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Delta sequences in the 5′ non-coding region of yeast tRNA genes

Jürg Gafner, Eddy M.De Robertis[1] and Peter Philippsen*

Department of Microbiology, and [1]Department of Cell Biology, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

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Two so far undetected tRNA genes were found close to delta (δ) sequences at the *sup4* locus on chromosome X in the genome of *Saccharomyces cerevisiae*. The two genes were identified from their abundant transcription products in frog oocytes. Hybridisation experiments allowed the mapping of the transcripts in cloned DNA and DNA sequence analysis revealed the presence of one AGGtRNA^{Arg} and one GACtRNA^{Asp} gene. tRNA^{Asp} genes with sequences similar or identical to GACtRNA^{Asp} exist in 14-16 copies per haploid yeast genome, whereas only one copy was detected for AGGtRNA^{Arg}. *In vivo* labelling of total yeast tRNA with ^{32}P followed by hybridisation revealed that the unique AGGtRNA^{Arg} gene is transcribed in *S. cerevisiae*. δ sequences are present 120 bp upstream from the first coding nucleotide in the case of AGGtRNA^{Arg}, 80 bp in the case of GACtRNA^{Asp} and 405 bp in the case of the known UACtRNA^{Tyr} *(sup4)* gene. δ sequences, as part of Ty elements or alone, were also found by other investigators at similar distances upstream of the mRNA start in mutant alleles of protein-coding yeast genes. Although protein-coding genes are transcribed by RNA polymerase II and tRNA genes by RNA polymerase III, the 5′ non-coding region of both types of genes could conceivably have a peculiar DNA or chromatin structure used as preferred landing sites by transposable elements.

Key words: δ sequences/frog oocyte injection/transposition/tRNA genes/yeast

Introduction

In the genome of *Saccharomyces cerevisiae* δ sequences occur either at both ends of the mobile repetitive element Ty or alone as so-called solo δ sequences (Cameron *et al.*, 1979). The ability to transpose was only detected for Ty elements (reviewed by Roeder and Fink, 1983; Williamson, 1983), and there is, so far, no indication for transposition of the ~330 bp long solo δ sequences. The origin of solo δ sequences at variable positions in the genome of *S. cerevisiae* can be explained by Ty transposition and subsequent homologous recombination between the terminal δ sequences, leaving behind a solo δ sequence (Farabaugh and Fink, 1980; Gafner and Philippsen, 1980).

Recently, we examined 30 *Saccharomyces* strains isolated from different parts of the world for the Ty and δ copy number (Stotz and Philippsen, in preparation). The copy number for Ty elements varied from 1 to 35 per haploid genome but the δ copy number (solo δ plus Ty-δ) seems to be more constant, since in all cases 30-50 *Eco*RI fragments were seen to hybridize to δ-specific probes. The distribution of these δ-carrying fragments in the genome is not known.

δ sequences at Ty termini play a functional role in the transcription of Ty elements, since Ty transcription starts in one of the terminal δ sequences, reads through the internal 5 kb and terminates in the other δ sequence (Elder *et al.*, 1980). We intended to test whether solo δ sequences have a similar potential in initiating transcription of adjacent regions and whether they could, therefore, be viewed as mobile promoters.

A DNA clone carrying four solo δ sequences was chosen to look for transcription products starting within δ sequences by microinjection of multiple copies into the nucleus of frog oocytes. Although the oocyte is a heterologous system it can, on occasions, provide the yeast geneticist with a highly efficient expression system for cloned segments of DNA (e.g., De Robertis and Olson, 1979; Nishikura *et al.*, 1982). To our surprise, instead of δ transcripts we detected two tRNA transcripts, the genes of which mapped in the neighbourhood of δ sequences. The probability of a δ sequence being located close to a tRNA gene is very low, although both types of sequences are repeated >100 times in the *S. cerevisiae* genome. The origin of the δ sequence in the 5′ non-coding region of these two tRNA genes will be discussed in the light of recent results on target site selection of mobile elements in yeast.

Results

Transcription products made in frog oocytes from DNA plasmids containing δ sequences

We injected H13 DNA carrying a 7-kb yeast *Hind*III fragment with four solo δ sequences cloned into the *Hind*III site of pBR322 together with [α-^{32}P]GTP into the nucleus of frog oocytes. This DNA directed the synthesis of two or more ~75 bases long transcription products, as seen after separation of the labelled RNA on a polyacrylamide gel (Figure 1, lane A). The transcription products made from two other yeast clones were separated on the same gel: pJDB248 in lane B, which carries the yeast LEU2 structural gene, a tRNA^{Leu} gene containing an intervening sequence and approximately one half of a TY element and with that a δ sequence (Beggs, 1978; Andreadis *et al.*, 1982) and pYT-C in lane C, which carries a yeast tRNA^{Tyr} gene with an intervening sequence (Goodman *et al.*, 1977). The bands in lane C belong to the 92 bases long precursor tRNA^{Tyr} with intervening sequence and the processed tRNA^{Tyr} with 78 bases (De Robertis and Olson, 1979). The strong transcript in lane B was not analyzed further, but, as judged from its length, it could be the tRNA^{Leu} precursor with the 32 bases long intervening sequence. Since the RNA was derived in each case from two oocytes which were injected with similar amounts of DNA and [α-^{32}P]GTP, it is obvious that transcription from H13 (lane A) is much stronger than from the other two yeast DNAs (lanes B and C).

To decide whether the H13 transcripts were δ-specific or not, we mapped their origin. We eluted the corresponding fraction of labelled RNA from a preparative polyacrylamide

*To whom reprint requests should be sent.

