Nucleotide sequences of yeast genes for tRNA^{Ser}, tRNA^{Arg} and tRNA^{Val}: homology blocks occur in the vicinity of different tRNA genes

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Three members of a collection of pBR322-yeast DNA recombinant plasmids containing yeast tRNA genes have been analyzed and sequenced. Each plasmid carries a single tRNA gene: pY44, tRNA^{Ser}; pY41, tRNA^{Arg}; pY7, tRNA^{Val}. All three genes are intronless and terminate in a cluster of Ts in the non-coding strand. The sequence information here and previously determined sequences allow an extensive comparison of the regions flanking several yeast tRNA genes. This analysis has revealed novel features in tRNA gene arrangement. Blocks of homology in the flanking regions were found between the tRNA genes of an isoacceptor family but, more interestingly, also between genes coding for tRNAs of different amino-acid specificities. Particularly, three examples are discussed in which sequence elements in the neighborhood of different tRNA genes have been conserved to a high degree and over long distances.

Key words: sequence homologies/tRNA genes/*S. cerevisiae*

Introduction

Studies in a variety of organisms have revealed that tRNA genes are genomically organized in different patterns. In prokaryotes, a tandem arrangement of tRNA genes resulting in multimeric transcription units is often observed (e.g., Nakajima *et al.*, 1981). In eukaryotes, by contrast, tRNA genes are predominantly organized as single transcription units (e.g., Feldmann, 1977). Nonetheless, in *Drosophila* or *Xenopus laevis*, sets of tRNA genes are found to be clustered at single chromosomal sites, but in no obviously regular manner. Such clusters can contain genes for several different species of tRNAs, some present in multiple copies (Clarkson *et al.*, 1978; Hovemann *et al.*, 1980; Robinson and Davidson, 1981) or they can represent redundant copies of the same tRNA gene family (e.g., Hosbach *et al.*, 1980). In *X. laevis* (Clarkson *et al.*, 1978), a clustered arrangement of tRNA genes seems to be advantageous for their amplification. In *Drosophila*, in addition to tRNA gene clusters, dispersed tRNA genes have been characterized (e.g., De Franco *et al.*, 1980). The only case so far studied in human cells shows that ~12 gene copies for the initiator tRNA are scattered throughout the genome (Santos and Zasloff, 1981).

In the yeast *Saccharomyces cerevisiae*, several tRNA gene families have been characterized (e.g., Goodman *et al.*, 1977; Valenzuela *et al.*, 1978; Page and Hall, 1981; Olson *et al.*, 1981; Broach *et al.*, 1981; Olah and Feldmann, 1981; Eigel *et al.*, 1981). The number of gene copies for a particular tRNA

varies between two for the minor and 18 for the major species. In all cases the multiple gene copies were found to be dispersed.

So far, little information is available on the structure of the different chromosomal regions in which dispersed tRNA genes are located. Furthermore, it is not known whether a distinct arrangement of the tRNA genes is of functional significance. Therefore, it is of interest to investigate, for example, what sequence similarities exist between different sites, both within the immediate neighborhood of these genes and in regions more distant from them. Are there common features of the flanking regions that might suggest elements conserved during evolution of the multigene families? Are there regions involved in the control of expression of the multiple gene copies? We have investigated these questions in the yeast system for which a good deal of genetic data and structural information is already available.

Here we report the DNA sequences of three different yeast chromosomal segments each carrying a single tRNA gene. These sequences have been compared with each other and with a number of sequences that have been determined recently in our laboratory (Olah and Feldmann, 1980; Eigel *et al.*, 1981; Feldmann *et al.*, 1981) or in other laboratories (Broach *et al.*, 1981; Page and Hall, 1981; Olson *et al.*, 1981; Valenzuela *et al.*, 1978; Goodman *et al.*, 1977).

