# Nucleotide sequence of a yeast $tRNA_{3A}^{Arg}$ gene and its transcription in a homologous in vitro system

Jorge Villanueva, Paulina Bull, Pablo Valenzuela and Alejandro Venegas*

*Laboratorio de Bioquímica, Dpto. Biología Celular, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile*

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Twelve bacterial clones containing complementary sequences to yeast $tRNA_3^{Arg}$ were isolated from a gene library. The size of the yeast *Bam*HI inserts ranges from 5.4 to 10 MDa. There are at least 6 copies of this gene in different loci of the yeast genome. Insert from clone pYAT-3 was mapped, and the presence of a $tRNA_{3A}^{Arg}$ gene was confirmed by DNA sequence. The coding region is colinear with the transcriptional product. Unlike other reported $tRNA_{3A}^{Arg}$ genes, this one is not linked to a $tRNA^{Asp}$ gene. In vitro transcription using a yeast extract produces a transcript of 76 ± 1 bases.

*Yeast $tRNA^{Arg}$ gene* *DNA sequence* *Scattered gene arrangement* *Homologous in vitro transcription*

## 1. INTRODUCTION

The use of recombinant DNA techniques has provided knowledge of the organization and structure of eukaryotic genes, a fundamental step in elucidating the mechanisms involved in gene expression and regulation, which are at least in part governed by specific sequences in the DNA.

The yeast tRNA genes have been attractive candidates for molecular analysis because they are relatively small and therefore easy to analyze, the gene products have been well characterized and many of them sequenced [1]. Molecular analysis of the tRNA genes and 5 S rRNA gene have allowed a direct comparison of the promoter and termination regions transcribed by yeast RNA polymerase III. In contrast to unique intragenic control region for the 5 S gene [2,3], an intragenic split promoter has been proposed for tRNA genes [4]. The termination mechanism seems to be similar in both cases, since it has been shown that transcription is halted in front of 4 T's for 5 S gene [5] and 5 in the case of a tRNA gene [6].

* To whom correspondence should be addressed

Some tRNA genes from yeast such as those coding for $tRNA^{Phe}$ [7,8], $tRNA_3^{Leu}$ [9], $tRNA^{Tyr}$ [10], $tRNA^{Ser}$ [11] and $tRNA^{Trp}$ [12] have been reported to contain intervening sequences or introns. These introns have turned out to be a common feature of most but not all eukaryotic genes [13]. Here, we analyze a $tRNA_{3A}^{Arg}$ gene (clone pYAT-3) from *S. cerevisiae*. It contains no intervening sequence and its coding region is identical to the two other reported $tRNA_{3A}^{Arg}$ genes [14], which are linked by a 10 base-spacer to a $tRNA^{Asp}$ gene. In our case, pYAT-3 has no such linkage.

## 2. MATERIALS AND METHODS

The library of cloned yeast tRNA genes used to isolate the $tRNA_3^{Arg}$ genes was obtained as described in [8]. Plasmid DNA was purified as in [15]. The clones containing hybrid plasmids were screened for the presence of sequences complementary to $tRNA_3^{Arg}$ by the colony hybridization technique [16]. The $tRNA_3^{Arg}$ was labeled with $Na^{125}I$ as in [17]. Restriction endonucleases, from Bethesda Research Laboratories or New England Biolabs, were assayed as indicated by the suppliers.

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Analytical and preparative gel electrophoresis in either agarose or acrylamide gels were carried out as in [18]. For hybridization, the restriction fragments were transferred to a nitrocellulose filter (Millipore) as in [19]. The filters were incubated with [$^{125}$I]tRNA$_3^{Arg}$ and processed as in [18]. DNA was end labeled with [$\gamma$-$^{32}$P]ATP (ICN) and polynucleotide kinase and sequenced as in [20]. In vitro transcription experiments were carried out as in [21,22]. Yeast extract was the kind gift of Dr P.A. Weil.