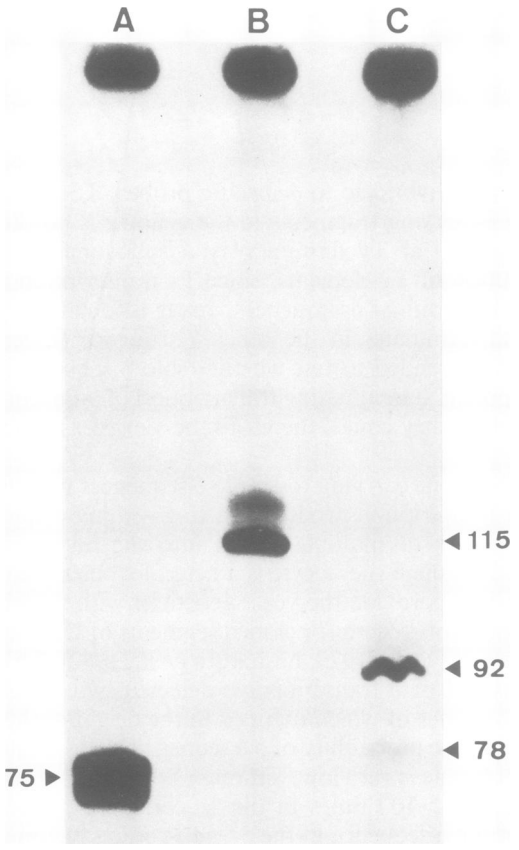

Fig. 1. Transcription products made from recombinant yeast DNA in frog oocytes. Three different plasmids **(A)** H13, the map of which is shown in Figure 2, **(B)** pJDB248 and **(C)** pYT-C were each injected into the nuclei of two frog oocytes and their radioactively labelled transcription products separated on an 8% polyacrylamide gel. The fast migrating RNAs seen in the autoradiogram are discussed in the text. The length (number of bases) of the pronounced products in **lanes A** and **B** were estimated by using the known length of the transcripts in **lane C** as markers (De Robertis and Olson, 1979). The slow migrating material at the top of each lane is most likely frog rRNA.

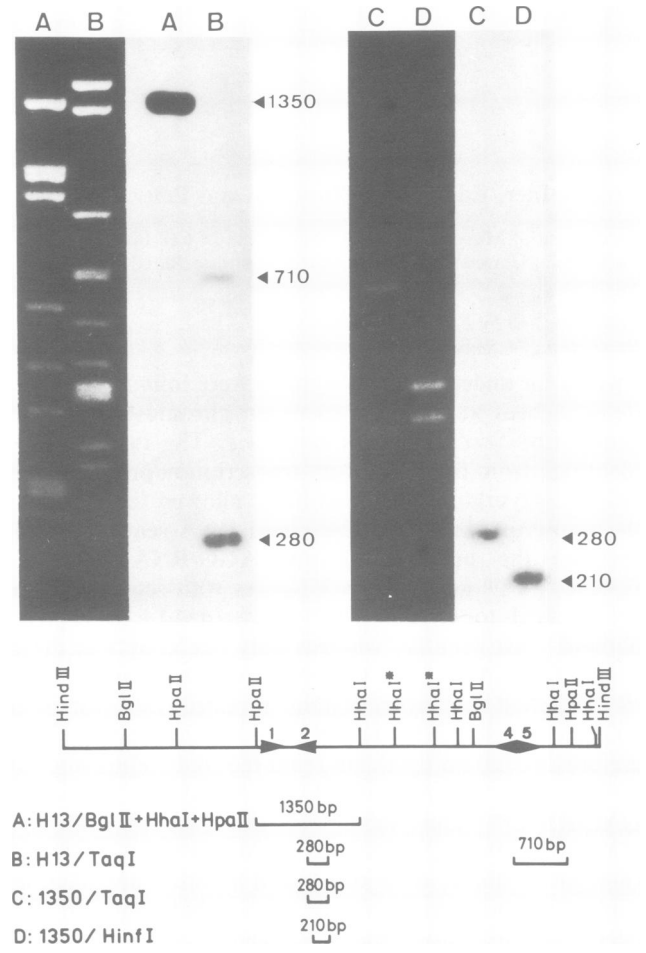

Fig. 2. Mapping of the frog oocyte transcripts from the H13 plasmid. The ^{32}P-labelled strong transcription products from H13 were electroeluted from an 8% polyacrylamide gel and hybridized to separated H13 DNA restriction fragments bound to nitrocellulose. **Upper part:** 1.2% agarose gels before DNA transfer and autoradiograms after the hybridisations with H13 DNA cleaved with *Bgl*II, *Hha*I plus *Hpa*II **(lane A)** and *Taq*I **(lane B)** and with the isolated 1350-bp *Hpa*II-*Hha*I fragment from H13 cleaved with *Taq*I **(lane C)** and *Hinf*I **(lane D)**. **Lower part:** restriction map of the yeast part in the H13 plasmid with the precise positions of the four solo δ sequences which are known from DNA sequence analyses (Gafner and Philippsen, in preparation). A more detailed map around the δ sequences is presented in the sequence strategies of Figure 3. The positions of the two *Hha*I sites marked with an asterisk could not be determined with certainty. The origin and length of those fragments from **lanes A** to **D** with homology to the probe are indicated.

gel after injection of 20 oocytes. This RNA was used as a hybridisation probe against appropriate DNA restriction patterns of H13 separated on agarose gels and blotted onto nitrocellulose paper (Figure 2). The interpretation of the hybridisation data was greatly facilitated by the available restriction map of H13 and substantial sequence data (Gafner and Philippsen, in preparation).

We first probed H13 DNA cleaved with *Bgl*II, *Hha*I and *Hpa*II and found strong homology to the 1350-bp fragment (Figure 2, lane A) which is encompassing δ1 and δ2. No homology to the 1050-bp fragment which contains δ4 and δ5 was detected. This result rules out that δ2 is the origin of the transcripts, since δ2 and δ5 have almost identical sequences, but leaves δ1 as a candidate, because of its substantial sequence divergence to δ4 and δ5 (Gafner and Philippsen, in preparation). This possibility was ruled out when *Taq*I-cleaved H13 DNA was probed. Strong homology was seen with the 280-bp fragment, which contains the right end of δ2 plus 190 bp adjacent sequences (Figure 2, lane B). This result was confirmed when the isolated 1350-bp fragment was probed after cleavage with *Taq*I or *Hinf*I (Figure 2, lanes C,D), again showing hybridisation to the 280-bp *Taq*I fragment and to a 210-bp *Hinf*I fragment which overlaps with the *Taq*I fragment. A weak but significant homology was also observed in lane B with the 710-bp *Taq*I fragment which contains the right part of δ4, the whole of δ5 plus 290 bp adjacent sequences. The first experiment (lane A) did not reveal any detectable homology to the probe in this part of H13. This could be explained by a cleavage, e.g., of *Hha*I, within the 75 bp of homology, which would prevent a stable hybridisation of the probe under the conditions employed.

