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Ty1 and delta elements occur adjacent to several tRNA genes in yeast

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A comparative analysis of a number of yeast DNA-pBR322 recombinant plasmids carrying repetitive sequence elements has revealed that Ty1 or delta elements occur in the vicinity of several tRNA genes. Four examples have been characterized in detail: three glutamate tRNA genes and a serine tRNA gene. The $tRNA_3^{Glu}$ genes occupy different chromosomal locations; two of these genes are found adjacent to Ty1 elements, and the third is found adjacent to an independent delta element. A delta unit is also found adjacent to a $tRNA_2^{Ser}$ gene. Next to one of the $tRNA_3^{Glu}$ genes, the delta element is joined to a truncated sigma element. Junctions between different delta units were characterized by the sequence analysis of two DNA segments that carry no tRNA genes.
Key words: repetitive elements/tRNA genes/*Saccharomyces cerevisiae*

Introduction

Dispersed repetitive elements have been characterized in a variety of eukaryotic genomes (for reviews, see Calos and Miller, 1980; Singer, 1982). Several of these repeated DNA elements have been cloned and their structure analyzed by hybridization techniques and by DNA sequencing. It is now well established that some repetitive elements like the *copia* element of *Drosophila* (Dunsmuir *et al.*, 1980) and the transposable yeast element Ty1 (Cameron *et al.*, 1979) are capable of transposition into different chromosomal sites.

The yeast transposable element Ty1 is present in ~35 copies per genome; it is composed of the internal epsilon element (~5.2 kbp long) and the terminal direct repeats of ~340 bp (delta elements) (Cameron *et al.*, 1979). Delta elements can also occur as independent units and are found in ~100 copies per genome (Cameron *et al.*, 1979). Farabaugh and Fink (1980) have shown that the *his4-912* mutation results from insertion of Ty1 into the non-coding region of the *his4* gene creating a 5-bp duplication; excision of the Ty1 element leaves an independent delta element. Gafner and Philippsen (1980) characterized two different loci in which the insertion of Ty1 elements also generated 5-bp duplications of target DNA.

By hybridization experiments, Cameron *et al.* (1979) have shown that a fragment carrying a tyrosine tRNA gene contains a delta unit. Tschumper and Carbon (1982) determined the sequence of a DNA segment containing a glutamine tRNA gene, a yeast chromosomal replicator region, and two delta units. Hybridization studies and DNA sequencing (e.g., Cameron *et al.*, 1979; Farabaugh and Fink, 1980; Gafner and Philippsen, 1980; Tschumper and Carbon, 1982) reveal that the various delta elements share only partial homology.

Recently, a new type of repetitive element (sigma) found adjacent to several tRNA genes in yeast has been described (Del Rey *et al.*, 1982).

We have investigated the molecular organization of several yeast tRNA genes to provide information on the structure of chromosomal regions in which dispersed tRNA genes are located. This information should be helpful in understanding, for example, the control of expression of the multiple gene copies, or the process of their dispersion over the genome. In the course of these studies, we observed that the flanking regions of several of these genes share great homologies and that these regions contain repetitive sequence elements (Eigel *et al.*, 1981; Baker *et al.*, 1982). An extension of the analyses including a number of newly isolated yeast DNA recombinant plasmids has led to the identification of DNA sequences homologous to Ty1 or delta elements in the vicinity of several tRNA genes.

Results

Comparative analysis of several yeast recombinant plasmids

Figure 1 summarizes the results of the analyses of six yeast recombinant plasmids. Hybridization experiments and DNA sequence analyses have revealed that pY5, pY20, and pY106 each carry a $tRNA_3^{Glu}$ gene which in pY5 and pY106 is flanked on its 5′ side by a Ty1 element, and in pY20 by an independent delta element. pY44 carries a $tRNA_2^{Ser}$ gene which is flanked by a delta sequence. pY151 and pY102 have been included in the analyses to obtain sequence information on Ty1 or delta elements in our yeast strain which have no tRNA genes in their vicinity.

