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Structural comparison of two yeast $tRNA^{Glu}_3$ genes

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ABSTRACT

DNA sequences in a 1.7 kb Pst fragment from yeast have been determined. This fragment is part of a yeast 7.4 kb Hind III segment cloned into pBR322 (pY 5). The fragment carries a single gene for a glutamate tRNA. The coding portion of this gene is identical in sequence to that of the $tRNA^{Glu}_3$ gene from pY 20 [1]. The flanking regions differ in their sequences, but possible secondary structures within the 5'-flanking regions bear similar features. Sequence homologies between pY 5 and pY 20 were detected far outside the tRNA genes. More surprisingly, extended sequence homologies were seen between the flanking regions of the pY 20 $tRNA^{Glu}_3$ gene and a $tRNA^{Ser}$ gene [2,3]. We have also checked the known tRNA genes for structural similarities. Hybridization studies indicate that portions of the Pst fragment are repeated within the yeast genome.

INTRODUCTION

In the preceding paper [1] we described the analysis of a yeast DNA fragment containing a $tRNA^{Glu}_3$ gene. Here we wish to report on the analysis of a 1.7 kb Pst fragment from a different plasmid (pY 5) which also carries a $tRNA^{Glu}_3$ gene. pY 5 was isolated from the same collection of recombinant plasmids as pY 20 [4].

The determination of the DNA sequences has allowed us to compare structural features of the regions in which the two glutamate tRNA genes are located. We have extended this comparison to the flanking regions of other tRNA genes.

Furthermore, we have obtained indications from hybridization experiments that sequences homologous to segments from the Pst fragment occur many times within the yeast genome.

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MATERIALS AND METHODS

Chemicals and enzymes were essentially the same as described in ref. 4. In addition, restriction endonucleases Sal I and Pst I (gifts from Dr. R.E. Streeck) were used. S1 endonuclease (800 units/ml) was a gift of Dr. F. Fittler.

Plasmid DNA. Clones pY 5 and pY 20 were from a collection of tRNA gene carrying yeast-pBR322 plasmids constructed as reported in ref. 4. Cells were grown in LB medium in 15 l fermenters [5]. Plasmid DNA was prepared by scaling up the procedure of Birnboim and Doly [6].

Labeling procedures and hybridizations. Preparation of yeast [^{125}I]tRNAs, hybridizations of these probes to DNA fragments on nitrocellulose filters [7], preparation of DNA fragments, gel electrophoreses, restriction mapping, DNA-DNA hybridization, and DNA sequencing were essentially the same as described [1,4].

RESULTS AND DISCUSSION

1. Characterization of pY 5 by restriction enzyme analysis and hybridization to tRNAs.

Plasmid DNA from clone pY 5 (total length of ca. 11.7 kb) was cut with Hind III, Eco RI, Bam H1, Sal I, and Pst I and combinations of these enzymes and analyzed on agarose gels in order to obtain the corresponding restriction map (Fig. 1A). The DNA fragments were also transferred to filters [7] and hybridized to [^{125}I]tRNA. Hybridization was limited to the 1.7 kb Pst I fragment of the inserted 7.4 kb yeast Hind III segment. A fine restriction map of this Pst fragment was determined for the following enzymes: Hpa II, Hha I, Hae III, Hinf I, Alu I, Sau 3A, and Taq YI (Fig. 1B). Hybridizations to [^{125}I] tRNA were then performed with this series of DNA fragments in order to better define the location of the tRNA gene.

2. DNA sequences from pY 5.

Hybridization with [^{125}I]tRNA was found to be limited to a region near one end of the 1.7 kb fragment. By sequencing this portion we found a $tRNA^{Glu}_{3}$ gene at the position indicated in Figs. 1 and 2. In order to allow comparisons of the surroundings of the $tDNA^{Glu}_{3}$ of pY 5 and pY 20 [1], we also analyzed extended