We concluded from these hybridisation data that the H13 transcription products made in frog oocytes share no homology to the four δ sequences but to sequences very close to δ2 and δ5, respectively.

Identification of frog oocyte transcripts

DNA sequencing showed that each of the two regions on the H13 DNA plasmid from which the frog oocyte transcripts

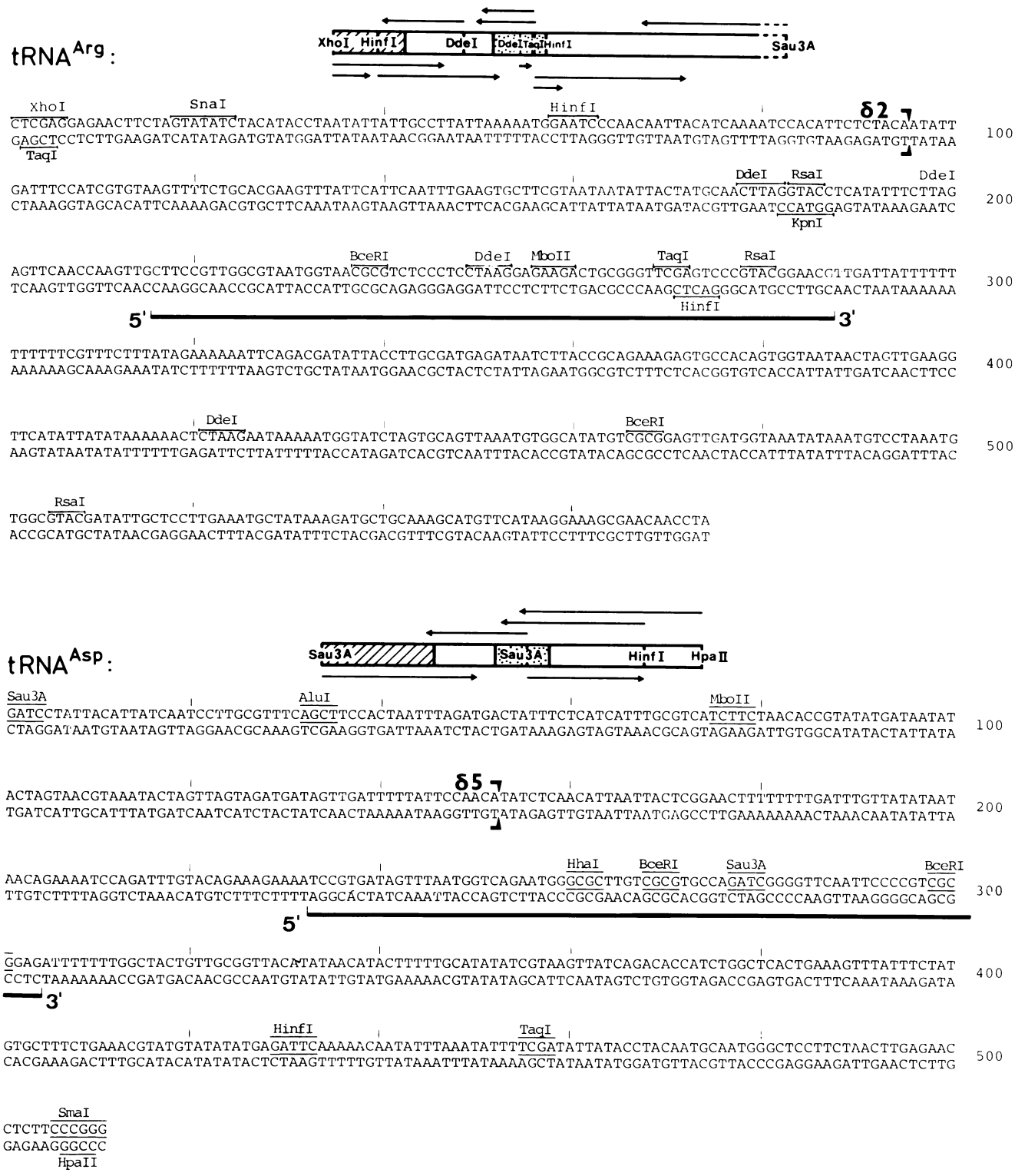

Fig. 3. DNA sequence analysis. Strategy: the DNA sequence strategy is indicated by arrows which give the length and orientation of the readable sequences for fragments labelled at the 3′ end. Several sequences were determined twice in independent experiments. The positions of δ sequences and tRNA genes are marked by shaded and dotted areas, respectively. DNA sequence: in the DNA sequence the restriction sites are indicated by bars. The tRNA gene sequences are underlined and the boundaries of the δ sequences marked by arrows.

originate contains a tRNA gene. The DNA sequence illustrated in Figure 3 was determined using the method of Maxam and Gilbert (1980). By visual inspection of the DNA sequence we detected in each homology region, close to a δ sequence, a transcription unit for a tRNA gene. 120 bp to the right of δ2 is the 5′ end of an AGGtRNA^{Arg} and 80 bp to the right of δ5 is the 5′ end of a GACtRNA^{Asp}. The respective sequences are marked in Figure 3 and the corresponding cloverleaves are shown in Figure 4. Together with the non-coded CCA end, both mature tRNAs should consist of 75 bases. Most common features for tRNAs, as described by Sprinzl *et al.* (1980), are found in these cloverleaf structures. The

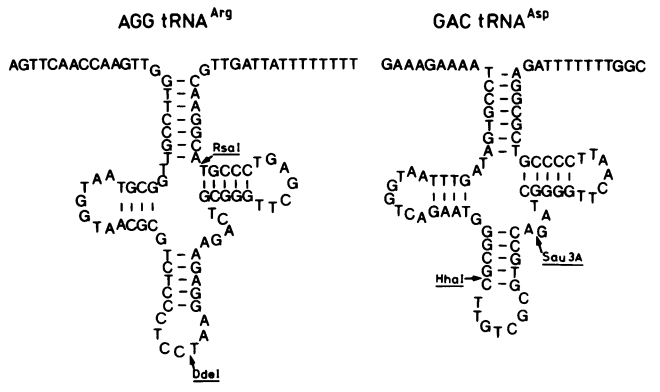

Fig. 4. Cloverleaf structure. The cloverleaf structure of the non-coding DNA strand is drawn for the $tRNA^{Asp}$ and $tRNA^{Arg}$ gene, respectively. The positions of the restriction sites used in the preparation of the hybridisation probes for the copy number determination are indicated.