Restriction maps and sequence data have recently been reported for pY5 (Eigel *et al.*, 1981), pY20 (Feldmann *et al.*, 1981), and pY44 (Baker *et al.*, 1982). pY106, pY102, and pY151 are from a newly constructed clone bank of recombinant yeast DNA-pBR322 plasmids. Fifteen recombinants (pY101 through pY114; and pY151) were selected from these clones which were identified by cross-hybridization to a DNA segment from pY5 that contains part of a delta sequence (see Materials and methods). These plasmids have yeast DNA inserts ranging between 1.2 and 10 kbp. By hybridizations, seven of these plasmids (pY105 through pY111) were found to contain tRNA genes in addition to the repetitive element(s). Differences in their restriction patterns (not shown) indicate that the cloned fragments have different chromosomal locations. The plasmids pY102, pY106, pY109, and pY151 were analyzed to different extents: restriction maps were determined for all four; those for pY106, pY102, and pY151 are shown in Figure 2. Subfragments from the yeast DNA inserts were used in hybridization experiments to locate the tRNA genes and/or the repetitive element(s). So far, DNA sequences have been determined from pY106, pY102, and pY151. The sequences are summarized in Figure 3.

Organization of the repetitive elements adjacent to tRNA genes

The best characterized example is pY5. We have recently determined the rest of the nucleotide sequence 5′-flanking the

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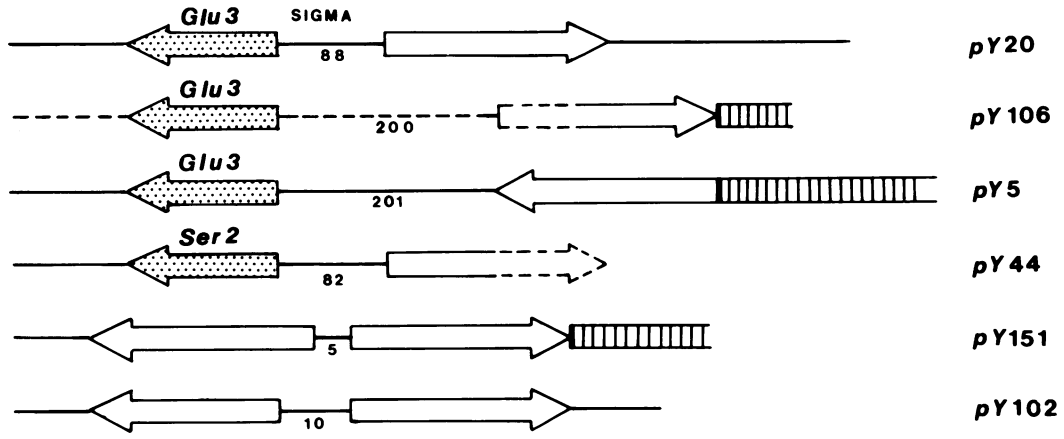

Fig. 1. Schematic representation of the relative position and orientation of several delta and Ty1 elements. The following symbols are used: single line = chromosomal DNA, open arrows = delta elements, hatched areas = epsilon sequences, stippled arrows = tRNA genes. The indicated orientation of the tRNA genes is concomitant with their direction of transcription; the mode of orientation of the delta elements is explained in the legend of Figure 4 and in the text. Dotted lines indicate that nucleotide sequences have not been determined completely. Sigma refers to the truncated sigma sequence (Del Rey *et al.*, 1982) adjacent to the tRNA gene in pY20. The numbers refer to the number of base pairs between a tRNA gene and a delta element or two delta elements, respectively.

