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**Analysis of yeast chromosomal regions carrying members of the glutamate tRNA gene family: various transposable elements are associated with them**

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Received July 26, 1988; Revised and Accepted October 27, 1988

Accession nos X08048–X08053 (incl.)

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**ABSTRACT**

We carried out an analysis on the genomic organisation of the tRNA<sup>Glu</sup> family in *S.cerevisiae*; eight clones were characterized by restriction mapping, hybridization and sequencing. These data taken together with our earlier findings show that the individual tRNA<sup>Glu</sup> copies are identical only in their structural part but embedded in entirely different genomic environments. All of the tRNA genes identified here are flanked by elements such as Ty, delta, sigma, and tau. In some cases, sequences from different elements form complex patterns indicating a sophisticated history of these chromosomal regions. A novel observation is that Ty and delta in the regions analyzed are exclusively associated with the tRNA genes. The observed patterns imply that the tRNA genes mark regions of multiple transposition and subsequent excision events, but that these have occurred after the individual tRNA gene copies had been fixed in their present locations. Transcription experiments by the use of micro-injection into *Xenopus* oocytes suggest that the elements flanking the tRNA genes exert a modulating effect on their expression.

**INTRODUCTION**

In the yeast, *Saccharomyces cerevisiae*, the major tRNAs are encoded by multiple (usually between 8 and 14) gene copies. The individual members of these gene families are scattered throughout the genome and constitute singular transcriptional units (1). Analysis of a variety of yeast tRNA genes (2) has revealed a unique feature in their genomic organisation: they are frequently associated with transposable elements such as delta, Ty1 or Ty2 (e.g.:3–8), sigma or Ty3 (9–11), and tau (12,13). In several cases, rather complex patterns are formed between two or more of these elements (e.g.:7,14). Also polymorphic situations have been identified (14,15) which differ by the presence or absence of particular elements. Whereas Ty or delta elements have been found in conjunction with a number of protein-coding genes (review,16), sigma or Ty3 (9–11) and tau (12,13), so far, have been encountered only in conjunction with tRNA genes. The apparently non-random occurrence of all these elements together with tRNA

genes is intriguing and challenges questions about their functional significance: (i) in evolutionary terms, the elements may play a role in the multiplication and mobilization of tRNA genes; (ii) the elements, because of their proximity to the initiation of tRNA transcription, may confer some special transcriptional properties on the adjacent tRNA genes, or they may play a role in the control of expression of certain tRNA genes or subsets of tRNA genes.

Suitably, these questions can only be approached by the analysis of a particular tRNA multigene family, a prerequisite for functional studies being the knowledge of its genomic organisation at the molecular level. We have earlier characterized three members of the tRNA<sup>Glu</sup><sub>3</sub> gene family (17,18) and have now analysed nine clones from a yeast cosmid library that carry tRNA<sup>Glu</sup> genes. This analysis shows that the tRNA<sup>Glu</sup> coding sequences are spread throughout most of the chromosomes and are embedded in different molecular environments. A similar situation is seen for five other tRNA genes that were localized on the cosmid clones. However, the tRNA flanking regions are extremely rich in all types of transposable elements, whereas no such elements could be detected in more distal regions; this is a particularly novel observation for the Ty and delta sequences. Furthermore, the complex patterns of elements associated with the tRNA genes suggest that the tRNA genes mark regions of a pronounced chromosomal plasticity. As judged from micro-injections into *Xenopus* oocytes, all of the tRNA<sup>Glu</sup> gene copies are actively transcribed. The level of expression, however, was found to vary proposing that the flanking regions -and possibly the different types of the elements- have a modulating effect in transcription.

### MATERIALS AND METHODS

Chemicals and enzymes. [ $\alpha$ -<sup>32</sup>P]dATP, [ $\alpha$ -<sup>32</sup>P]dCTP, and carrier-free <sup>32</sup>P-phosphate were purchased from Dupont (New England Nucl. Corp.). Na<sup>125</sup>I was from Radiochemical Centre, Amersham. Restriction endonucleases were obtained from Boehringer Mannheim GmbH or Biolabs, respectively, and used according to the manufacturers' instructions. T4 DNA ligase, *E.coli* DNA polymerase, DNA polymerase (Klenow fragment), and calf intestine alkaline phosphatase were from Boehringer Mannheim GmbH.