$AGGtRNA^{Arg}$ is a minor $tRNA^{Arg}$ in yeast, and the sequence has not been reported yet. The $GACtRNA^{Asp}$ is the major aspartate tRNA, the sequence of which was determined by Gangloff *et al.* (1972); no differences were found when compared with our DNA sequence except that the CCA at the tRNA 3′ terminus is not coded for.

The transcription of the two tRNA genes most likely stops in the cluster of adenines located at the 3′ end of both genes on the coding strand. This cluster is a common transcription stop signal for RNA polymerase III transcripts in eucaryotes (Brown and Brown, 1976; Maxam *et al.*, 1977; Valenzuela *et al.*, 1977).

Determination of the gene copy number for $AGGtRNA^{Arg}$ and $GACtRNA^{Asp}$

The copy number of these two tRNA genes in the genome of *S. cerevisiae* was determined by hybridisations against total genomic DNA cleaved with different restriction enzymes after blotting of the separated DNA from the agarose gels onto nitrocellulose filters. Restriction fragments containing part of the tRNA genes, either 90% of $AGGtRNA^{Arg}$ or 59% of $GACtRNA^{Asp}$ plus unique DNA sequences of *S. cerevisiae*, were the source for the hybridisation probes as indicated in the maps of Figure 5. This limitation in homology was unavoidable because the probes had to be free of δ sequences. The probes were labelled after cleavage with appropriate restriction enzymes by filling in of the cohesive ends in the presence of [α-^{32}P]deoxynucleotide triphosphates with Klenow polymerase (see legend to Figure 5 for details). The copy number of the two tRNAs genes were then obtained by counting the bands in the corresponding autoradiograms. $GACtRNA^{Asp}$ exists in 14–16 copies, whereas $AGGtRNA^{Arg}$ has a single copy in the haploid genome of *S. cerevisiae*. In the latter case, the restriction fragments with homology were as long as expected from the known restriction map around the $AGGtRNA^{Arg}$ gene. Occurrence of single copy tRNA genes which are used for the expression of essential genes are interesting because they cannot be involved in non-lethal deletion events.

In vivo transcription studies of tRNA genes in yeast

It remained to be tested whether these two tRNA genes are transcribed in *S. cerevisiae* or not. *In vivo* ^{32}P-labelled total tRNA was isolated from yeast cells and purified over a 5% polyacrylamide gel as described by Rubin (1975). This ^{32}P-labelled tRNA was hybridised to equimolar amounts of cloned restriction fragments from the $AGGtRNA^{Arg}$ and $GACtRNA^{Asp}$ gene region. The p18 clone used also contained, in the 12-kb yeast insert, the previously known $UACtRNA^{Tyr}$ gene *(sup4)*. The autoradiogram (Figure 6) shows the presence of an $AGGtRNA^{Arg}$ gene transcript in addition to those from the $tRNA^{Tyr}$ and $tRNA^{Asp}$ genes. The detection of hybridisation specific for the single copy $AAGtRNA^{Arg}$ gene clearly shows that this locus is normally transcribed in yeast. No definite conclusion can be drawn as to the *in vivo* activity of the $GACtRNA^{Asp}$ next to δ5, because identical tRNA molecules are synthesized at several loci in the genome. The $tRNA^{Tyr}$ gene in this region is known to be active since all eight $tRNA^{Tyr}$ genes of *S. cerevisiae* (Olson *et al.*, 1977) can mutate to active suppressor tRNAs (Hawthorne and Leupold, 1974).

The signal strengths in Figure 6 should approximately reflect the ratios of the three tRNA types in the yeast tRNA pool, because the majority of the tRNAs with homology to the three genes had bound during the first hybridisation. This was concluded from a second hybridisation of the used probe to a new nitrocellulose strip with DNA which revealed only very weak signals. We estimate, therefore, from the autoradiogram in Figure 6 that the single copy $AGGtRNA^{Arg}$ gene is more efficiently (of the order of 2 to 4 times) transcribed than the average of the eight $UACtRNA^{Tyr}$ genes or the average of the 14–16 genes with $GACtRNA^{Asp}$ homology.

Discussion

With the aid of yeast DNA injections into frog oocytes we discovered two tRNA genes, each with a 72-bp coding region, left of the $tRNA^{Tyr}$ *(sup4)* locus on chromosome X in the genome of *S. cerevisiae*. The existence of more than one tRNA gene in this region had already been mentioned as a possibility by Olson *et al.* (1979). The new genes were localised with the Southern method and characterised by DNA sequence analysis. One gene codes for $GACtRNA^{Asp}$, the tRNA sequence of which is already published (Gangloff *et al.*, 1972). It exists in 14–16 copies per haploid genome and we established the first locus for this tRNA. The other gene codes for $AGGtRNA^{Arg}$ and is a single copy gene. Piper (1978) had described a single copy recessive lethal suppressor $UCGtRNA^{Ser}$ gene, but it still showed extensive homology to other $tRNA^{Ser}$ genes. Based on homology data, the $AGGtRNA^{Arg}$ gene seems, therefore, to be the first single copy yeast tRNA gene.

The region between the *sup4* and the *CYC1* locus on chromosome X, which includes the $AGGtRNA^{Arg}$ gene, shows an enhanced frequency of deletion events as compared with other regions in *S. cerevisiae* (Rothstein, 1979; Liebman *et al.*, 1981). These deletions are probably triggered by an unusual accumulation of δ and Ty sequences which allows, for example, excisions by homologous recombination. None of the mapped deletions however, is lacking the $AGGtRNA^{Arg}$ region, in line with the single-copy and therefore essential nature of this gene.