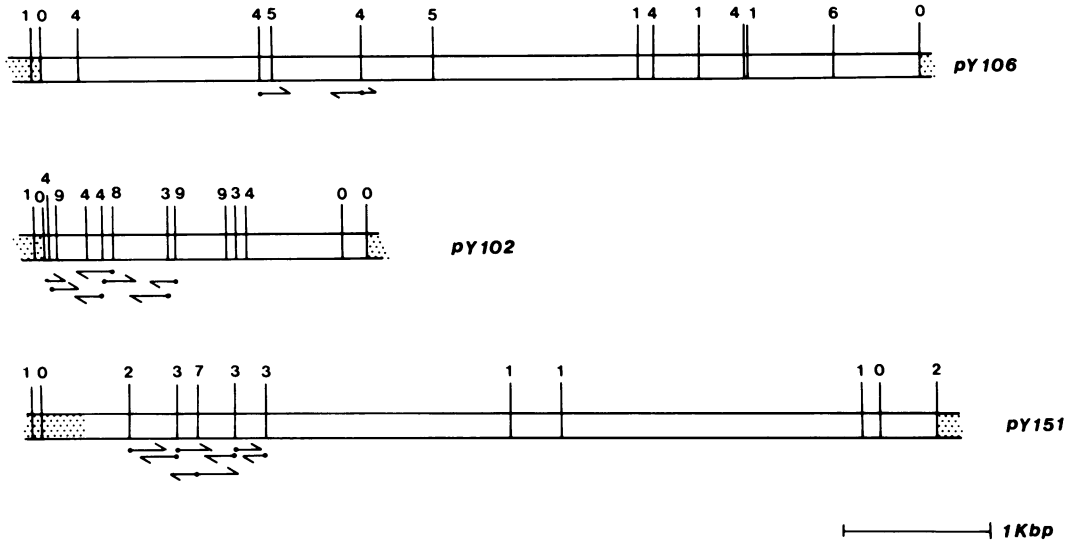

Fig. 2. Restriction endonuclease cleavage maps of pY106, pY102, and pY151. pBR322 DNA is indicated by the stippled regions. The arrows below the lines indicate the direction and the extent of sequencing. The following abbrevations for restriction endonucleases are used: *Hind*III = 0; *Eco*RI = 1; *Bam*HI = 2; *Sau*3A = 3; *Taq*I = 4; *Bgl*II = 5; *Pst*I = 6; *Acc*I = 7; *Rsa*I = 8; *Hae*III = 9.

glutamate tRNA gene. This part of the sequence is documented in Figure 3. We have shown previously (Eigel *et al.*, 1981) that repetitive sequences occur in the vicinity of the $tRNA_3^{Glu}$ gene in pY5; a comparison of the DNA sequence in Figure 3 with the sequences of Ty1 elements and independent delta elements (e.g., Farabaugh and Fink, 1980; Gafner and Philippsen, 1980) revealed that the tRNA gene is preceded by a sequence that shares great homologies with the known Ty1 sequences. One delta element is completely contained in the sequenced portion of pY5 (positions 703 through 1127 in Figure 3), it ends some 200 nucleotides upstream from the beginning of the structural part of the tRNA gene. From hybridization experiments (not shown) we have indications that the delta element flanking the Ty1 element on the other extremity is located near the border of the yeast DNA insert in pY5 (see Eigel *et al.*, 1981).

In pY20, we have identified an independent delta element which is located some 80 nucleotides from the 5′ end of the $tRNA_3^{Glu}$ gene. For convenience, this part of the sequence (Feldmann *et al.*, 1981) has been included in Figure 3. 72 bp of the sequence separating the tRNA gene and the delta are homologous to a truncated sigma element, as reported recently by Del Rey *et al.* (1982). The nucleotide sequence adjacent to the other side of the delta elements has no homology with a Ty1 element. Therefore, in this case, the delta sequence is similar to an independent delta element as defined, for example, by Gafner and Philippsen (1980); however, 5-bp repeats are not present at the ends of this element.

pY106 contains part of a Ty1 element and a $tRNA_3^{Glu}$ gene, the locations of which were determined by hybridizing subfragments of pY106 to delta sequences and epsilon sequences (derived from pY5), or to tRNA: the left end of the yeast DNA insert in pY106 (Figure 2) consists of ~1.4 kbp of the Ty1 element which is followed by the tRNA gene. Sequence data from *Taq*I fragments of this region show that the structural part of the $tRNA_3^{Glu}$ gene in pY106 is identical to the