Results

Analysis of the DNA sequences from pY44, pY41, and pY7

The three plasmids analyzed here belong to a collection of yeast DNA recombinants that carry tRNA genes (Olah and Feldmann, 1980). Figure 1 summarizes their restriction maps and the locations of the tRNA genes as they were deduced from restriction analyses and filter hybridizations (see Materials and methods). The three plasmids each contain only a single tRNA gene, the DNA sequences of which are given in Figure 2.

pY44 carries a tRNA^{Ser} gene. Its structural part is colinear with the tRNA sequence as determined previously (Zachau *et al.*, 1966); no intervening sequence is present. As judged from a comparison with the flanking sequences of three other tDNA^{Ser} sequences (termed B, F, and G) which were reported recently (Page and Hall, 1981), the gene in pY44 has a chromosomal location different from these.

The DNA fragment from pY41 contains a tRNA^{Arg} gene which, in its structural part, is colinear with the RNA sequence as determined by Weissenbach *et al.* (1972); no intron is found. The tRNA^{Arg} gene of pY41 occurs as a single transcription unit, in contrast to a tRNA^{Arg} gene, which is arranged in a dimeric transcriptional unit together with a tRNA^{Asp} gene in yeast (Beckman *et al.*, 1979).

pY7 contains an intronless gene for tRNA^{Val}. The tRNA sequence had been determined by Baev *et al.* (1967) and has been revised by Bonnet *et al.* (1971). Our data agree with the revised tRNA sequence (Bonnet *et al.*, 1971).

In all three cases studied here, the structural genes are followed by a cluster of Ts (in the non-coding strand) which are thought to be termination signals for RNA polymerase III

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Fig. 1. Restriction endonuclease cleavage maps of pY44, pY41, and pY7. The first line in each array represents the *Hind*III inserts in pBR322 (pBR322 DNA indicated in black). The second line in each array shows the fine restriction map of a subfragment carrying the tRNA gene (drawn in black). The arrows below indicate the direction and the extent of sequencing. The maps are arranged in such a way that the transcription of the tRNA genes is from left to right (cf. Figure 2). The cleavage sites for different enzymes are represented by arabic numerals: 0 = *Hind*III; 1 = *Eco*RI; 2 = *Pst*I; 3 = *Sau*96; 4 = *Sau*3A; 5 = *Taq*YI; 6 = *Alu*I; 7 = *Hpa*II; 8 = *Hae*III or *Bsp*I; 9 = *Hin*fl.

(e.g., Korn and Brown, 1978; Silvermann *et al.*, 1979; Garber and Gage, 1979). The base compositions of the flanking regions show that these tRNA genes are embedded in relatively A + T-rich sequences, as was observed in other cases (e.g., Feldmann, 1976). For these three genes, the average G + C content within 120 nucleotides adjacent to the coding regions is only 29%. These sequences contain several longer runs of Ts in one or the other strand, similar to the transcription termination signal.

Comparative analyses of yeast tDNA segments

Our data, and previous sequence information for several tRNA genes (Goodman *et al.*, 1977; Valenzuela *et al.*, 1978; Olah and Feldmann, 1980; Page and Hall, 1980; Olson *et al.*, 1981; Broach *et al.*, 1981; Eigel *et al.*, 1981), were used as a basis for an extensive comparison of the regions adjacent to the tRNA coding sequences. We have applied the rules developed by van Ooyen *et al.* (1979) in their comparison of hemoglobin genes for lining up pairs of tRNA flanking sequences. The result is shown in Figure 3. The sequences we compared differ considerably in size: sequences determined

previously for the tRNA^{Tyr} (Goodman *et al.*, 1977), the tRNA^{Phe} (Valenzuela *et al.*, 1978), or the tRNA^{Ser} (Page and Hall, 1981) genes extend only some 20–40 nucleotides into the region 5' to the tRNA coding segments; sequences determined by Olson *et al.* (1981) and in our laboratory extend up to several hundred nucleotides into the flanking regions. A further difficulty that arises is to optimize the alignment because of the enormous number of combinations that have to be tested. We carried out the comparison by visual inspection of the sequences in combination with a computer search (Neumaier, 1981). In Figure 3 we list the examples which were most obvious; in most of the other pairs compared we could not detect any homology. The number of gaps introduced was minimized. The homologies of the segments are expressed as the number of matching nucleotides per number of positions compared (gaps included). In three cases [*SUQ5* (Olson *et al.*, 1981)/*Ser*₂; *Glu*₃/*Ser*₂; *Glu*₃/*SUQ5*] extended homologies could be detected over the entire sequences that were available; for the pairs *Val*₁/*SUP RL1* (Olson *et al.*, 1981); *Met*₃/*Glu*₃; and *Arg*₂/*Val*₁ homologous nucleotide blocks were seen only within some 40–90 nucleotides of the

pY44 (tDNA^{Ser2})