## 3. RESULTS AND DISCUSSION

### 3.1. *Isolation of the bacterial clones carrying yeast tRNA$_3^{Arg}$ genes*

A previously described yeast tRNA bank cloned in pBR315 consisting of 400 colonies [8] was screened by hybridization to [$^{125}$I]tRNA$_3^{Arg}$. Nineteen positive bacterial clones were detected and named pYAT-1 to pYAT-19. None of them hybridized against yeast tRNA$^{Leu}$, tRNA$^{Phe}$, tRNA$^{Trp}$, tRNA$^{Met}$ or tRNA$^{Ser}$. This suggests a non-clustered organization for the genes described here. Further characterization of these clones by gel electrophoresis of the *Bam*HI fragments and Southern hybridization with [$^{125}$I]tRNA$_3^{Arg}$ reduced this number to twelve. These results are in fig.1. The size of the positive fragments ranged from 5.4 to 10 MDa. Nine of these clones contained only one yeast insert, the other 3 contained 2 inserts. In order to determine the actual number of different tRNA$_3^{Arg}$ genes present in the yeast genome, the *Bam*HI fragments were treated with a second endonuclease, *Eco*RI or *Hin*dIII and analyzed by agarose gel electrophoresis (not shown). Only 6 copies of this gene occurring at different loci were cloned. The sizes, in MDa, of the *Bam*HI fragments for these 6 different copies were: 10 (clone 17), 7.3 (clone 12), 7.3 (clone 2), 6 (clones 14, 15 and 19), 5.7 (clones 1, 13 and 18), 5.4 (clones 3, 11 and 16). These results suggest a dispersed organization for these genes.

### 3.2. *Physical map and sequence analysis of one yeast tRNA$_{3A}^{Arg}$ gene*

We selected plasmid pYAT-3 for further analysis because it contained a small positive fragment (5.4 MDa). By analytical digestion with *Eco*RI followed by Southern hybridization, a fragment of 2.5 MDa containing a sequence complementary to tRNA$_3^{Arg}$ was detected. This frag-