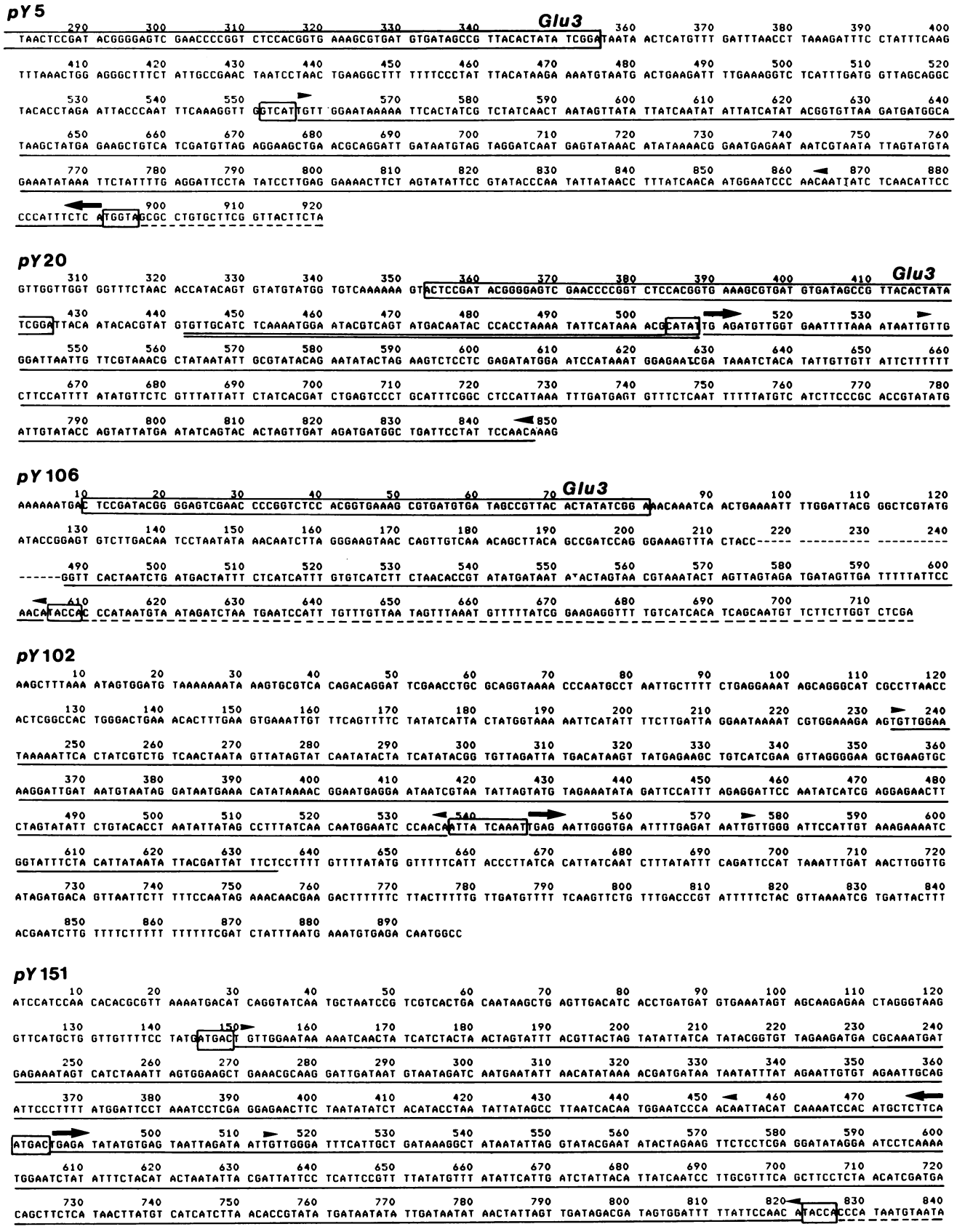

Fig. 3. Nucleotide sequences of yeast DNA fragments from pY5, pY20, pY102, pY106, and pY151. The orientation of the nucleotide sequences (upper strand) is concomitant with that chosen in Figures 1 and 4. Delta sequences are underlined, the long arrows indicate the beginning and the relative orientation of these elements; the short arrows mark the positions of short inverted repeats (cf. Figure 4 and the text). Epsilon sequences of Ty1 elements are underlined with a broken line. The sigma sequence in pY20 is doubly underlined. The 5 bp next to delta or epsilon sequences and the tRNA genes (specified by the symbols) are boxed.

other $tRNA_3^{Glu}$ genes in pY5 and pY20; its 5′-flanking region is different.