Cloning procedures, DNA preparations and analyses. To construct a yeast cosmid library, total high molecular weight DNA from a wild-type yeast strain (C836) was partially digested with *Sau*3A, and the resulting restriction fragments were size fractionated in two steps: (i) centrifugation in a NaCl gradient (1.25 to 5 M) at 39000 rpm for 3 h in a SW41 rotor of the Beckman ultracentrifuge; (ii) electrophoresis of single fractions on a 0.4% agarose gel. Fragments in the 35-45 kb size range were then ligated to the independently isolated arms (i.e. the 10.0-kb *Cla*I-*Bam*HI fragment and the 7.7-kb *Bst*IIIE-*Bam*HI fragment, respectively) of the cosmid vector pYc3030 (19). The procedures for ligation (20), in vitro packaging into phage lambda and transduction (21) of *E.coli* strain 490A followed standard protocols.

Appropriate fragments from selected cosmid clones were subcloned into pBR322 or M13 derivatives and DNA prepared by standard procedures (20). Restriction analysis was performed by standard procedures (20) and by the fast procedure according to Rackwitz et al. (22). Sequence comparisons and alignments were performed on a MicroVaxII workstation in conjunction with sequence data from GenBank and EMBL Bank, respectively.

## RESULTS

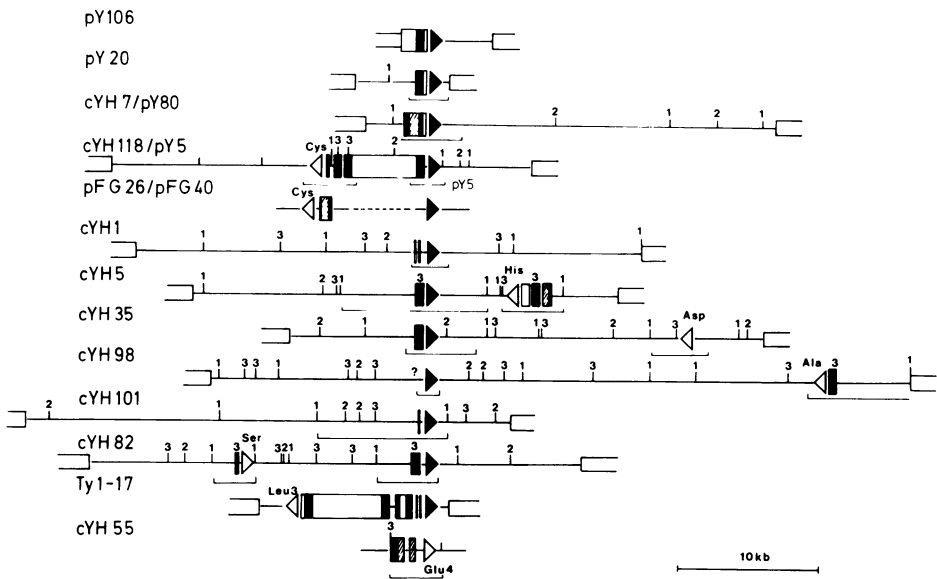
### Construction of a yeast cosmid library, isolation and characterization of clones specific for tRNA genes and elements.

To accommodate the whole yeast genome in a minimal number of clones and, likewise, to gain access to extended genomic regions, we constructed a cosmid library using the shuttle cosmid vector pYc3030 derived from the cosmid vector pHC79 (19). High molecular weight DNA was partially digested with Sau3A and size fractionated; fragments in the 35 - 45 kb size range were then cloned into the BamHI site of pYc3030.

$2 \times 10^5$  cosmid clones were screened by colony hybridization (20) for the presence of a tRNA<sup>Glu</sup><sub>3</sub> gene using an appropriate DNA segment from pY5 (17) as a probe. 153 positive clones were then characterized through restriction analysis with BamHI, ClaI, HindIII, SalI, and XhoI. Redundant clones were sorted out and a final set of nine unique clones (designated cYH1, cYH5, cYH7, cYH35, cYH55, cYH82, cYH98, cYH101, and cYH118; Fig. 1) were subjected to further analysis. In addition to hybridizations with the tRNA<sup>Glu</sup><sub>3</sub> probe, restriction fragments from the various cosmid clones were hybridized with <sup>125</sup>I-labelled total yeast tRNA to localize further tRNA genes (not shown). Furthermore, authentic probes for delta, sigma, tau, and the internal region of Ty1 (for details, see legend to Fig.1) were used in similar hybridizations to localize these particular elements. Appropriate restriction fragments spanning regions that hybridized to one or several of the above probes were then subcloned into pBR322. Subcloned regions are indicated in Fig.1 and the corresponding plasmids are distinguished by their nomenclature: for example, pYH35Glu3 contains a 4.3 kb HindIII fragment from cYH35, carrying a tRNA<sup>Glu</sup><sub>3</sub> gene together with the flanking regions, etc. Restriction maps were established for the plasmid clones by the use of the following enzymes: BamHI, BglII, ClaI, EcoRI, HindIII, PstI, PvuII, SalI, ScaI, SmaI, StuI, XbaI, and XhoI (data not shown). Finally, regions of interest were subcloned into M13 derivatives (23) and their sequences determined by the Sanger technique (24).