AGAAAATGTTAGAAATTTTAAACATCGATAAAATGATATTGTGTAGAAACACGGAATCCCTTTTGTAGATTCTATATCTTTGGGTATGACTCTAGTATTATCTGTATATCTAATATTATA CCTTTTACAATCTAAAATTGTAGCTATTTTACTATAACACATCTTTGTGGCTAAGGGAAAACATCTTAAAGATATAGAAACCCATACTGAAGATCATATAGACATATAGATTATAATAT	120
GTCTCTAAACACGAGTGGAAATCCCAACAAATTATGCACAAATTCAGCAAAATCCCAAGTTTCATACATGTGTTGATATTTATAGATAATCTAATGACGTTATTTATTTCTGGTAAAGTTAT CAGAGATTGTGTCACTAGGGTGTGTAATACGTGTTTAAAGTAGCTTAAAGGTCCTAAGAGTATGACAACTATAAATCTATTAAGTATTATGCTGGCAAATAAAGACCATTCGAATA	240
GATATCATGTAAAAACAGGCAACTTGGCCGAGTGGTAAAGGCGAAGAGTTAGAAATCTTTGGGCTTTGCCCGCGCAGGTCGAGCTCTGCAGTTGTCGTTATTTTTTGGACAGTAATA CTATAGTACATTTTGTCCGTGAACCGGCTACCAATTCCGCTTTCTAATCTTTAGAAAACCCGAAACGGGCGCTCCAAGCTCAGGACGTCAACAGCAATAAAAAAACCTGTCAATTAT	360
AGAGTACGTAAGTCAATGGAATATGTGGTAAAGCATCACGTTTATGTTTTTAAAGAGTTGGCATAGAAGTGGCCCTCTTCGTGAACGACTTATACCCGGTAAAAAGTATGCTATAT TCTCATGCAATTCAGTTACCTTATACACCAATTTTCGTAGTGCAAATACAAAATAATTTCTAACCGTATCTTGAACGGGAAGAAGCATTGCTGAATAGTGGGCAATTTTCATACGATATA	480
GAGTGTATCGGTCGAGTGATTTCTTCTAGCTCAATTTAAAGCATGAAGATGAGGATCTCGCTTCATTTTCAAGGATGTTTGTATGGGATATGATATGAATGTACCCGGCACTT CTCAACATACCCGCTCACATAAAGAAAGGTACGAGTAATTCGATTCAATTCATGTACGACGCAAGTAAGAAGTTTCCATCAAAACATCGGCATATGATATACAGTACGAGTGGGCGTGA	600
TTGTAAAGAAAATTTTGAGTTTATGCTTTTCCGAAAAATTTTACCAGTACGCAATTTGAAAAGTGCATTATTAATTTTGTAGCGTAAAAACCCAGGATTAACAGGTAAGTGTAGGTTG AACATTTCTTTTTTAAACCTCAAAATACAGAAAGGCTTTTAAAAATGGCTACTCGTAACCTTTTCAACGTAATAATTTAAACCATCGCATTTTGGTGGCTAATTGTGTCATCACATCCAAC	720
TAAATTAAGATCATATTCTTTCTGTTTACATCCAGTTTTACGTGTTGGCCGCTATGGTAAATTTCTAGTATCAGTTACACATATTTCAATTGAAAGTATGGCTAAACAAAGACAACT ATTTAAATCTAGTATAGAAAGGACAGATTTTGTAGGTAAGGATGCAACACCCGGCATAACCATATAAAGATCATAGTCAATGTGTATAAAGTAACTTTTCATACCGATTTGTTCTGTTTGA	840

pY 41 (tDNA^{Arg2})