The $tRNA_2^{Ser}$ gene of pY44 (Baker *et al.*, 1982) is flanked by a delta element which is located some 80 nucleotides from the 5′ end of the gene (175 bp of this element are contained in Figure 2, Baker *et al.*, 1982). This is similar to the distance between the delta and the gene in pY20. Since, however, the sequence data do not extend far enough, we cannot decide whether the delta in pY44 is an independent delta element or whether it is part of a Ty1 element.

For pY109, which has a yeast DNA insert of ~10 kbp, data from hybridization experiments and preliminary sequence data have shown (Eigel and Feldmann, unpublished results) that this plasmid carries a complete Ty1 element and adjacent to this a tRNA gene; further sequence analyses will be necessary to find out the nature of the tRNA gene.

Analysis of two plasmids carrying parts of Ty1 or delta elements without tRNA genes

The analyses of two yeast DNA fragments have revealed the following (Figure 1): pY151 carries a Ty1 element; one of its flanking deltas is contained in the sequenced portion (positions 488–824 in Figure 3). These two deltas share ~80% homology and are somewhat different in length (336 and 330 bp, respectively). The independent delta is flanked by a direct 5-bp repeat (A-T-G-A-C).

The pY102 carries two delta sequences in an inverted orientation. One of these deltas (position 233–537 in Figure 3) has a deletion of 27 bp. The nucleotide sequence of the other delta (starting at position 547 in Figure 3) begins like a normal delta element, but the homology is abolished after some 90 bp. This situation parallels the finding of a 'divergent' delta near a $tRNA^{Gln}$ gene in yeast (Tschumper and Carbon, 1982). The two deltas in pY102 are interspersed by 10 bp (A-T-T-A-T-C-A-A-A-T). The inverted orientation of the two deltas together with the truncation results in a DNA sequence that could be arranged in a rather extended hairpin structure.

Comparison of the various delta elements

The DNA sequences of the delta elements which we have determined in this study were compared with each other and with the ones previously published (Farabaugh and Fink, 1980; Roeder *et al.*, 1980; Gafner and Philippsen, 1980; Tschumper and Carbon, 1982). This comparison confirms that the various delta sequences are only partially homologous (80–90%). The differences include insertions or deletions as well as transitions and transversions. There are, however, regions within the delta elements that are highly conserved in sequence: if delta sequences are arranged as shown in Figure 4, a block of 27 (or 28) nucleotides with only partial homology is seen to be followed by a constant block (TGTTGG....). The inverted sequence of this latter block (....CCAACA) occurs at the other end of nearly all of the delta elements. It is noteworthy that identical or similar sequences are found at the ends of sigma (TGTTGT....ACAACA) (Del Rey *et al.*, 1982), *copia* (TGTTGG....ACAACA) (Dunsmuir *et al.*, 1980), and SNV (TGTGGG....ACAACA) (Shimotohno *et al.*, 1980).

Discussion

We have presented evidence that sequences homologous to Ty1 elements are found in the vicinity of tRNA genes in yeast. These particular examples have been described here: a $tRNA_3^{Glu}$ gene in pY5, another $tRNA_3^{Glu}$ gene in pY106, and an as yet unidentified tRNA gene in pY109. The best investigated case is pY5 for which extensive DNA sequences are available. Furthermore, (independent) delta elements were found adjacent to the $tRNA_3^{Glu}$ gene in pY20 and the $tRNA_2^{Ser}$ gene in pY44.

With reference to their direction of transcription, the genes adjacent to different delta sequences are oriented differently (Figure 1). The tRNA genes in pY20, pY106, and pY44 have the same orientation with respect to the deltas. An analogous configuration is seen for the delta element adjacent to the $tRNA^{Gln}$ gene present in a replicator region (Tschumper and Carbon, 1982) and also in the *his4-912* mutant (Farabaugh and Fink, 1980) for the delta element (and hence the whole Ty1 element) next to the mutant *his4* gene. The Ty1 element in pY5, however, has the opposite orientation with respect to the tRNA gene. Different configurations as observed here between the tRNA genes and the deltas, have been encountered also between tRNA genes and adjacent sigma elements (Del Rey *et al.*, 1982).

A unique situation is the occurrence of sequences of two different repetitive elements next to the glutamate tRNA gene in pY20: a truncated sigma element (Del Rey *et al.*, 1982) and a delta element (cf. Figure 1). The junction between the two elements can be identified at positions 1411/1412 (Figure 3).