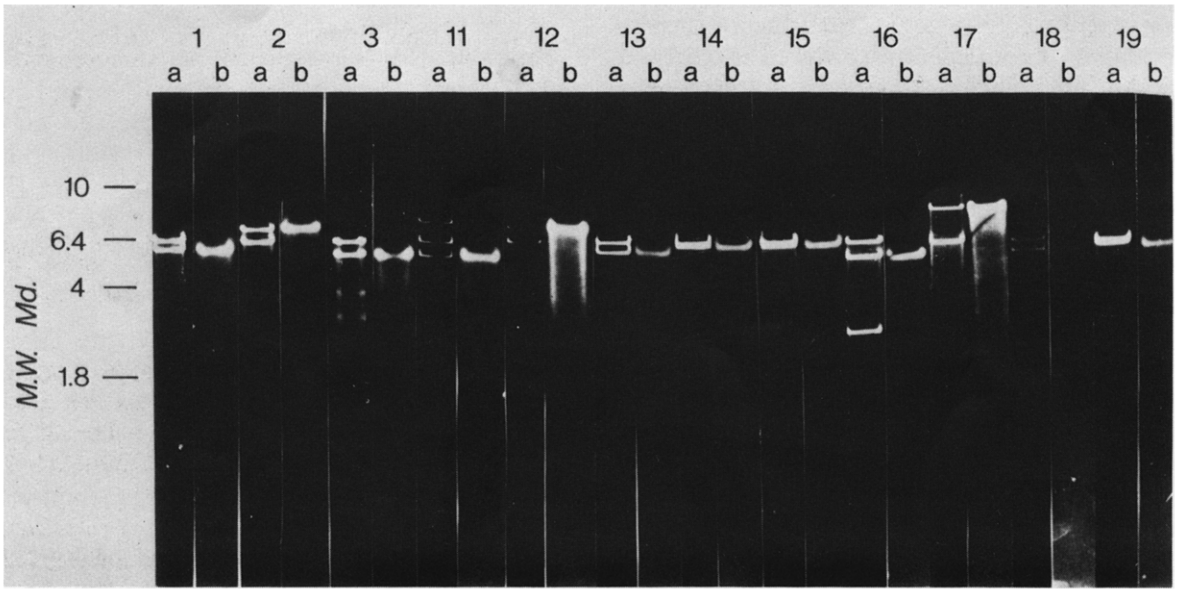


Fig.1. Agarose gel electrophoresis of hybrid plasmids digested with *Bam*HI and hybridization to yeast [$^{125}$I]tRNA$_3^{Arg}$. DNA fragments were resolved by electrophoresis in 0.8% agarose. (a) Ethidium bromide staining pattern. (b) Autoradiogram of hybridization with yeast [$^{125}$I]tRNA$_3^{Arg}$.

ment was mapped using several restriction enzymes and contained at one end a 400 bp region corresponding to the vector pBR315. The results are in fig.2a. The $tRNA_3^{Arg}$ gene was located in an *AluI–AluI* fragment of 380 bp by hybridization experiments (not shown). This fragment contains almost all the gene except the first base. An *AvaI–AluI* fragment of 550 bp is enlarged in fig.2b to indicate the restriction sites used for the sequencing strategy (fig.2c). A region of 643 bp was sequenced and is shown in fig.3. The gene is located between positions +1 and +72 (underlined).

This gene has no intervening sequence, and the 3′-terminal CCA is not coded for by the DNA, like two other yeast $tRNA_{3A}^{Arg}$ described in [14]. The 5′-flanking region is AT-rich, similar to other 5′-ends of tRNA genes [7,9,23]. A sequence similar to that described as ACT-TA box [24] was found centered at position −10 (underlined). The gene finishes in a long cluster of T's, interrupted only in 4 positions, which suggests an immediately contiguous termination signal for this gene.

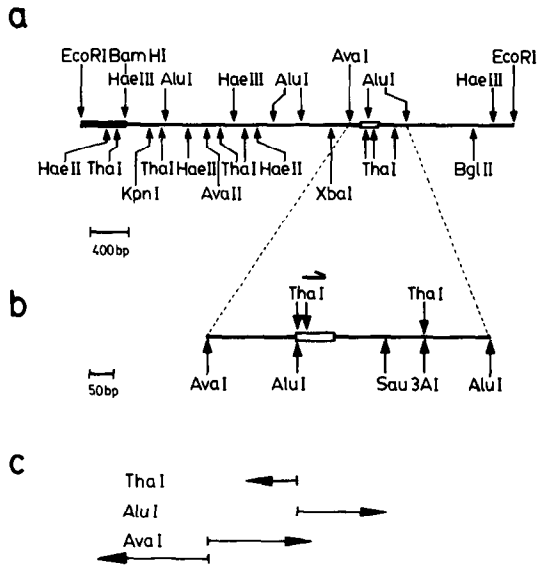


Fig.2. Restriction endonuclease map of the fragment of pYAT-3 containing a $tRNA_3^{Arg}$ gene. The yeast $tRNA_3^{Arg}$ gene and the pBR315 region are shown by an empty and a filled box, respectively. Transcription direction is to the right. (b) Enlargement of the *AvaI–AluI* fragment containing the tRNA gene. (c) Summary of the sequence strategy indicating the enzymes used.


Fig.3. Nucleotide sequence of yeast $tRNA_{3A}^{Arg}$ gene and flanking regions. The gene (underlined) starts at position +1. A putative ACT-TA box is also underlined.

Analysis of this sequenced region by a computer program for searching tRNA sequences [25] shows that only this tRNA gene is present. This differs from the case reported in [14] in which a $tRNA^{Asp}$ gene was found linked to the 3′-end of the $tRNA_{3A}^{Arg}$ gene by a 10 base-spacer. Interestingly, this spacer is identical to the 3′-flanking sequence of pYAT-3. Moreover, the sequence identity continues after the $tRNA^{Asp}$ gene for a few bases. This distribution suggests two possibilities about a more ancient arrangement of the genes:

(i) The $tRNA^{Asp}$ gene became inserted into the 3′-flanking sequence of a $tRNA^{Arg}$ gene at position +82. This would imply that the region cloned in [14] represents a more recent arrangement;

(ii) Originally the yeast tRNA genes were strongly clustered, and later the genome was expanded producing the present scattered organization with the exception of a few linked genes such as those reported in [14]. In the case of pYAT-3, the $tRNA^{Asp}$ gene would have been precisely deleted.