CCTCGAGGAAAGCCTCTGGCATATTATAGGGGTATTATCCTTTAGAAAAATGGAATGAAAGAACACCACAAAATTGTCTATGTTGCACAATTGCGTATACCATTGCATATAATTA	120
GGAGCTCCCTTCGGAAGCCGTATAATATATCCCATATAAGGAAATCTTTTTTACCTTACTTTCTTAGTGGGTGTTTAAACAGTACAAACGTGTAAACGCATATGTAACGATATTAAT	
TCGCGAAAAATCATCAATATGTATGGAAGAAATGATCATCTCCACCCCTTTCCACCCGGCAATAATAATTTGAATATAATAATAATGATGAGTGGGCAATTATGGGAAATATAAAGATGAAAT	240
ACAGCTTTTAGTAGTTATACACCAATCTAGTAGTAAGTGGGAAAAAGTGGCGCTTATTATAAACTTATATTATTAAGTACTACCCGTAATAACCCCTTTTTCATTCTTTAA	
TGCTTTTAGTACGTGTATCGATAAAATCTTTAATTTTCTGACAATCTTTCTTCGTGGCCCAATGGTACGGCGTCTGGTACGAACCGAAGATTCAGGTTCAAGTCTGGCGGGGA	360
ACGAAAATCATGCATAGCTATTATAGAAATAAAAGAGTCTAGACAAAGGAGACCGGGTTACCAAGTCCGCGAGACCGATGCTTGGTCTCTTAAGTCCAAGTTCAGGACCGCCCT	
AGATTTTTTTTTTAACATTGAGTAGAAATACAAAGTCTTTATGCTATTCTAAAGGGCTATAGGCGGCAATAATTTGCTTTTAACTTATTGTCATGACATCTTAATCACAACTTA	480
TCTAAAAAAAATTTGTAACCTCATCTTTAATGTTTCCAGAAATACGATAAGATTCCCGATATCCGCGGATTATAAACGAAAATGGAATAACAGTACTGTAAGATTAGTGTGTGAAT	
GCCTCCCTTTTGTATTTTCCAACCTTTTCAAGTCTTAAATTTTCCCAACCGAAAAAATGGAAGATC	
CGGAGAGAAAAACATAAAAGGTTTGAAAAAGTTCAGATTTTAAAGGGTGGCTTTTAACTTCTAG	

pY 7 (tDNA^{Val1})

ATTGAGAGCTACATTAAAAGATTCTATTATTAGAGGAAAGTTCTGTACGGTTGACCTGAAAAAGAAATGCTCTTCTATAAAAGATTACATAAAATGTTTAGTCGATAAAACCATAAA 120
TAACCTCGAATGTAAATTTCTCAAGATAAATATCTCTTCAAAGGACATCGGCACTGAAGATTTTCTTATCAGGAGAAGATTTTCTAATGTATTTACACAAAGCAGCTATTTGGGTTAT

TATGTTTTCAGACATCACAAAATAACAGTTGTTGGTGTCTAGTCGGTATATGGCATCTGCTTAACACACAGAACCTCCCAAGTTGCATCTGGCGGAAATCACTTTTATCTCAAGCGTTTAC 240
ATATCAAAGTCGTGTATGTTTATTGTCTCAAAGCACCAGATAGCCAATACCGTAGACGAATGTGCGCTCTGCAGGGGCTAAGCTAGGACCCGCTTAGTAAAAATAGAGTCGGCAATG

AGAAAAGTTTTTAAACCCCCTCTGGCGCCCA
TCTTTCAAAAATTTGCGGGCAGACCCTGGGT

Fig. 2. DNA sequences from subfragments of pY44, pY41, and pY7. The sequences are oriented in such a way that transcription of the genes (boxed) is from left to right.

immediate 5'-flanking regions, although much longer sequences were compared (see Figure 3).