Fig. 4. Comparison of various delta elements. Data concerning Ty1 in *his4-912* are taken from Farabaugh and Fink (1980) and those concerning Ty1-B10 from Gafner and Philippsen (1981). Data concerning pY5 (Eigel *et al.*, 1981), pY20 (Feldmann *et al.*, 1981), and pY44 (Baker *et al.*, 1982) are from the respective references. pY102 and pY151, left and right, respectively, refer to the delta sequences as they are documented in Figures 1 and 3. Depicted are only the extremities of the deltas. The elements (upper strand) are arranged in a way such that the beginning of the element (long arrow) is defined by a block of 27(28) nucleotides which is followed by a block of six constant nucleotides (T-G-T-T-G-G...) (short arrow); the inverted repeat of the constant block (...C-C-A-A-C-A) at the other end of the element is also marked by a short arrow. The orientation of the deltas defined here was also used in Figures 1 and 3.

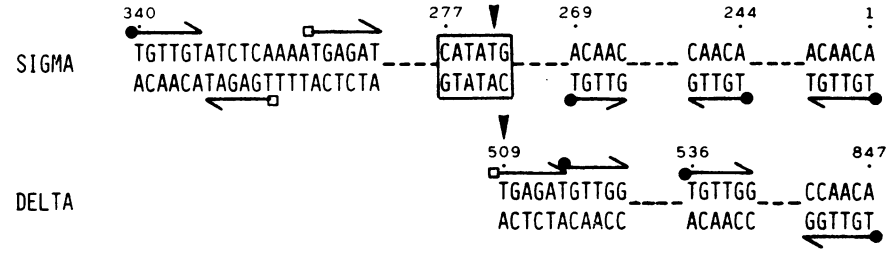

Fig. 5. Repeated sequence motifs in sigma and delta, and the junction point between the two elements in pY20. The horizontal arrows indicate the sequence motifs (direction 5′→3′). The numbers refer to the ones in the original sigma (Del Rey *et al.*, 1982) or to the ones of the delta in pY20 (cf. Figure 3), respectively. The hexanucleotide sequence is boxed; the vertical arrow marks the point of connection between the two elements.

The sigma segment in pY20 corresponds to position 340 (T-G-T-T-G...) through 273 (...C-A-T-A-T) in the original sigma (Del Rey *et al.*, 1982). It would be interesting to find out by which mechanism this junction has occurred. Our data are not sufficient, however, to propose a model. We may only point out some peculiarities which are seen in comparing the (original) sigma and the delta sequence in pY20 (Figure 5). The junction has obviously occurred within a sequence which in the original sigma is a 6-bp sequence of dyad symmetry (C-A-T-A-T-G) resembling the recognition site of a type II restriction endonuclease. The short sequence motifs which we have observed in the deltas (see Figure 4) occur several times in both delta and sigma. These sequences may be considered to be of significance in a potential interaction of the two elements.

The finding of complete Ty1 elements in the vicinity of tRNA genes on the one hand, and independent delta elements on the other hand, suggests that Ty1 can be transposed into the 5′-flanking region of tRNA genes but can be excised by 'delta-delta-recombination' (Cameron *et al.*, 1979; Farabaugh and Fink, 1980; Gafner and Philippsen, 1980) which was observed to occur for several Ty1 elements. The possibility that the tRNA genes are part of a transposition unit in those cases where only delta elements are seen in their vicinity, cannot be excluded but seems not very likely. The available data are not sufficient to decide whether or not Ty1 has a preference for particular tRNA genes. It is striking, however, that by screening only a few hundred clones three examples are found for glutamate tRNA genes. Statistically, the occurrence of both a delta and a glutamate tRNA gene in a DNA segment of 3 kbp on average would be in the order of magnitude of 10^{-5}.