A survey of codon usage in sequenced genes shows that the $AGGtRNA^{Arg}$ is a minor $tRNA^{Arg}$, whereas $GACtRNA^{Asp}$ is the most frequently used $tRNA^{Asp}$. The aspartate codon GAC is 44 times more often used in abundant yeast genes than the arginine codon AGG (Bennetzen and Hall, 1982). The transcription efficiency of the single $AGGtRNA^{Arg}$ gene compared with the 14–16 $tRNA^{Asp}$ genes does not quite

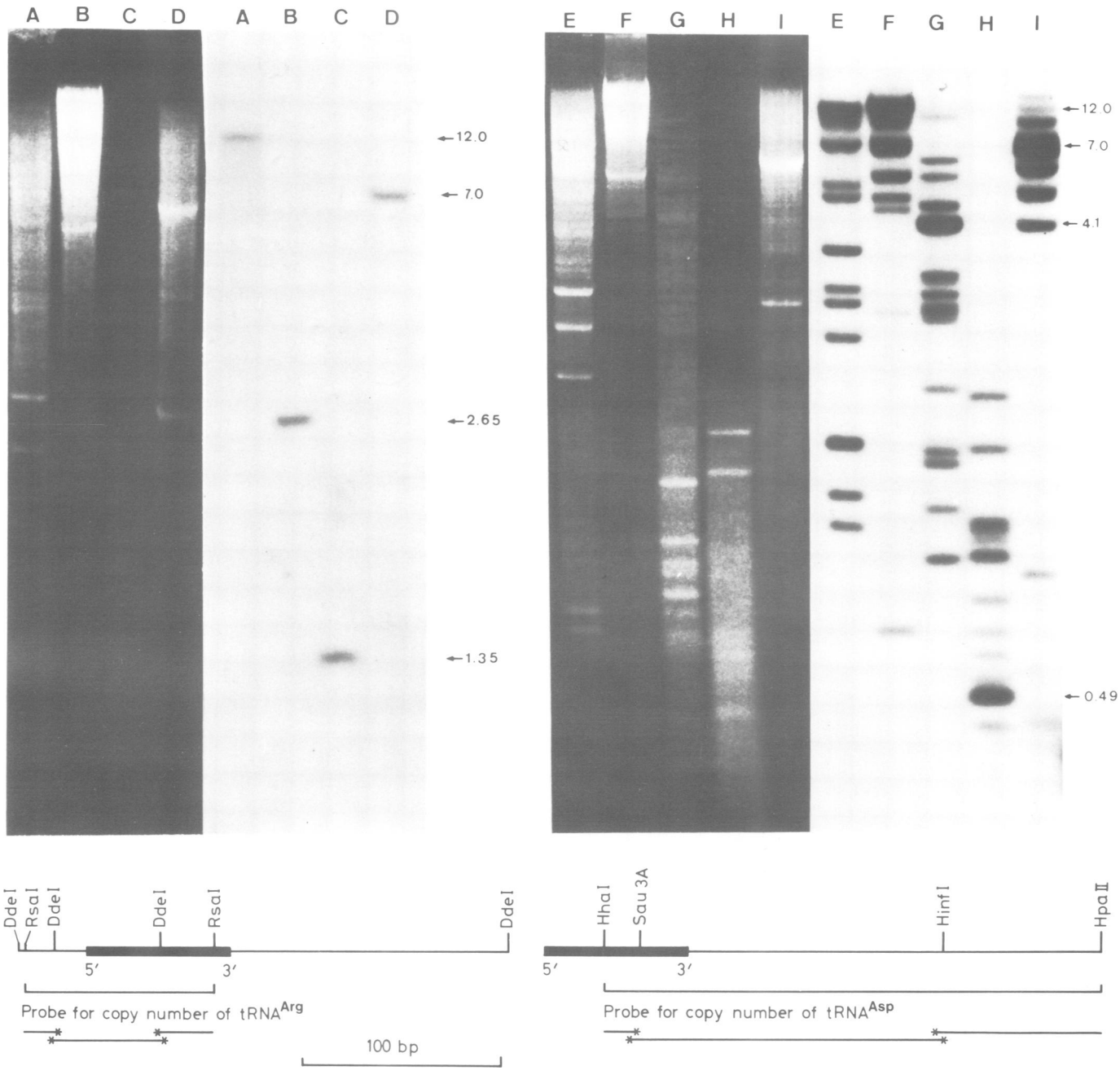

Fig. 5. Determination of tRNA gene copy numbers. The probe specific for AGGtRNA^{Arg} was hybridised against restriction patterns of S288C DNA obtained with **(A)** *Eco*RI, **(B)** *Xho*I, **(C)** *Hpa*II plus *Hha*I and **(D)** *Hind*III. The probe specific for GACtRNA^{Asp} was hybridised against restriction patterns of S288C DNA obtained with **(E)** *Eco*RI, **(F)** *Xho*I, **(G)** *Hpa*II, **(H)** *Hinf*I and **(I)** *Hind*III. The gels and autoradiograms are shown in the upper part. The scheme in the lower part explains the strategy for the preparation of the hybridisation probes. Due to the adjacent δ sequences, the choice of proper restriction fragments was limited. The thick lines are the tRNA genes. tRNA^{Asp} probe: the 251-bp *Hha*I-*Hpa*II fragment contains 42 bp of the 72 bp long GACtRNA^{Asp} gene. It was isolated and cleaved with *Hinf*I and *Sau*3A. The cohesive ends were filled in with Klenow polymerase in the presence of all four [α-^{32}P]deoxynucleotide triphosphates. Probably, only the 18 bases in the non-coding strand of the *Hha*I-*Sau*3A fragment was able to give hybridisation signals with other tRNA^{Asp} genes because the adjacent fragment starts with a snap-back structure of five G·C pairs (see cloverleaf model of Figure 4). This was probably the reason why a first hybridisation with a labelled *Sau*3A fragment failed to give any signals. tRNA^{Arg} probe: the 95-bp *Rsa*I fragment contains 64 bp of the 72 bp long AGGtRNA^{Arg} gene. It was isolated, cleaved with *Dde*I and the cohesive ends filled in with Klenow polymerase in the presence of α-^{32}P-labelled dATP and dTTP. The asterisks mark the labelled ends.

reflect this differences as determined in hybridisations using *in vivo* ^{32}P-labelled total yeast tRNA. It seems that the tRNA^{Arg} gene is even transcribed 2 to 4 times more strongly than an average GACtRNA^{Asp} gene (see Figure 6). The hybridisation of the oocyte transcription products from the injected clone with one copy of GACtRNA^{Asp} and one copy of AGGtRNA^{Arg} also revealed an excess of tRNA^{Arg} (see Figure 2). Unfortunately, these relative differences do not allow a comparison of the promoter strength of the same gene in yeast and in *Xenopus* oocytes because the transcription of the GACtRNA^{Asp} gene next to δ5 cannot be studied in yeast independently of the other homologous tRNA^{Asp} genes.