We have subdivided the examples into three "classes" (cf. Figures 3A, B, and C): Figure 3A shows homologies between 5'-flanking regions of different serine isoacceptor genes. In these cases, the tRNA coding sequences are either identical [e.g., *SUP17* (Broach *et al.*, 1981)/*SUQ5* (Olson *et al.*, 1981)], or they differ by 13% at the most [e.g., *SUQ5/Ser₂* (Olson *et al.*, 1981)]. Figures 3B and 3C show pairwise homologies in the flanking regions adjacent to genes specifying tRNAs for different amino acids. In these cases, the homologies within the structural parts of the genes are in the range 42–59%. These values were calculated either by comparing the structural parts of the sequences applying the same principle as described above, or by comparing the nucleotides in equivalent positions of these tRNAs (Gauss and Sprinzl, 1981); both of these approaches gave similar values. Figure 3B shows the largest homology we have observed in the 5'-flanking regions of two different tRNA genes. The three consecutive portions (termed a, b, and c in Figure 3B) of the ~250-bp long segment preceding the genes for tRNA^{Glu} (pY20) and tRNA^{Ser} match to a somewhat different extent:

regions (a) and (c) show ~ 53% homology, but region (b) has 76% homology.

Discussion

Organization of the yeast tRNA genes and comparative analysis of the adjacent sequences

Comparisons of the DNA sequences previously obtained for multiple copies of yeast tRNA genes (Goodman *et al.*, 1977; Valenzuela *et al.*, 1978; Page and Hall, 1981; Olson *et al.*, 1981), have not revealed serial homologies in the flanking regions. In several cases, however, sequencing has not been carried out very far into the segments adjacent to the tRNA coding regions. But even in the case of two tRNA^{Glu} genes for which we have determined rather long sequences (Feldmann *et al.*, 1981; Eigel *et al.*, 1981) we could not detect significant homologies. Therefore, we were rather surprised to find (Eigel *et al.*, 1981) profound similarities in the flanking regions of two yeast genes specifying tRNAs for different amino acids, namely a minor tRNA^{Ser} species (Olson *et al.*, 1981) and a glutamate tRNA (Feldmann *et al.*, 1981). This prompted us to compare other published sequences and our own data. The degree of similarity seen in the examples in

Fig. 3. Homologous flanking regions from several yeast tRNA genes. **A:** Alignment of sequences adjacent to serine isoacceptor tRNA genes. **B:** Alignment of the 5'-flanking sequences of a glutamate and a serine tRNA gene. **a, b, and c** denote consecutive segments of these regions; the numbering refers to that used in the respective sequences (see below). **C:** Alignment of sequences adjacent to genes specifying tRNAs for different amino acids. In the comparisons of the 5'-flanking regions the beginning of the respective structural parts of the tRNA genes is indicated by a bar on the right side of the shown sequence. In the comparison of the 3'-flanking region the end of the structural part is indicated by a bar on the left side. Matched positions are indicated by the dots. The tDNA sequences are taken from the following references: Glu₃ (pY5) (Eigel *et al.*, 1981); Glu₃ (pY20) (Feldmann *et al.*, 1981); Ser₂ (pY44), Arg₂, and Val₁ (this paper); *SUQ5* and *SUP RL 1* (Olson *et al.*, 1981); *SUP16*, *SUP17*, and *SUP19* (Broach *et al.*, 1981); Tyr (pYTG) (Goodman *et al.*, 1977); Met₃ (Olah and Feldmann, 1980). The percentage homology given for each pair was calculated according to van Ooyen *et al.* (1979); for Figure 3B, see text.

Figure 3 differs to some extent. Some of the flanking regions could only be matched to give significant blocks of homology by introducing relatively large gaps in one or other of the sequences. Only in a few cases, however, could flanking regions of multiple gene copies for a particular tRNA be matched by this procedure: one example is the pair *SUP16/SUP17* (Broach *et al.*, 1981) where slight homology is seen. Also the 5'-flanking regions of *SUQ5* and *SUP17* show only limited similarity (i.e., in the immediate vicinity of the coding portion). This is striking because the two latter sequences appear to be isolated from the same locus in two different yeast strains.

It is, therefore, the more surprising that in three cases the 5'-flanking regions of different tRNA genes can be matched to a rather high degree and over long distances. The most compelling example is the one presented in Figure 3B. It may be added that the homologies observed here seem not to be restricted to these two sequences: the highly conserved region (b) is also contained in a repetitive yeast DNA segment which we have cloned and sequenced recently (Eigel and Feldmann, unpublished results). This finding substantiates our previous notion (Feldmann *et al.*, 1981; Eigel *et al.*, 1981) that the 5'-flanking regions of several tRNA genes do contain repetitive elements.