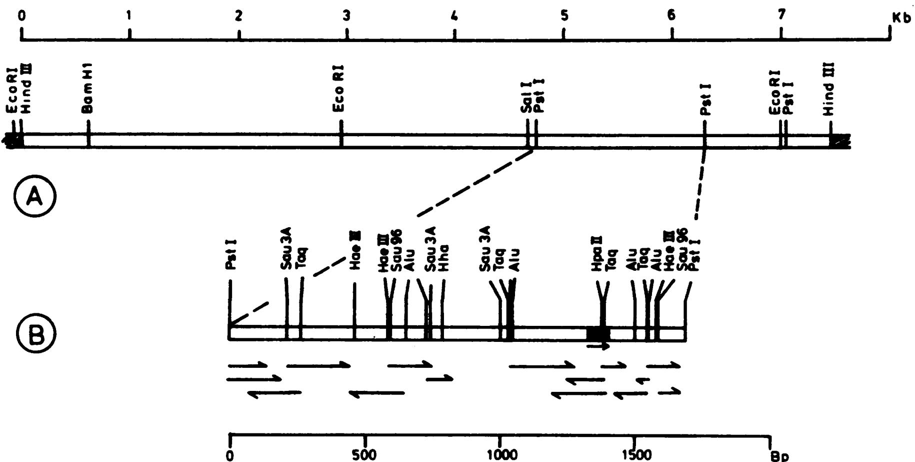

Fig. 1: Restriction endonuclease cleavage map of pY 5.
A: The 7.4 kb Hind III insert in pBR322 (hatched ends)
B: Fine restriction mapping of the 1.7 kb Pst fragment and DNA sequencing strategy.
The arrows indicate the direction and the extent of sequencing. The location of the tRNA gene is shown in black.

portions of this fragment. DNA sequencing was performed by the Maxam-Gilbert technique [8]; the strategy is indicated in Fig. 1B. In the portions sequenced, the upper strand has a base composition of 29.11% A, 20.99% G, 16.66% C, and 33.27% T. The (G+C) content of the double-strand is 37.62% which is close to the value for total yeast DNA (~ 38%) [9]. The $tRNA^{Glu}_{3}$ gene is located between position 1342 and 1413 (Fig. 2). As in the case of pY 20 [1], we have analyzed the DNA sequences of pY 5 for several structural features by computation; we have also compared the two sequences.

No further tRNA gene was detected in pY 5 by inspection of the DNA sequences so far determined. There are a number of internal sequences homologies ($\geq$10) in the pY 5 Pst fragment which are indicated by arrows (no. 3 through 6) in Fig. 2. In addition, we found inverted repeats in several portions of the sequence which could be used to form possible secondary structures. The stem and loop structures between positions 80 and 180 are immediately followed by a "clover-leaf" structure (not shown). An-

