA genes. These observations are consistent with the genetic mapping data for known yeast nonsense suppressors, which appear not be clustered in the yeast genome (Hawthorne and Mortimer, 1963). As an interesting aside, we note here that one of the tRNA₂^{Ser} clones was located on the yeast genetic map by virtue of its proximity to the yeast *cyc-1* gene. A 9kb *EcoRI* fragment that had been identified and cloned from strain B596 by virtue of its hybridization to a *cyc1*-specific probe (Montgomery, *et al.*, 1978) was found to hybridize to labeled yeast tRNA₂^{Ser} (Marlis Worthington, personal communication). A detailed comparison of the restriction sites within the SerB fragment and the clone pYe.cyc 1-9 (Montgomery *et. al.*, 1978) showed them to be identical. Subsequent to this identification all of the DNA which lies between SerB and the *cyc-1* gene has been sequenced (David Leung and Michael Smith, personal communication). The distance between the two genes is 2240 base pairs.

DNA sequences were obtained for each of three tRNA₂^{Ser} genes, designated SerB, SerG, and SerJ. Each has the DNA anticodon sequence AGA, corresponding to the IGA anticodon found in the mature tRNA₂^{Ser}. This codon is theoretically capable of translating the codons UCU, UCC, and UCA. As such, a G-to-C transversion mutation in the second position of the anticodon should allow the mutant tRNA₂^{Ser} to act as a suppressor of UGA nonsense codons. In spite of this, to date no tRNA₂^{Ser}-derived UGA suppressor has been isolated. This may be explained by the possibility that the mutant tRNA would also translate the codons UGU, and UGC, which specify cysteine, and thus prove lethal for the cell. Alternatively, it may be that tRNA₂^{Ser} is not used for the translation of UCA codons *in vivo*, this function being entirely relegated to tRNA_{minor}^{Ser} with the U*GA anticodon. In accordance with this hypothesis, UAA suppressors derived from the minor tRNA_{UCA}^{Ser} class have been isolated (Ono, *et. al.*, 1979; Olson, *et. al.*, 1981). Nonetheless, if tRNA₂^{Ser}-derived UGA suppressors can in fact be obtained and have not been identified for some unknown reason, we can predict the genetic map position of one such suppressor to be 2240 base pairs from the *cyc-1* locus

on yeast linkage group X. This suppressor would correspond to the SerB tRNA gene.

In most respects the tRNA₂^{Ser} genes are structurally analogous to other yeast tRNA genes. Within that sequence that codes for the mature tRNA, the genes are precisely homologous to one another and match the tRNA sequence; just adjacent to the 3' end of the SerJ gene (in the direction of transcription) one finds an A+T-rich region presumed to play a role in termination of transcription. Although the DNA sequence 3' to the SerG gene does not show such an A+T-rich region, the sequence is limited in extent. It may be that the presumed termination region is found slightly further downstream.

We will mention briefly the lack of homology between sequences located 5' to the three tRNA₂^{Ser} genes. These bear no discernable relation to one another nor to those 5' to other yeast tRNA genes. The possible significance of the generally observed 5' nonhomologies with regard to control of transcription and recognition of the tRNA gene promoter by RNA polymerase III has been discussed elsewhere (Koski, et al.; DeFranco, et al., 1980).

Finally, we note that the tRNA₂^{Ser} genes have no intervening sequences, in contrast to the genes that encode yeast tRNA^{Tyr} (Goodman, et al., 1977), tRNA^{Phe} (Venegas, et al., 1979), tRNA^{Trp}, tRNA₃^{Leu} (Kang, 1979), and tRNA_{UGC}^{Ser} (Etcheverry, et al., 1979), all of which have intervening sequences. It is of interest that tRNA₂^{Ser} is among the largest of yeast tRNA's, due to the presence of a large extra arm. It may be that this extra coding sequence is a derivative of a pre-existing intervening sequence. In this context it should be mentioned that yeast tRNA₃^{Leu} has both an intervening sequence and a large variable arm.

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