### Genomic organization of members of the tRNA<sup>Glu</sup><sub>3</sub> family.

Fig. 1 is a schematic representation of the cosmid clones carrying members of the tRNA<sup>Glu</sup><sub>3</sub> gene family including plasmid clones (pY5 (17), pY20 (18),



**Fig.1: Schematic representation of genetic and restriction maps of clones carrying members of the tRNA<sup>Glu</sup> gene family, other tRNA genes, and transposable elements.** pY5 (17), pY20 (18), pY80 (14), and pY106 (5) are yeast/pBR322 recombinants; cYH1, cYH5, cYH7, cYH35, cYH55, cYH82, cYH98, cYH101, and cYH118 are cosmid clones (see Materials and Methods); pFG26 and pFG40 are taken from ref.13, and Ty1-17 from ref.8. For simplicity, only the restriction sites for BamHI (=1), SalI (=2), and XhoI (=3) are listed. Filled triangles represent tRNA<sup>Glu3</sup> genes, indicating the direction of transcription. Other tRNA genes (and their transcriptional orientations) are represented by the open triangles. The transposable elements are symbolized in the following way: solid boxes, delta sequences; small open boxes, sigma sequences; hatched boxes, tau sequences; large open boxes, Ty sequences. As indicated by underscoring, appropriate fragments from the cosmid clones were subcloned into pBR322 for further analysis and sequencing. Sequence specific DNA fragments used as probes for hybridizations were subcloned into M13 derivatives and labelled with <sup>32</sup>P by nick-translation (20): (i) tRNA<sup>Glu3</sup> gene: 294-bp MboII-RsaI fragment from pY5, pos. 1268 through 1492 in ref.17, cloned into the SmaI site of M13mp8; (ii) delta element: 660-bp PvuII-BglII fragment from pY109 (15) cloned into HindII/BamHI of M13mp8; (iii) Ty internal segment: 1.7-kb ClaI-PstI fragment from pY109 (15) cloned into BamHI/PstI of M13mp8; (iv) sigma element: 328-bp HaeIII-HpaII fragment from pY66 (14) cloned into SmaI of M13mp10; (v) tau element: 292-bp AccI-ClaI fragment from pGC106 (12,13) cloned into SmaI of M13mp8. Total yeast tRNA was labelled with (125)I as described earlier (17). The tRNA genes and the short elements are not drawn to scale.

pY106 (5), and pY80 (14)) that have been characterized earlier. cYH7 and cYH118 are extensions of pY80 and pY5, respectively. pFG26/pFG40 (12,13) and Ty1-17 (8) are included for comparison. The nucleotide sequences of the

**a**

1 CATTGCACATGTGTGGGAAAGCAACTAAAATATCATCTGTTTTCCGGCATTGCGTGTAGTAGTATATATCACATGCGGTGAAGAAGATGACATAAAAATTGAGAAAACAGCATC  
 121 AAATATAATGAACGTCGAAATGCAAGGATGGTAAATGTAATGAGATAATGAACATAATGAACGGAGGAGAAATAATCGTAATATATATGTGAAAATACCGAATCCATTTTGAGG  
 241 TTCCCAATCTCGAGAAAGCACTGTAGTAAACATAATATAGTTTTTAAACAAAGCAATCTCAACAAATATCAAGATTCACCTAAACATTCAAAATATATATATGATGAAAATAAT  
 361 GATATAAGGATGGGAAAATATATTCAGATTAATGAAGGTGATGCAATTTGATCAATTAAGAGTACTAGGGAATGAAAAGAGCATAACTATATATATATAAAAATACCGATT  
 481 CCCTTTGGCGATTACTCTATCCCATGAGGAGAATACCCTAGTATATCTGCATCAITTAATAACCACTTATACAGCTTATAAAGAGTCCAAAACCTCATATGGCCCAATTTCTC  
 601 GGCAATATGATCCCTATAGCTGTAAAGTTTTCCATATAGTAATTTACCTTGTAAATGAAATTTCCACAATATTTGCGATATAGTGAACGGCTATCACATCAAGCTTTCCAGG  
 721 TGGAAACCGGGTTCGACTCCCGTATCGGAGTTTTGTCTAAGTGGAAAGTTTTTCCAGTTAAGTTATATGAGCAGTGCACATAAGGAACACCGAACAAGTTGCAATTTAA  
 841 GGCACCAGATGAAGAAAATGAGAACCTAAATTCGGCTGACATAGTAAAACTGGTTTTATCTGCAATGAAAAGTTATATAGATTTATGCTCATATATAAATAGAAATTTAGAATAAG  
 961 ATAATCCCTATTTATTTTCGAGATC 985