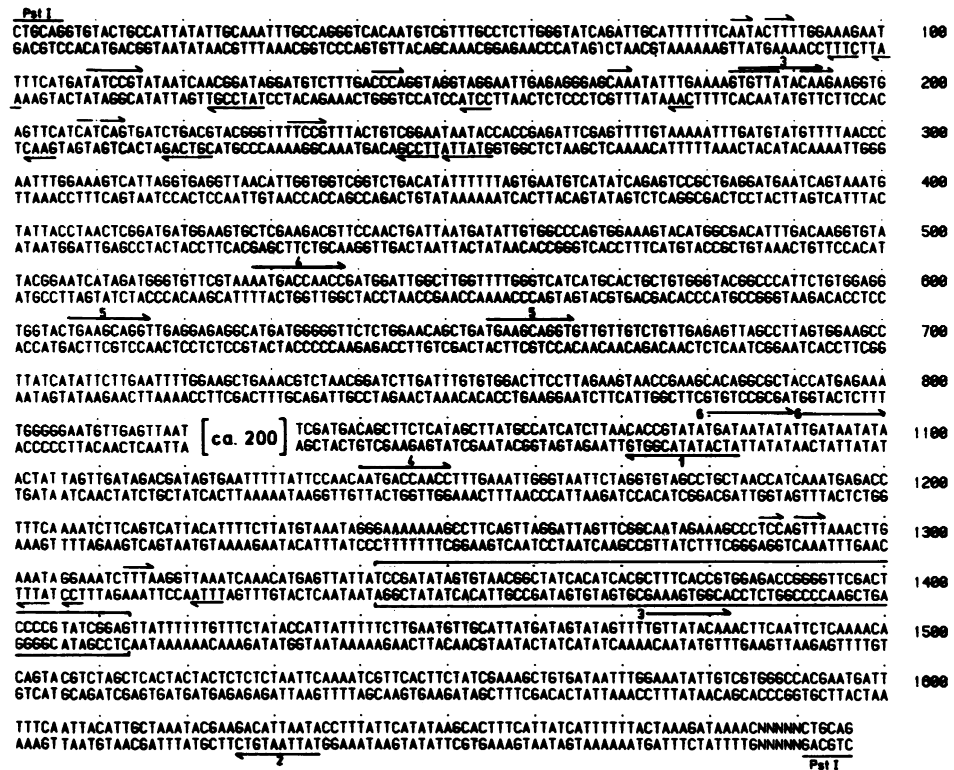

Fig. 2: DNA sequences from the 1.7 kb Pst fragment of pY 5. The sequence is oriented in a way such that transcription of the tRNA gene (boxed) is from left to right. Numbered arrows (3-6) above the sequence indicate internal homologies. Arrows no. 1 and 2 pertain to sequences shown in Fig. 5A. Inverted repeats are designated by arrows above and below the sequence (cf. Fig. 3).

other stem and loop structure precedes the $tRNA^{Glu}_{3}$ gene in the 5'-flanking region.

3. Comparison of the $tRNA^{Glu}_{3}$ genes from pY 5 and pY 20.

The sequence of the Bam x Hind III fragment from pY 20 carrying a $tRNA^{Glu}_{3}$ gene is presented in the preceding paper [1]. The two genes from pY 20 and pY 5 (Fig. 2) are identical in sequence within the coding portion for $tRNA^{Glu}_{3}$ and correspond to the tRNA sequence reported by Kobayashi et al. [10]. No intervening sequence is present. The Wobble position in the anticodon

of tRNA$^{Glu}_{3}$ is 5-thiouridine-5-acetic acid methyl ester, the presence of which is thought to restrict codon recognition of the glutamate tRNA to GAA [10]. According to the DNA sequence, this minor nucleotide would be derived from a uridine residue by modification.

The 5'- and 3'-flanking regions are considerably different in the two sequences. The only homology is a putative transcription termination signal - a block of six T's - beginning three bases after the 3'-terminus of the structural gene (the -CCA end of the tRNA is not encoded). In the sequence from pY 5, a block of T's is repeated 15 bases downstream from this signal.

The absence of serial homologies in the flanking regions of the glutamate tRNA genes parallels the situation described earlier for the phenylalanine [11] or tyrosine [12] tRNA genes in yeast. Also in Drosophila [13] or in Xenopus laevis [14,15] redundant genes for the same tRNA species are surrounded by differing sequences. The only exception so far reported, was found for five clustered glutamate tRNA genes in Drosophila [16] which show extended sequence homologies also in the flanking regions. This is believed to be the result of unequal crossing over during the evolution of these genes.

Despite the absence of sequence homologies in the 5'-flanking regions of the two tRNA$^{Glu}_{3}$ genes, secondary structures can be formed from the sequences between positions -10 and -60 that bear some similarity. This is seen in the comparison of Figs. 3A and

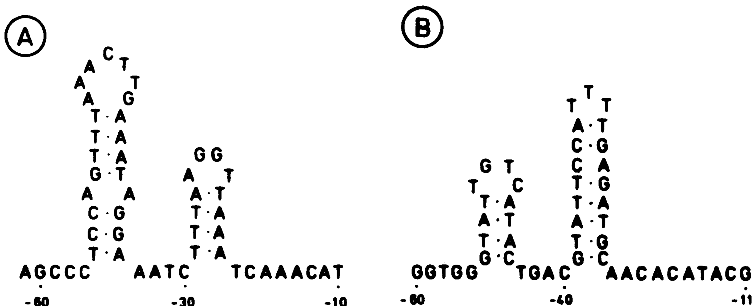

Fig. 3: Possible secondary structure in the 5'-leader sequences of the tRNA$^{Glu}_{3}$ genes.
A: pY 5. B: pY 20 [1].

3B. Even one of the formerly described secondary structures preceding the $tRNA^{Met}_3$ gene [4] has some resemblance to the ones described here.

As found for other tRNA genes in yeast [4,11,12] the $tRNA^{Glu}_3$ genes are nested within AT-rich regions. This was previously observed in the case of pY 20 [1], and is also true for the sequence from pY 5 shown here.