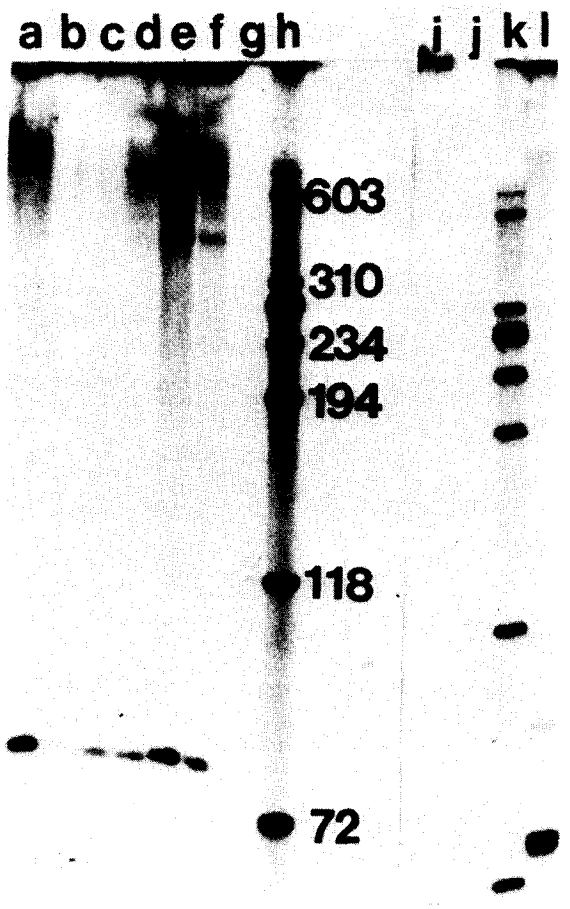


Fig.4. In vitro transcription of pYAT-3 using a yeast extract. Autoradiogram of a 7% acrylamide-8 M urea gel of transcription products. Lanes a, e, f, 2, 20 and 40 μg/ml of pYAT-3. Lane i, 6.8 μg/ml of pBR315. Lane l, 20 μg/ml of pYPT-6. Incubations done at 25°C for 1 h. Lanes b–d, 15, 30 and 60 min incubation of 10 μg/ml pYAT-3. Lanes g, j, no DNA added. Lanes h, k, φX174-*Hae*III standard. Numbers indicate fragment sizes in base pairs.

### 3.3. *In vitro transcription experiments*

When performing a transcription assay with a yeast extract [21], the synthesis of an RNA band of 76 ± 1 bases was obtained, which increased linearly when incubating for longer time periods (fig.4, lanes b–d). No high $M_r$ precursor, nor processing of the transcript at the times analyzed, was observed. Obviously, the long cluster of T's is working as a transcription termination signal, in opposition to that found for the linked tRNA genes, which are transcribed as a large precursor [14]. This is not surprising, since in their case the spacer does not contain a stretch of 4 consecutive T's. There is an optimal DNA concentration at 2 μg/ml (fig.4, lane a), a value similar to that found for other yeast tRNA genes (unpublished).

This gene has a lower in vitro transcriptional efficiency when compared to other yeast tRNA genes, such as some $tRNA^{Phe}$ genes (unpublished). For instance, transcription of clone pYPT-6, carrying a $tRNA^{Phe}$ gene, is shown in fig.4 lane 1. pBR315 DNA is included in lane i as a control.

Clone pYAT-3 was also assayed using a HeLa extract [22], and the product obtained was of the same size (not shown), suggesting that both transcription systems recognize the same starting and stopping signals in this case. We have evidence that this does not always hold true (unpublished).

Our results, together with others [26], strengthen the concept that different arrangements and expression levels may be found for a given gene family in yeast.

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