**b**

1 AGATCTGTTAATAAAATTTAGTAGATAACAATGACACATCTCATTACCGGTTAAGATTGACGTCATTCTGAGTTACATGATCTAGGACCTGTGCTCATTATTTGCTCACTAATCTAA  
 121 TTCTCCGCCCTTTCATATTGCTATCTTAATCTATATCCAAAATTTTTTGGCAAAATCCAGATTGGCTTTGATTTGGCATCGGTGGCTTCTTCAAAATCTCTCCCGTGAAT  
 241 CAACTGCACATACCCTAACTAGTTCAGTTAGTCCCTGCTGAGGACTGATAATAAATAATTAAGCCCACTCTCAGTACATAATTTGGTAGTACTTCATCATAATAGCTTGGC  
 361 GGTACCATTGGCCCGTACTGGG.....GAGCTAAAAAAGCTGACAGTCAATGTGTGACAGATAGTATACCGAGCCAGGAATCGAAGATAGAGACAATATTCCTAA  
 481 TCAAAAATGCTCATTATGTCAAGTGTGAGCATTGGTGAAGATTCAGTGGGAAAATTTGCGAATAATTTGATAATCGTTGTAATGAGTCAATATCATCTGTTATTAAG  
 601 TATCCGGTGTATTAGTATATCATCCATCAGCGTGAAGAATGATGAGAAAGTGCAGTCAATCAATAATGAAGCTGAAAATGGCGAGATGATGTAATAGGCAATAAAA  
 721 AATATAAGACGGAAGAAAATAACCGTAATAGCATTATGTAATAAATAGGGAATTTGAGGATTCCTATATCTCGAGGAGAACCTCTAGTATATTAACATACAGCAATATCATAGCCCTGA  
 841 TCAACAATAAAAATCCAGCAATTAACAATATCATCCATTTCTCAGTGCATCTCACTAGTAGGCGTTTTTGGAAATAATGCTAACATTTGGTAAGGTATGAAATAAAATGGCAT  
 961 ATCCCTCGTATGTTGCGCCGATATAGTGAACGGCTATCACATCACGCTTTCCAGCGTGGAGACCGGGTTCGACTCCCGCTATCGGAGATATATTAATATTTTAAATTTGACTCTA  
 1081 AATGAGAACATTTTTTAAAGCATCGGTGTGTAACAAGCTT 1124

**c**

1 TATACTAGACTCTACTCGAGATATAGTAATCTATAAGGGAATCGGATTTCTAAATATTACGSTATTTTTATTTCCTTTTACCTGTTTCTATGTTGTCGTTCAATAATCATAACAT  
 121 ATTAATCTAGTCTTAGTTCATCAATCTGATGACTTTTTCTCAATCTCTGATGATCTTTTTTGGCAGTATATGATAAAAATAAAGTAAATAAATAACAGCCATTGATGACATTTGG  
 241 AAATTTAATCTAATAAGTATGATGCTTTAAAACATTAATATAGCTATTTAGCTATTTGAAGCCAGAAAATTTTACAGATTAACAACCGATATAGTGAAGTGAAGGTTTCA  
 361 CCGTCGACACCGGGTTCGACTCCCGTATCGAGTTCTTTTTCTTTACCTCTTGAANAAGAGTGAAGAACTCAATAAATCTTGGCTGAAGTATTTCAATCAAGTTGTCGAAGG  
 481 TTATTATTTTTAATGACTATATCTGCTCAACACACAGGTTACACCATAATACTCTATTTACTGGACAGG 555