A more specific observation we may mention, is the occurrence of two homologous DNA sequences of opposite polarity that are located about 280 base pairs preceding and about 200 base pairs following the $tRNA^{Glu}_3$ genes. These sequences are compared in Fig. 4A. Although these homologies do not appear to be incidental, we have no compelling explanation for this finding at

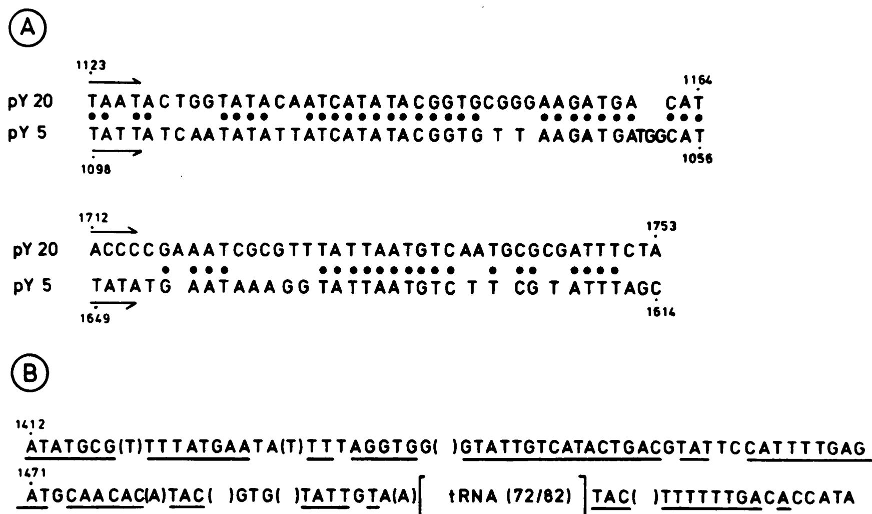

Fig. 4: Sequence homologies.
A: Comparison of two regions flanking the $tRNA^{Glu}_3$ genes from pY 20 and pY 5 (cf. text). The arrows indicate the 5'→3'-orientation. Identical nucleotides are indicated by dots.
B: Comparison of the 5'-leader and 3'-trailer sequences in pY 20 with those from a $tRNA^{Ser}_{UCA}$ gene [2,3]. Underlined are identical nucleotides in the upper strand of the $tRNA^{Glu}_3$ gene, (N) = nucleotides occurring in pY 20 only, ( ) = nucleotides missing in pY 20. The numbering refers to the sequences from pY 20 [1]. 72 is the length of the $tRNA^{Glu}_3$ gene, 82 is the length of the $tRNA^{Ser}$ gene.

present.

4. Comparison of the $tDNA^{Glu}_{3}$ with other tRNA gene sequences.

So far, only five extended tRNA gene sequences from yeast are available. These include a $tRNA^{Met}_{3}$ gene, the two $tRNA^{Glu}_{3}$ genes discussed here, and the gene sequences for two pairs of minor serine tRNA species [2,3]. One of the latter corresponds to SUP-RLI, the gene product of which is the only $tRNA^{Ser}_{UCG}$: the precursor to this tRNA contains an intervening sequence [2]. The second of the latter corresponds to SUQ 5, the product of which is a $tRNA^{Ser}_{UCA}$, but this gene has no intervening sequence [2]. The sequences of these tDNAs have been determined [3]. By comparing all of these sequences, we found that there is a rather extended homology in the flanking regions of $tRNA^{Glu}_{3}$ (pY 20) and $tRNA^{Ser}_{UCA}$ [3] even though the structural genes for these two tRNAs are completely different [1,3]. There are two stretches of nucleotides within the 5'-flanking region which are nearly identical (14 out of 16 in one portion, and 36 out of 40 in a second portion), and there are 12 identical base pairs within the 3'-flanking region (Fig. 4B). It is rather surprising that regions adjacent to two non-homologous tRNA genes seem to be highly conserved in sequence, and it is tempting to speculate about their functional significance. The situation here is similar to that of the mating type sequences, in which regions of homology precede and follow non-homologous sequences [17,18]. In mating type interconversion, the homologous sequences seem to be involved in an "illegitime" transposition effect [17,18]; similar events have recently been examined by Scherer and Davis [19]. It remains questionable, however, whether or not the sequence homologies found in our case, comparably much shorter, could serve a similar mechanism.