We think that the close vicinity of tRNA genes and δ sequences is worth discussing further, as this may be a general

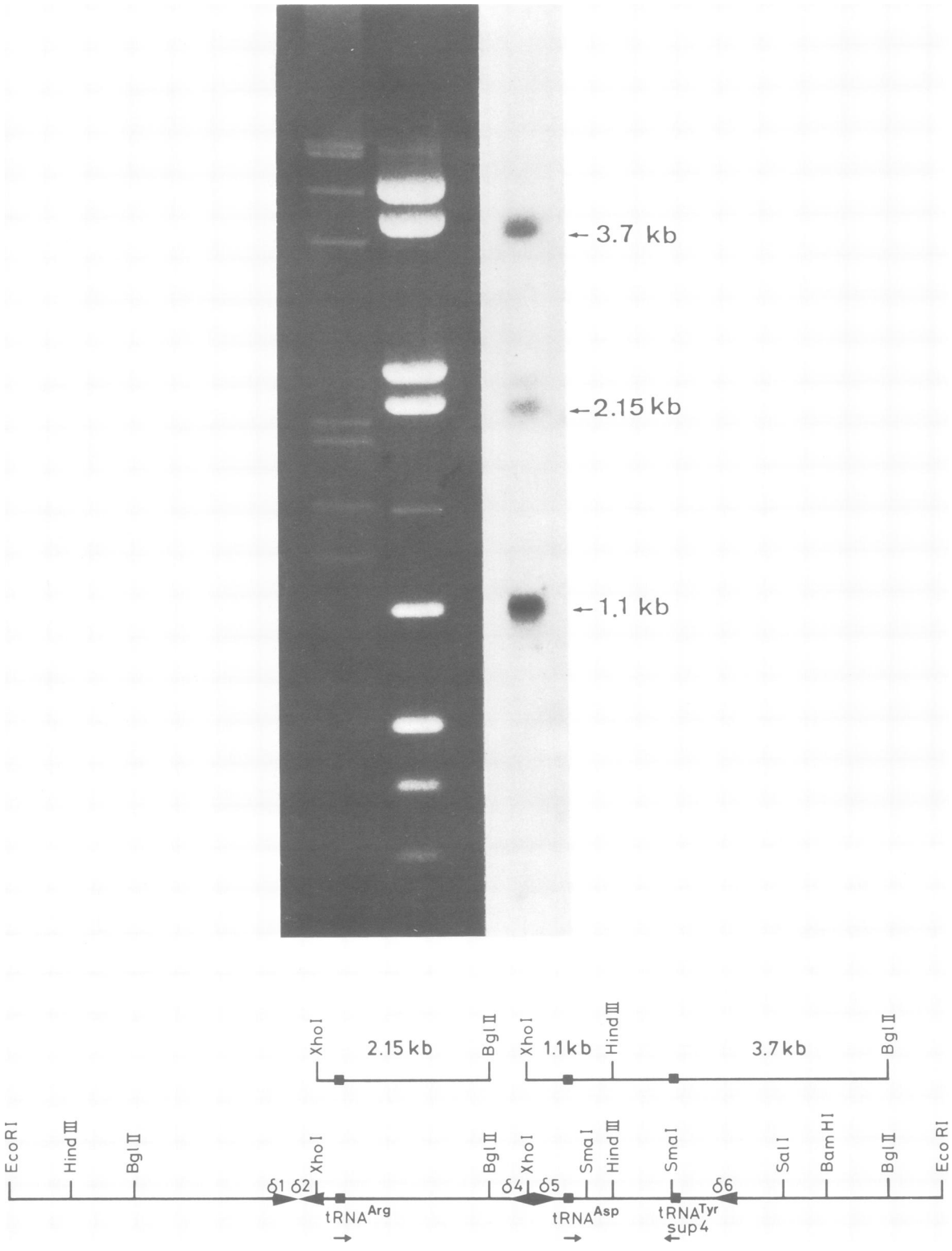

Fig. 6. Transcription studies with *in vivo* ^{32}P-labelled total tRNA of yeast. p18 DNA which carries the 12-kb *Eco*RI restriction fragment of the *sup4* region from S288C was totally cleaved with *Bgl*II, *Eco*RI, *Hind*III and *Xho*I (*Sal*I was also added, but it cleaved only very little). The fragments were separated on a 0.7% agarose gel, blotted to nitrocellulose and hybridised with purified ^{32}P-labelled total yeast tRNA. The gel and the autoradiogram are shown in the upper part and the map with the tRNA gene carrying fragments in the lower part. Thick arrows in the map represent δ sequences (Cameron *et al.*, 1979; Gafner and Philippsen, in preparation) and boxes tRNA genes, the transcription direction of which are indicated by thin arrows. The weak, unmarked signal in the autoradiogram is due to *Sal*I cleavage in a minor part of the 3.7-kb tRNA^{Tyr} fragment yielding this signal at 2.3 kb. The intense band in the gel at the same position is largely the *Bgl*II-*Xho*I fragment with δ1 and δ2. λ DNA cleaved with *Hind*III and *Eco*RI was used as size standard (**left lane**). When total yeast tRNA was labelled *in vitro* with ^{121}I according to Cory and Adams (1977) only the fragments with the tRNA^{Asp} and tRNA^{Tyr} gene gave strong hybridisation signals, most likely because the AGGtRNA^{Arg} is a bad substrate for iodination. This result was obtained with a ^{121}I-labelled probe kindly provided by H.Feldmann and with a probe prepared in our laboratory.