**d**

1 TGAAACCTTGAATGAGCTTACAGTCTTCACTCTTTTTAAAGTTGTTATTTTTTATACTATATACATTATTTCTAAAATCATTATAAATACGTTAAAACGGCAT  
 121 TATACTTTGATAGATTTCCAGAAAACCAACGAATTTGATAGTACCAACTCCAGGATGAATCGGATCGCATCACTATTCCTGGGCAAGAAAAAGAGTAAAGTATAGCT  
 241 GTTACTAGTATAATATCACACAGTGTATCTGTACACATAATATTATAGCTTTAAACAGCAATGAATCCAACTCCCCACAACATTCACAAATATCCATGTAATGAAGAGTTCT  
 361 TTAATATTACATGAGTAACTCCATTAGCTTTCAATTTCTAATCCTTTGTAACCGATATAGTGAACGGCTATCACATCAAGCTTTACCGTGGAGACCGGGTTCGACTCCCGTAT  
 481 CGAGTTTTTCATATATTATTTAATCTAGCAGTACTAGCGTGTAAATGTTACTCATTTTTTTGTTGAATCATTATGGTCAGTACTTTTCCATAGATATGATTTATCATAGCGCT  
 601 TTTCTGCTAACTACAGATGTAATCGTGAATGTATGATGTCGATGCAAGTAAATAATGTTGCTCTTTTTGATCGCTGTCAATTAACGGGAAAAAACTTGAAGGTA  
 721 AAAGAAACGGTCTTTACTATATTCAACCAACCTATCTGACCAAGGATGAAGAGCTTAGGACTATACAACCTGAAATGTAAGAAAATGTAAGCAAAACAGAGACAGCTTC  
 841 TGCTGTGGTGTCTTATTTCATTTATTTACCTAGGCTATTTTGAATCTATTTTCACTTCCAGCGCTTGTCTAGGATGAGCCATATAAAGTACTCAACCCCTCGCAAGAG  
 961 TGTTTTGATGTCGGTGTGTTTACGTCAGATACCCTTTTTGATAAAGTCACTCATGATCCGAAAAACAGAAATTTCTGGCGCTTTTATTAAG 1060

**e**

1 AAAAATAGAACAATGATATGATGCTTTTTAATAACCTGATAAAGCTCTAGCGGGAAGAATATAAAGCAGGTAATAAATTCGATATAGTGAACGGCTATCACATCACGCTTC  
 121 ACCGTGAGACCGGGTTCGACTCCCGTATCGAGTTCTTTTTGAAGAAATTTGAAAATTTTTTCTTCC 194

**f**

1 GAGAAGTTTAAAATTTGTTGATGTAATACAGGTGATGCTGCAGAGAAAGATATCGGATGATGTTTTGAATTTACCCTCATTAATAGCAGACAGGTTTTGCAGACAGAGGCA  
 121 TTCTGGTATAGTAAAGTGTGGGAGGGTAAAGGCACTTTTTGGAGGCTCAAGAAATTTGTGCGTGTAGCCACTAGCAATGTTGTAAGTTTCGCATGATGATTTATAAGGGAT  
 241 GATTTGCAATCGGCAAGGCAATTTATGAAACAGAGTGGGTGAGTGGTATCGGTTGAGAGAGTCTCTGTGGT.....GGTTAGCCCTGGCGGCTG  
 361 CACAGGCACTGAACACTAGGAAAAGCTACTTTGGGGCCGATGTAANAATAATCTATCAAAAACACTAGTACAGAACAGTTGAGTTAGAGCGGTGCTATATATCATATGGAAGATGAT  
 481 TCCTTGTATTTGGTAAGTACTACTTAGGTGTGAATATTAGTCCCTATAATTCGATATAGTGAACGGCTATCACATCACGCTTTCCAGCGGAGACCGGGTTCGACTCCCG  
 601 TATCGAGCTTTTTTTTATGATTAAGTAAATGATTTTTTTGAAGTGTGTAGTAGTCACTTTATCCATAAATAATAATACACAGAAAATTAATCTGTTTGTCTCAATTT  
 721 AGTTTGCATCTGTGCTAGATGTTCAATAATGGAACCTTTACAATACCGATCGGTGGGACATTTGAAAATAACAATCGTGGCATGCTTAAATTTTTTTGAGGTCATGAGCT  
 841 AAATAGACTGG 851

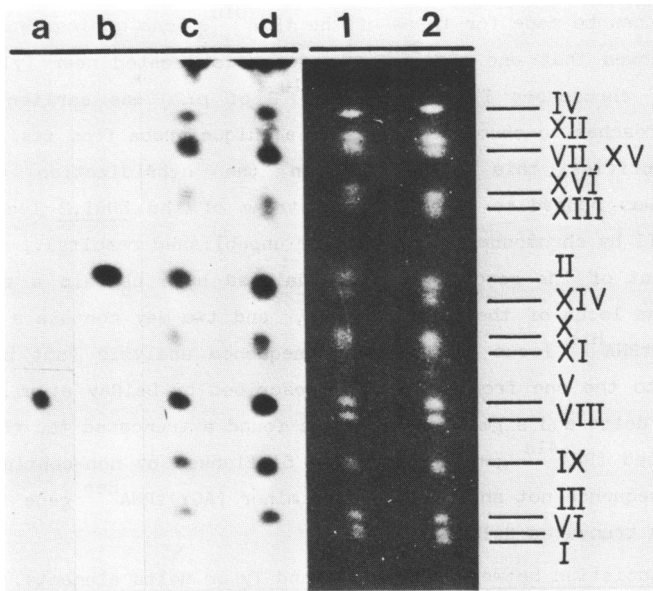
**Fig.2: Nucleotide sequences of tRNA<sup>Glu</sup>3 genes and flanking regions from Fig.1.** The tRNA<sup>Glu</sup>3 genes are boxed; their orientation corresponds to transcription from left to right. Sequences pertaining to elements are indicated by underscoring (delta, —; sigma, - - -; tau, ....). a, pYH5Glu3; b, pYH82Glu3; c, pYH35Glu3; d, pYH1Glu3; e, pYH98Glu3; f, pYH101Glu3.