In comparing the 5'-flanking regions of yeast tRNA genes without introns, we observed a constant trinucleotide TGA, at position -43/-41, which is part of a short "consensus sequence" between positions -44 through -37. We then examined other available tDNAs and found comparable sequences in four out of eight cases in the Xenopus tRNA gene cluster [14,15], in four out of six in the 9 kb tRNA gene cluster from Drosophila [13], and in four out of five Drosophila glutamate tRNA genes [16]. Most of

the sequences were centered around position -40 in the 5'-flanking regions, a few occurred further upstream. It may well be, that this occurrence is only incidental; but on the other hand, sequences outside the coding regions of the tRNA genes may indeed have control functions not yet understood (see also [20]).

5. Genomically redundant sequences in pY 5.

Hybridization experiments similar to those reported in ref. 1 were carried out with subfragments of the 1.7 kb Pst fragment of pY 5. Both, Eco RI and Hind III digested yeast DNA separated on agarose gels displayed multiple hybridizing bands when probed with subfragments that do not contain the $tDNA_3^{Glu}$. The radioautographs are shown in Fig. 5. The band pattern obtained for DNA sequences from the "left" end of the Pst fragment is clearly different from that observed for DNA sequences in the middle portion of this fragment. In both cases, multiple bands (up to 12) are visible here. In contrast, maximally two bands were observed when the probe contained outlying sequences of pY 20 [1]. These hybridization experiments with the subfragments from pY 5 and pY 20 [1] were performed using the same Eco RI and Hind III digests resolved in parallel lanes in the same gel. The results obtained here indicate that sequences from pY 5 are frequently reiterated in the yeast genome. Further experiments will be

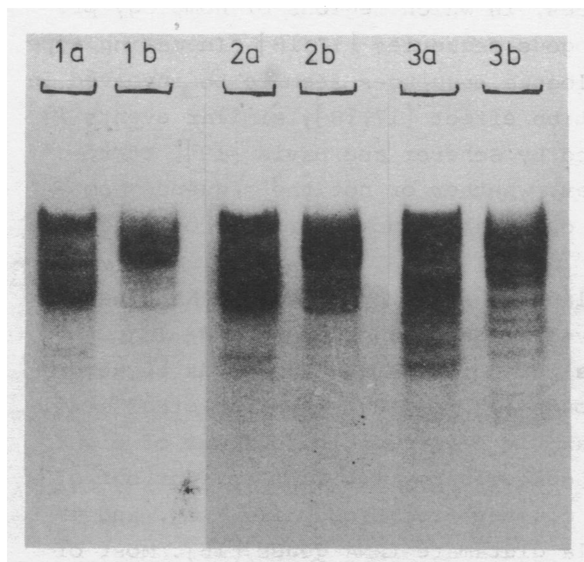

Fig. 5:
Hybridization of subfragments from pY 5 to yeast DNA restricted with Eco RI (a) or Hind III (b), respectively, and resolved on 0.8% agarose gels.
Probes used were the following Sau 3A fragments (cf. Figs. 1 and 2)
1: Positions 1-215
2: Positions 215-741
3: Positions 741-ca. 1000

necessary to obtain more detailed information concerning their arrangement and possible function.

CONCLUDING REMARKS

We have extensively analyzed and compared two $tRNA_3^{Glu}$ genes contained in two different cloned yeast DNA fragments. Hybridization studies together with DNA sequence analyses have shown that these are the only tRNA genes contained in these relatively large DNA fragments. Other large cloned fragments from our collection also contain only single tRNA genes [1,4,21]. These and earlier findings [11,12] lead to the impression that tRNA genes in the yeast genome are more dispersed than in higher eukaryotic systems.

Comparisons of the DNA sequences of the fragments in which these two $tRNA_3^{Glu}$ genes are located, have revealed that they do not share extended homologies; however, some structural features may be conserved. On the other hand, we saw extended sequence homologies in the flanking regions of two genes for completely different tRNAs (the $tRNA_3^{Glu}$ from pY 20 and a $tRNA_{UCA}^{Ser}$).

Despite the lack of homology between the immediate flanking regions of the two $tRNA_3^{Glu}$ genes, other portions from the DNA fragments seem to be conserved and repeated within the yeast genome. In the case of pY 20 [1], hybridization of such segments occurred to two apparently different loci. Sequences homologous to segments from the pY 5 Pst fragment seem to be reiterated many times within the yeast genome. These observations may be interesting in the context of recent reports of dispersed repeated elements in yeast [e.g. 19].

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