Table I. Mobile sequences adjacent to the 5′ end of tRNA genes

tRNA gene	Distance between the element and the 5′ end of the coding region	Type of element in the 5′ non-coding region	Chromosomal location	References
$tRNA^{Asp}$	80 bp	Solo δ flanked by 5 bp duplication	Chromosome X R	Gafner, 1982
$tRNA^{Ser}$	82 bp	Solo δ, maybe at the ends of a TY	Unknown	Eigel and Feldmann, 1982
$tRNA^{Glu}$	88 bp	Solo δ not flanked by 5 bp duplication	Unknown	Eigel and Feldman, 1982
$tRNA^{Leu}$	89 bp	Ty	Chromosome III L	Andreadis *et al.*, 1982
$tRNA^{His}$	90 bp (430 bp)[a]	Solo δ not flanked by 5 bp duplication	Unknown	Fink, personal communication
$tRNA^{Arg}$	120 bp	Solo δ flanked by 5 bp duplication	Chromosome X R	Gafner, 1982
$tRNA^{His}$	167 bp (507 bp)[a]	Solo δ not flanked by 5 bp duplication	Unknown	Fink, personal communication
$tRNA^{Glu}$	200 bp (approx.)[b]	Ty	Unknown	Eigel and Feldmann, 1982
$tRNA^{Glu}$	201 bp	Ty	Unknown	Eigel and Feldmann, 1982
$tRNA^{Gln}$	311 bp	Solo δ flanked by 5 bp duplication	Chromosome VIII R	Tschumper and Carbon, 1982
$tRNA^{Tyr}$	405 bp	Solo δ flanked by 5 bp duplication with one mismatch	Chromosome X R	Gafner, 1982

[a]The distances given in parentheses (430 bp and 507 bp) also include a σ element which seems to be, in both cases, a recent insertion.
[b]Only determined by restriction digests and not by DNA sequence analysis as in all other cases.

phenomenon in yeast. δ sequences are present in the 5′ non-coding region of both the two new tRNA genes (Figure 3) and of the known nearby $tRNA^{Tyr}$ gene (Gafner and Philippsen, in preparation) with the following distances to the first tRNA coding nucleotide: 120 bp for the AGGtRNA^{Arg} gene, 80 bp for the GACtRNA^{Asp} gene and 405 bp for the UACtRNA^{Tyr} gene. The average δ-tRNA gene distance should be of the order of 30 kb for the ~100 δ sequences (Cameron *et al.*, 1979; Stotz and Philippsen, unpublished data) and 300 tRNA genes (Schweizer *et al.*, 1969; Feldmann, 1976) in the 10^7 bp of the haploid *S. cerevisiae* genome. The close sequence arrangement of tRNA genes and δ sequences is therefore remarkable and deserves an explanation.

The δ2, δ5 and δ6 sequence adjacent to the $tRNA^{Arg}$, $tRNA^{Asp}$ and $tRNA^{Tyr}$ gene, respectively, are flanked by a 5 bp duplication, in the case of δ6 with one mismatch (Gafner and Philippsen, in preparation). Such solo δ sequences most likely signal a previous Ty transposition event followed by a recombination between the terminal direct δ repeats of a Ty element, which leaves a solo δ sequence in the chromosomal DNA (Farabaugh and Fink, 1980; Gafner and Philippsen, 1980). Strain SL403-4D, which is known for the frequent deletions in the *sup4* region, carries two Ty elements in the *sup4* region, one at the position of δ2 and the other at the position of, or close to, δ6 (Liebman *et al.*, 1981; S.Picologlou and S.Liebman, personal communication). We assume, therefore, that Ty elements initially jumped into the 5′ non-coding region of the three tRNA genes.

These selections of target sites 5′ to transcribed sequences are not unique cases for Ty transpositions. Ty elements have been found in the 5′ non-coding region of the protein-coding genes *HIS4, CYC7* and *ADH2* with distances to the first transcribed nucleotide ranging from 8 to 192 bp (Farabaugh and Fink, 1980; Roeder *et al.*, 1980; Errede *et al.* 1980a, 1980b; Montgomery *et al.* 1982; Williamson *et al.* 1981). In a study which focussed on the target site selection of Ty elements, Eibel and Philippsen (in preparation) were able to show that Ty elements select preferentially or even exclusively the 5′ non-coding region as transposition target; five from 300 *lys2* mutants isolated with a non-biased mutant selection scheme had a Ty insertion and all five in the 5′ non-coding region of the *LYS2* gene. Sequences in front of RNA polymerase II and III transcribed genes seem therefore to be preferred landing sites for Ty elements.

Several recently published DNA sequences around a number of tRNA genes in *S. cerevisiae* reinforce this hypothesis. Eigel and Feldmann (1982) described one RNA^{Ser} and three $tRNA^{Glu}$ genes with δ or Ty elements 82–201 bp upstream from the genes 5′ end. Andreadis *et al.* (1982) found a Ty element 82 bp upstream from a $tRNA^{Leu}$ gene. Tschumper and Carbon (1982) identified a δ sequence 311 bp upstream from a $tRNA^{Gln}$ gene. Two $tRNA^{His}$ genes have δ sequences in their 5′ non-coding region (G.Fink, personal communication). No preference for either of the two possible orientations of Ty or solo δ sequences was observed. The data are summarized in Table I. Since Ty elements have several structural features in common with mobile elements in other eukaryotes (Temin, 1980; Eibel *et al.*, 1980), it would not be surprising if similar arrangements between transposable sequences and genes are found in many eukaryotes.

Another mobile sequence in *S. cerevisiae*, called σ, loves 5′ regions of tRNA genes, but shows more precision in the site selection as compared with Ty, since it inserts either 16 or 18 bp upstream of the first coding nucleotide and sometimes between an existing δ and a tRNA gene (Del Rey *et al.*, 1982; Eigel and Feldmann, 1982; Sandmeyer and Olson, 1982). 5′ Non-coding regions may be in general preferred landing sites for mobile sequences, because of their special DNA or chromatin structure. 3′ Non-coding regions of genes as landing sites for Ty or σ cannot be completely excluded although no case has been reported yet.