newly determined tRNA<sup>Glu</sup><sub>3</sub> genes are documented in Fig.2. Extensive analyses of the data yielded the following information.

(i) The coding regions of the single tRNA<sup>Glu</sup><sub>3</sub> genes are identical; none of the copies contains an intervening sequence. However, their molecular environments are entirely different. Computer analyses of contigs of sequences surrounding the tRNA coding regions, from which sequences pertaining to whole or rudimentary elements had been eliminated prior to search, did not reveal any significant homologies. Furthermore, we could not detect any particular features (e.g., common sequence motifs, inverted repeats, etc.) in the regions "empty" of elements.

(ii) The analysis enabled us to record the whole spectrum of transposable elements associated with the single members of a tRNA gene family. Variations were found ranging from complex patterns formed by several of the elements, in some cases containing complete Tys, to divergent or remnant sequences of the elements: three of the tRNA<sup>Glu</sup> copies (pY106, cYH118/pY5, and Ty1-17) carry Ty elements inserted into the 5' flanks. pY20 and pY80, which represent an 'allelic' pair with respect to tau, contain an intact delta. Divergent or truncated deltas were found next to the tRNA<sup>Glu</sup><sub>3</sub> genes in cYH5, cYH35, cYH82, cYH1, and cYH101. In cYH98, no elements could be detected in the region around the tRNA<sup>Glu</sup><sub>3</sub> gene; however, these may become visible only after more extensive sequence analysis. The constellation of the tRNA genes and the elements in cYH118/pY5 is particularly interesting. The sandwich-like arrangement of two oppositely oriented tRNA genes bracketing a region with several elements is paralleled in Ty1-17 (8) (involving a tRNA<sup>Glu</sup><sub>3</sub> and a tRNA<sup>Leu</sup><sub>3</sub> gene; see Fig.1) and in pY66 (14) which involves a tRNA<sup>Gln</sup> and a tRNA<sup>Ser</sup><sub>2</sub> gene; further examples of this type do occur (our unpublished results). Furthermore, it appears that these regions constitute 'hot-spots' (8,26) involving Ty and delta elements that lead to multiple rearrangements, and hence polymorphic situations in different isolates. An explicit example has been recently discussed by Warmington et al. (26) for the hot-spot on the right arm of chromosome III which lies upstream of a tRNA<sup>Lys</sup> gene. The locus in cYH118 seems to undergo similar rearrangements, since Genbauffe et al. (12,13) characterized the same locus (pFG26/pFG40; see Fig.1) from a different strain (M1417-1c) to show that Ty and delta were lost in this case but a tau element had been incorporated.

(iii) By using pulsed field gel electrophoresis (27) in combination with Southern hybridizations, we show (Fig.3) that the tRNA<sup>Glu</sup> gene copies are spread throughout most of the yeast chromosomes. From earlier genomic



**Fig.3: Pulsed field gel electrophoresis of chromosomes from strain C836 and hybridization with tRNA<sup>Glu</sup> probes.** Yeast chromosomes were prepared as described in ref.27. 1% agarose (LMP, Sigma) gels and the electrophoresis apparatus devised by Ziegler et al. (29) were used to fractionate the chromosomes (20 mM TEB buffer, pH 8.3; 13°C; 250 volts; 60 sec pulse time; 24 h). The assignment of the bands to single chromosomes follows those made in ref.30. 1 and 2, EtBr staining of gel; approximately the double amount of cells of lane 1 (and c) was used for lane 2 (and d). **a**, probe specific for tRNA<sup>Glu</sup> locus on chromosome V (1 kb HindIII fragment, 250 bp downstream from the tRNA<sup>Glu</sup> gene of pY20 (18), tested by genomic hybridizations to be unique); **b**, probe specific for tRNA<sup>Glu</sup> locus on chromosome II (unique 500-bp HindIII-BamHI fragment from cYH18/pY5, 2.2 kb downstream from the tRNA<sup>Glu</sup> gene; cYH18 was shown to be located between DUR1,2 and MET8 on chromosome II by chromosomal walking from CDC28 towards the right telomere (Stucka and Feldmann, unpublished); **c** and **d**, tRNA<sup>Glu</sup> specific probe, see legend to Fig.1.