The relatively close association between Ty1 or delta elements and several tRNA genes suggests the obvious possibility that these elements confer some special properties on the adjacent tRNA genes. As has been shown in the *his4-912* mutation, the insertion of Ty1 abolishes transcription (Farabaugh and Fink, 1980). Similarly, the presence of these elements may influence tRNA gene transcription. The effect on transcription by RNA polymerase III, however, might be quite different from that on transcription by RNA polymerase II. At least, *in vitro* transcription experiments with the two tRNA genes from pY5 and pY20 (Baker and Feldmann, unpublished results) have indicated that both genes are actively transcribed, despite their different molecular environment. The occurrence of two types of repetitive elements, delta (or Ty1) and sigma, in the vicinity of tRNA genes suggests that both of them could be part of a (common) regulatory control involving tRNA genes. This is supported by the finding that delta and sigma are capable of interaction. A further indication that there might be a functional relation between tRNA genes and repetitive elements comes from an investigation which we are carrying out at present. Hybridization experiments have suggested (Eigel and Feldmann, unpublished results) that the 5′-flanking regions of other yeast tRNA genes such as $tRNA_3^{Met}$ (Olah and Feldmann, 1980), $tRNA_1^{Val}$, and $tRNA_2^{Arg}$ (Baker *et al.*, 1982) contain repetitive sequences different from those described above.

Materials and methods

Carrier-free [^{32}P]phosphate and [^{125}I]Na were purchased from the Radiochemical Centre, Amersham; [α-^{32}P]dATP and [α-^{32}P]dCTP (400 Ci/mmol) were obtained from New England Nuclear Corp. Alkaline phosphatase and polynucleotide kinase were from Boehringer Mannheim GmbH; T4 DNA ligase was a gift from R.E. Streeck. The following restriction endonucleases were used in this work: *Bam*HI, *Hind*III, *Hpa*II, *Sau*3A, *Taq*I, *Alu*I, *Rsa*I, *Bgl*II, *Pst*I, *Acc*I (Boehringer Mannheim GmbH); *Eco*RI, *Hin*fI, *Hae*III (prepared in our laboratory). Yeast tRNA was purchased from Boehringer Mannheim GmbH. Yeast DNA (*Saccharomyces cerevisiae* strain C836) was prepared as described earlier (Pirro and Feldmann, 1975).

Plasmid DNA

pY5, pY20, and pY44 were from our collection of recombinant plasmids carrying yeast tRNA genes (Olah and Feldmann, 1980). Two clone banks of *S. cerevisiae* C836 DNA were constructed: *Hind*III fragments or partial *Sau*3A fragments, respectively, were ligated (Pech *et al.*, 1979) to pBR322 DNA that had been linearized with *Hind*III or *Bam*HI, respectively, and purified by gel electrophoresis. Ligated DNA samples were then used to transform (Lederberg *et al.*, 1974) *Escherichia coli* strain 490A. The clones, pY101 through pY114, and pY151, were identified by colony hybridization (Grunstein and Hogness, 1975) to nick-translated (Rigby *et al.*, 1977) *Sau*3A fragments from pY5 containing repetitive sequences (Eigel *et al.*, 1981). The clones were further characterized by the method of Birnboim and Doly (1979); plasmid DNA was prepared by scaling up their procedure. DNA fragments were prepared by the procedure recently described (Olah and Feldmann, 1980). All operations for cloning and culturing of strains with recombinant plasmids were carried out under L2/B1 conditions ('Richtlinien zum Schutz vor Gefahren durch *in vitro* neukombinierte DNA').

Restriction and hybridization analysis

Restriction mapping of cloned DNA was achieved in the usual manner by digestion with various restriction endonucleases and combinations of these enzymes; or by secondary digestion of isolated fragments with appropriate restriction enzymes, both followed by electrophoresis in agarose slab gels. The technique of Southern (1975) was used for the transfer of DNA fragments to nitrocellulose filter paper. Filters were hybridized with nick-translated (Rigby *et al.*, 1977) DNA fragments or [^{125}I]tRNA (Olah and Feldmann, 1980), respectively.

DNA sequencing

DNA sequences were determined by the method of Maxam and Gilbert (1980). Details of the procedure used here, were as described previously (e.g., Feldmann *et al.*, 1981).

Computer analysis

DNA sequences were analyzed with a MINC 23-RT11 computer system (Digital Equipment). The program of Staden (1980) adapted to this system was used.

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Note added in proof

Ty1 and delta elements have also been found integrated in the 5′ non-coding region of two tRNA genes at the *SUP* 4 locus of *S.cerevisiae* (J.Gafner and P.Philippsen, personal communication).