The finding of mobile repetitive elements in the 5′ non-coding region of tRNA genes raises the question of whether insertions into these regions have any function. The effects of Ty insertions into the 5′ non-coding region of structural genes on gene expression have been characterized and can lead to different alterations. There are cases reported where a wild-type activity is lost completely or is much reduced (Chaleff and Fink, 1980; Roeder *et al.*, 1980; Eibel and Philippsen, in preparation). A regulated gene expression can turn into a constitutive or overproducing gene expression (Williamson *et al.*, 1981; Errede *et al.*, 1980a, 1980b; Montgomery *et al.*, 1982). Mutants can be isolated from some of these Ty insertions, which have the internal part and a δ sequence of the Ty element excised, leaving behind a solo δ sequence. These δ insertion mutants are either cold sensitive or

lack expression (Chaleff and Fink, 1980; Roeder and Fink, 1980; Ciriacy and Williamson, 1981; Eibel and Philippsen, in preparation).

Do δ sequences adjacent to tRNA genes hinder their expression? At least for the AGGtRNA^{Arg} gene we could demonstrate that the δ2 sequence starting 120 bp adjacent to the 5′ end does not drastically hinder or reduce the synthesis of tRNA^{Arg}. In fact, it seems that this is a very active tRNA in yeast. At present there are insufficient data to draw conclusions about the influence of insertion sequences in the 5′ non-coding regions of tRNA genes. It is known, however, that the 5′-flanking region is in some cases important in controlling the rate of transcription of tRNA genes (De Franco *et al.*, 1980). A detailed study on the effect of δ sequences *in vivo* in yeast would need a single copy tRNA gene such as AGGtRNA^{Arg} with and without insertion elements in the 5′ non-coding region.

Materials and methods

Strains and media

Escherichia coli K12 strains HB101 (*hsdR$^-$hsdM$^-$ recA13 supE44 (su2$^+$) lacZ4 leuB6 pro A2 thi-1 (B1$^-$) SmR)* was obtained from R.W.Davis (Davis *et al.*, 1980) and used for all plasmid constructions. The DNA of the original clones of the *sup4* region from *S. cerevisiae* S288C carrying a 12-kb *Eco*RI restriction fragment in λ (λgt-ScS1) or in pBR322 (p18) was kindly provided by J.R.Cameron and described by Cameron *et al.* (1979). H13 is the 7-kb *Hind*III restriction fragment from the λgt hybrid subcloned into the *Hind*III site of pBR322. S288C is a widely used *S. cerevisiae* laboratory strain and most likely isogenic with the type strain X2180-1A of the Yeast Genetic Stock Center at the University of California, Berkeley.

Nucleic acids, enzymes and chemicals

Plasmid vector DNA was isolated according to established procedures described by Davis *et al.* (1980). Restriction fragments from λcI857 and pBR322 DNA were used as size standards in gel electrophoresis (Philippsen *et al.*, 1978; Sutcliffe, 1979).

Nucleic acids smaller than 600 bp were eluted from polyacrylamide gel slices by electroelution as described by Smith (1980). DNA >600 bp was isolated from low melting agarose gels. The gel slice containing the desired DNA restriction fragment was cut out from the gel with as little excess of DNA-free agarose as possible and put into an Eppendorf tube. The gel slice (0.3 – 1.0 ml) was melted in the tube in the presence of 400 μl phenol pH 7 at 65°C until the mixture was homogeneous and white (5 – 10 min). The phenol phase was separated from the water phase by centrifugation in an Eppendorf centrifuge at room temperature and the upper water phase with the DNA re-extracted several times with phenol until the two phases were clear. The DNA was precipitated after addition of one tenth volume of 5 M NaCl with 2 volumes −20°C ethanol and the precipitate washed several times with 70% ethanol before drying under vacuum. The DNA yield of this procedure is higher than 90% as determined with radioactively labelled restriction fragments ranging in size from 21 kb to 0.5 kb. This DNA was used for restriction enzyme digestions, ligations, transformations and DNA sequencing.

The *in vivo* labelling of yeast cells with ^{32}P and the isolation of total yeast tRNA was essentially as described by Rubin (1975). Restriction endonucleases, T4 DNA ligase, Klenow polymerase, were purchased from Bethesda Research Laboratories, Biolabs or Boehringer Mannheim. [^{32}P]-, [^{121}I]- and [α-^{32}P]dNTPs were obtained from Amersham.

DNA sequence analysis

The DNA sequence was determined using the method developed by Maxam and Gilbert (1980). Restriction fragments were 3′ end-labelled with the Klenow fragment of DNA polymerase I and appropriate [α-^{32}P]deoxynucleotide triphosphates. If necessary, the fragments were cleaved with another restriction enzyme in order to obtain DNA fragments labelled only at one end. After the chemical degradation, the mixtures were separated on 8% and 18% denaturing polyacrylamide gels, 40 cm long, 30 cm wide and 0.4 mm thick.

Transfer of DNA to nitrocellulose and hybridisations with radioactively labelled DNA or RNA

The transfer of restriction fragments separated on agarose gels onto nitrocellulose paper and the hybridisation with radioactively labelled DNA or RNA was performed as described by Southern (1975) and Maniatis *et al.* (1982) with minor modifications. We used SSPE-buffer during the transfer and hybridisations (1 x SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, 1 mM Na_2 EDTA, pH 7.0). We omitted a prehybridisation or a hybridisation in the presence of Denhardt's solution. For the determination of the copy number for tRNA genes, the hybridisations (DNA to DNA) were performed in 3 x SSPE 0.2% SDS at 60°C overnight. Afterwards, the nitrocellulose papers were washed once for 30 min at 52°C in 3 x SSPE, 0.2% SDS twice for 15 min in 1 x SSPE at room temperature, dried and exposed to X-ray film. When tRNA was used as probe, the hybridisations were performed in 3 x SSPE, 50% formamide at 42°C overnight. The nitrocellulose papers were afterwards washed once in 3 x SSPE, 50% formamide for 30 min at 42°C, twice in 1 x SSPE for 5 min at room temperature, dried and exposed to X-ray film.

Oocyte microinjection

Fully grown *Xenopus* oocytes were microinjected into the nucleus with 30 nl of recombinant DNA plasmids at ~200 μg/ml and 10 mCi/ml of [α-^{32}P]GTP (400 Ci/mmol, Amersham). After incubation at 19°C for 24 h, RNA was extracted and analyzed in 8% polyacrylamide-7 M urea gels as described previously (De Robertis and Olson, 1979; Nishikura *et al.*, 1982).

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