hybridizations with labelled tRNA (17) a total of 14 copies was derived. On this basis and with the relative intensities of the hybridization pattern in Fig.3 one would arrive at the following estimate for the genomic distribution of the tRNA<sup>Glu</sup> genes: chromosomes IV, XVI, XIII, X, and III, one copy each; chromosome IX, one to two copies; chromosome II, two copies; chromosomes VII and XV two copies together; chromosome V, two to three copies. Chromosomes XIV, VIII, VI, and I, seem to be devoid of tRNA<sup>Glu</sup> genes. The total number of copies counted in this way (minimum 13, maximum 15), agrees very well with the earlier number of 14 copies. So far, precise

assignments can be made for three of the tRNA<sup>Glu</sup><sub>3</sub> gene copies: Warmington et al. (8) showed that one tRNA<sup>Glu</sup><sub>3</sub> gene copy is located near Ty1-17 on the left arm of chromosome III. The tRNA<sup>Glu</sup><sub>3</sub> of pY20 was earlier mapped by genetic approaches to chromosome V (18); a unique probe from its 3' flanking region identifies this chromosome in the hybridization experiment. cYH118/pY5 was mapped to a region downstream of the DUR1,2 locus (28) on chromosome II by chromosomal walking (our unpublished results).

(iv) Five out of the genomic regions analysed here contain a second tRNA gene near the locus of the tRNA<sup>Glu</sup><sub>3</sub> gene, and two may contain a third tRNA gene. The tRNA<sup>His</sup> locus of cYH5, by sequence analysis (not shown), is homologous to the one from clone pYD2 described by DelRey et al. (9); in addition to delta and sigma sequences, we found a truncated tau (Fig.1). The newly detected tRNA<sup>Ala</sup> gene in cYH98 is 5'-flanked by non-contiguous delta sequences (sequence not shown), and the minor (AGY)tRNA<sup>Ser</sup> gene in cYH82 is flanked by a truncated delta (31).

### Specific association between tRNA genes and Ty or delta elements.

The above analyses strongly suggested that the 5' flanking regions of the tRNA genes are preferred locations for Ty and delta elements. In order to establish that no such elements occurred in other portions of the cloned chromosomal segments, we conducted hybridization experiments under variable conditions of stringency (data not shown). For example, an authentic delta probe monitored all of the delta sequences associated with the tRNA genes. The truncated or remnant delta sequences became detectable only under conditions of low stringency, in some cases as extremely faint bands, but no further signals occurred. By similar hybridizations with probes from internal Ty segments, only the Ty elements listed in Fig.1 could be detected. Therefore, we conclude that Ty and delta sequences are absent from regions outside the immediate tRNA flanking portions on the cosmid clones analyzed here.

### Transcription of tRNA genes by micro-injection into *Xenopus* oocyte nuclei.

To test whether the different tRNA gene copies are actively expressed, we injected appropriate plasmid constructs containing the tRNA genes together with extended flanking regions into Xenopus oocyte nuclei. It has been shown earlier that the oocytes will transcribe exogenous tDNA and mature the precursors reliably (review, 35); variable sizes of the plasmids do not seem to interfere with transcription rates (our unpublished results). From the results in Fig.4 it is evident that the tRNA<sup>Glu</sup> genes are transcribed in the oocyte system, but that the extent of expression varies. It is relatively





**Fig.4: Micro-injection into *Xenopus* oocyte nuclei of plasmids carrying tRNA genes, and analysis of the RNA by electrophoresis on 10% polyacrylamide gels.** The micro-injection experiments, the analysis and the quantitation of transcription products followed the protocols detailed in refs.32 and 33. 20 nl of plasmid DNA (concentration 100 -200 ug/ml; amounts normalized for molar ratios) were injected; groups of 40 to 50 oocytes were used for each experiment. The experiments of each series were repeated at least once giving nearly the same results. The following plasmids with inserts containing different tRNA genes together with their flanking regions (see text and legend to Fig.1) were used (size of insert in kb): **a**, pY20 (1.9); **b**, pYH7 (5.5); **c**, pY5 (1.7); **d**, pYH1Glu3 (2.3); **e**, pYH35Glu3 (4.3); **f**, pYH98Glu3 (1.8); **g**, pYH101Glu3 (9.8); **h**, pYH55Glu(GAG) (3.3) (7); **i**, pYH35Asp (4.6). Standard, labeled yeast tRNA.

low in those cases in which no element or only delta remnants are present (e.g., pYH1Glu3, pYH101Glu3, pYH35Asp; Fig.4), whereas it is elevated in cases in which delta, tau, or Ty sequences are associated with the tRNA genes. It thus appears that the elements in certain constellations might have a stimulatory effect on the expression of the adjacent tRNA genes. This observation parallels our earlier findings which indicated that the transcriptional level of yeast tRNA<sup>Lys1</sup> constructs micro-injected into oocytes was enhanced by the insertion of Ty sequences (32).