Evolutionary and functional aspects

In *Drosophila*, the clustered genes for particular tRNAs frequently show significant homologies in the sequences adjacent to the coding regions (e.g., de Franco *et al.*, 1980; Hosbach *et al.*, 1980; Robinson and Davidson, 1981). This has been taken as a strong indication that the multiple gene copies have been derived, together with their flanking regions, from common ancestral sequences. The observed patterns may have arisen from gene duplication and unequal crossing-over (e.g., Hosbach *et al.*, 1980).

By contrast, the results so far obtained in comparative studies of yeast tRNA gene families suggest that a more complex reorganization of sequences has occurred in the process of dispersion of these genes. The novel patterns in tRNA gene arrangement described here indicate, however, that in some cases sequence elements in the vicinity of tRNA genes have been conserved to a high degree. It will be of interest to find out how these patterns originated and what the functional significance of such an arrangement might be. It is possible, for example, that in certain cases the tRNA genes, together with their flanking regions, have been conserved. On the other hand, one could argue that different tRNA genes have been combined with similar DNA segments during evolution.

If such reorganizations have indeed occurred, the possibility exists that sequence elements in the flanking regions had functional significance in this process. A role for these sequences may be assumed in the control of gene expression (see e.g., Garber and Gage, 1979; Sprague *et al.*, 1980; de Franco *et al.*, 1980). In order to study this problem, we are carrying out *in vitro* transcription experiments with our yeast tRNA genes using HeLa cell extracts containing RNA polymerase III activity (Manley *et al.*, 1980).

Materials and methods

[³²P]Phosphate (carrier-free) was purchased from New England Nuclear Corp., Na [²⁵I] from the Radiochemical Centre, Amersham. Yeast tRNA, alkaline phosphatase, and polynucleotide kinase were obtained from Boehringer Mannheim, GmbH. SeaKem and SeaPlaque agarose were products of Marine Colloids Inc., Rockland, ME. Acrylamide and N,N'-bismethylenacrylamide were from Serva, Heidelberg. All chemicals used were of analytical grade.

The following restriction enzymes were used in this work: *Eco*RI, *Bam*HI (gifts of T. Igo-Kemenes); *Hae*III (gift of U. Hänggi); *Sau*96, *Sau*3A, *Hinf*I, *Taq*YI, *Alu*I, and *Pst*I (gifts of R.E. Streeck); *Hind*III and *Hpa*II were prepared as described earlier (Feldmann *et al.*, 1981).

pY44, pY41, and pY17 are recombinant plasmids from a collection we previously described (Olah and Feldmann, 1980); they contain *Hind*III fragments from *S. cerevisiae* C836 DNA cloned into pBR322.

Preparation of DNA and labeled tRNA

Plasmid DNA from the clones mentioned above was prepared by scaling up the procedure of Birnboim and Doly (1979). Cells obtained from 10 l cultures were used as starting material. The operations were carried out according to the "Richtlinien zum Schutze vor Gefahren durch *in vitro* neukombinierte DNA". [¹²⁵I]tRNA was prepared as described earlier (Olah and Feldmann 1980).

Restriction mapping and hybridization techniques

Restriction mapping of the cloned DNA was achieved in the usual manner by digestion with various restriction endonucleases and combinations of these enzymes; or by secondary digestions of isolated fragments with appropriate restriction enzymes, both followed by electrophoresis in agarose slab gels. Length standards employed were the ones previously described (Grosskopf and Feldmann, 1981) or pBR322 DNA digested with *Hpa*II or *Taq*YI. The technique of Southern (1975) was used for the transfer of DNA fragments to nitrocellulose filters and subsequent hybridization with labeled tRNA.

DNA sequencing

DNA fragments for sequencing were prepared as described earlier (Olah and Feldmann, 1980). Labelling of the fragments with [³²P]ATP and DNA sequencing followed the protocol of Maxam and Gilbert (1980). The label and cut method was applied, as well as the strand separation of labeled fragments. As far as possible, the sequence of the one strand was determined starting from different restriction sites. 8% and 20% polyacrylamide gels of 0.2 or 0.5 mm thickness were employed. Partial cleavage at purine residues was achieved by using the reaction described by Gray *et al.* (1978).

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