#### DISCUSSION

With the present study, it is possible to compare 12 different copies of the yeast tRNAGlu3 gene family at the molecular level. The data show that the individual gene copies are spread throughout most of the chromosomes and that their molecular environments are substantially different. A remarkable

feature is that nearly all of the tRNA<sup>Glu</sup><sub>3</sub> genes are affiliated with one or more of the transposable elements, exclusively located in the upstream regions; this is also true for the additional tRNA genes located on the cosmid clones. The arrangement of these sequences is highly individual, and in several cases rather complex patterns are formed. Furthermore, the sequences that were detected range from full-size intact elements to rather divergent or heavily truncated elements. On thorough inspection, one can even find sequences down to the size of 18 or 20 bp that reveal significant homology to sequences of one or the other element.

The general picture that emerges from this study is that the tRNA genes mark highly dynamic regions and that the observed patterns of elements are the result of multiple and complex interactions not only involving Ty and delta but in addition sigma and tau. With respect to Ty and delta, the patterns are a direct reflection of the proposed Ty cycle (34): Ty elements undergo a process of delta-delta recombination, resulting in single delta elements. These solo delta elements then slowly 'decay' in the genome to produce the observed remnants which may provide fertile substrates for the evolution of new, unique DNA sequences. It has been noted earlier that sigma (9-11) and tau (12,13) elements are highly specific for tRNA genes. Our data strongly suggest that the majority of the Tys and deltas are also frequently located in the vicinity of tRNA genes. Until today, some 50 out of a total of about 400 tRNA loci (2) have been analyzed to a sufficient extent to reveal the occurrence of these elements associated with them. Altogether, 6 Ty elements and some 30 delta sequences (either intact or rudimentary) have been identified. An extrapolation of these figures would arrive at a total number of delta sequences that is substantially higher than that estimated from hybridization experiments (e.g.:16), probably around 300.

The close association of the yeast tRNA genes with transposable elements has led to the suggestion that the tRNA genes might be part of transposition units, and that transposition (or recombination) could account for the presence of dispersed copies of identical tRNA genes in yeast (e.g.26). Direct proof for such a mechanism would be to show that two members of a given tRNA gene family have extended homologies in their flanking regions. The results of our analysis of the tRNA<sup>Glu</sup><sub>3</sub> genes, i.e. the diversity of their chromosomal contexts, do not favour such a model. By contrast, our data support the view that all movements of the elements occurred after the tRNA genes had been fixed in their present locations.

The preferred targeting of the elements into the tRNA flanking regions has to consider their potential to interfere with the expression of these genes.

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It has been shown in several cases that Ty transpositions into promoter regions of protein-coding genes drastically modify their transcription (review, 16). In the case of the tRNA genes, the accumulation of the elements in their vicinity appears to be non-hazardous to the cell, at the least. The more attractive view is that the elements in these positions might not only be tolerable but even advantageous.

In many cases, non-coding sequences flanking the tRNA genes have been found to modulate the level of transcription; the effects seem to be brought about by sequences that are rather individual for different organisms or even for different sets of tRNA genes (review, 35). The transcription experiments, in which we utilized micro-injection of various plasmids carrying tRNA genes into *Xenopus* oocyte nuclei, point to the possibility that the elements may exert a modulating effect on tRNA gene transcription in yeast. However, we are aware of the fact that the oocytes represent a heterologous system and that this type of experiment cannot distinguish between possible effects brought about by the immediately flanking regions or different types of the elements. Neither *in vitro* nor *in vivo* experiments in the homologous system can directly differentiate between expression rates of the individual tRNA gene copies but have to rely on constructs employing a reporter gene. We are currently carrying out *in vivo* transcription studies in the yeast system by the use of an artificial tRNA gene to investigate the role of the elements in tRNA gene expression.

#### ACKNOWLEDGEMENTS

The expert technical assistance of Mrs. C.Schwarzlose, Ms. U.Pilz, and Ms. K.Beer is gratefully acknowledged. We would like to thank our colleagues W.Lorentz and G.Weichhold from this laboratory for their help with the pulsed field gel electrophoresis. The Deutsche Forschungsgemeinschaft (SFB 304, München) and Fonds der Chemischen Industrie have supported this work.

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