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Isolation and structure of a yeast initiator $tRNA^{met}$ gene

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ABSTRACT

Sixteen bacterial clones containing yeast initiator $tRNA^{met}$ genes have been isolated. The size of the BamHI fragments encoding these genes ranges from 4,000 to 23,000 base pairs. The nucleotide sequence of one member of this group has been determined. It has no intervening sequences.

INTRODUCTION

In yeast, with a few exceptions intervening sequences have only been found in genes of tRNAs specific for non-polar amino acids. For instance, chromosomal genes coding for $tRNA^{phe}$, $tRNA^{tyr}$, $tRNA_3^{leu}$, $tRNA^{trp}$ and $tRNA^{Ser}_{UCG}$ contain intervening sequences (1-5) and others like those for elongator $tRNA^{met}$ (6), $tRNA^{arg}$, $tRNA^{asp}$ (7) and $tRNA^{glu}$ (8) do not. Here we describe the isolation of 16 clones containing putative yeast initiator $tRNA^{met}$ genes and the nucleotide sequence of one member of the group. From DNA sequence analysis we find that this gene has no intron. The sequence allows us to solve two base ambiguities and to establish a difference of one base respect to the reported tRNA sequence (9).

MATERIALS AND METHODS

The yeast tRNA gene bank was isolated from a yeast gene library as described previously (10). The yeast tRNA gene bank (400 clones) was screened for the presence of sequences complementary to $tRNA_I^{met}$ by colony hybridization (11). Purified $tRNA_I^{met}$, kindly provided by Dr. S.Gilham, was labeled with $Na^{125}I$ according to the procedure of Tereba and McCarthy (12). Plasmid DNA was isolated and purified by the method of Meagher et al. (13). Analytical and preparative gel electrophoresis in either acrylamide or agarose gels were carried out as described elsewhere (14). Restriction endonucleases were obtained from New England BioLabs or from Bethesda Research Laboratories and assayed as indicated by suppliers. For hybridization, DNA fragments resolved in agarose gels were transferred to nitrocellulose filters (Millipore) as described by Southern (15). The filters were incubated with ^{125}I-or ^{32}P-labeled $tRNA_I^{met}$ and processed as described

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previously (14). For DNA sequencing, appropriate restriction fragments were isolated from gels by electroelution, labeled with ^{32}P-γ-ATP (ICN) and polynucleotide kinase (P.L.Biochemicals) and processed according to the method of Maxam and Gilbert (16).

RESULTS AND DISCUSSION

Isolation of Yeast $tRNA_I^{met}$ Genes

Of the 400 colonies composing the yeast tRNA gene bank (10), approximately 40 gave positive signals when their DNA was hybridized to ^{125}I-$tRNA_I^{met}$. This result suggests that multiple copies of this gene exist in the yeast genome. In order to measure the size of these yeast fragments, plasmid DNA was isolated from these clones and digested with BamHI endonuclease, separated by gel electrophoresi and hybridized to labeled $tRNA_I^{met}$. Twenty-three out of 40 colonies gave positive signals. An analysis based only on the size of the BamHI positive fragments reduced to 13 the number of probable different clones. The sizes found were: 23, 17, 13.5, 11.5, 10.5, 9.8, 8.6, 8.4, 8.0, 5.4, 5.0, 4.7 and 4.0 kilobase pairs (results not shown). A similar number, about 12 $tRNA_I^{met}$ genes, has been reported in human (17).

Physical Map and DNA Sequence of a Yeast $tRNA_I^{met}$ Gene

In order to perform a detailed analysis of a $tRNA_I^{met}$ gene, the hybrid plasmid pYMT-5 which gave the strongest hybridization signal and contained one of the smaller yeast inserts (5000 bp) was chosen. The yeast fragment was subjected to digestion with several restriction enzymes. The DNA fragments were subsequently ordered by partial and double digestions and ^{32}P-end labelling (results not shown) and the physical map deduced is presented in Figure 1. Also, the sequence strategy is shown by arrows. The sequence experiment was done twice and the results fully confirmed. The gene sequence is displayed in Figure 2. Like other $tRNA^{met}$ genes (6,17), the DNA is colinear and agrees with the nucleotide sequence of $tRNA_I^{met}$ reported by Simsek *et al.* (9), as shown in line b of Figure 2, except for the first nucleotide of the coding sequence. A guanine instead of an adenine was found in the DNA. This change does not alter the structure at the amino acid stem because G-U pairing is also possible. As in the other yeast tRNA genes (1-5), the CCA at the 3' end of the tRNA is not coded for by the DNA.

The region before the gene is very AT-rich. No palindromes are found in the putative promoter regions. Some direct repeated sequences (boxed in line a of Fig. 2) of unknown function exist. A particular sequence of 9 base pairs at the 5' flanking region is repeated inside the coding region, near the beginning

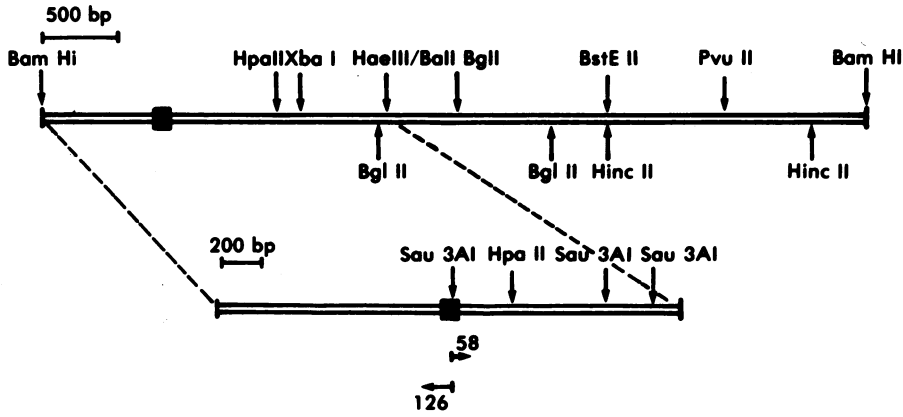

FIGURE 1. Restriction endonuclease map of the yeast DNA fragment of pYMT-5 which contains a $tRNA_I^{met}$ gene. The endonuclease cleavage sites were determined by combined enzyme digestions, analysis of the size of the resulting DNA fragments by agarose or acrylamide gel electrophoresis and hybridization to ^{32}P-$tRNA_I^{met}$ data. The yeast $tRNA_I^{met}$ gene is shown by a filled box. The arrows indicate the base pairs sequenced in each direction.

of the gene (underlined in line a and d of Fig. 2). Other repeated sequences occurring at similar positions have been observed in other tRNA genes. Curiously the first nucleotide transcribed is always contained within the first repeat (17). We are currently carrying out in vitro transcription experiments

a. ...TTGTCATAGTTGAATAAAATAGTAGAATAAAAACATTCATATATCTACGTGCAAGCGTCAGATTGTAATGTT...
...AACAGTATCAACTTATTTTATCATCTTATTTTTGTAAGTATATAGATGCACGTTCGCAGTCTAACATTACAA...

m^{1}_{m} m^{2}_{2} t^{6} $m^{7}m^{55}$ m^{1}

b. 5'p-AGCGCCGUGGCGCAGUGGAAGCGCGCAGGGCUCAUAACCCUGAUGDCCUCGGAUCGAAACCGAGCGGCGCUACCA-3'OH

c. AG GCG AGACT AG A CG GTTCGA C C G CA

d. ...GGCGCCGTGGCGCAGTGGAAGCGCGCAGGGCTCATAACCCTGATGTCCTCGGATCGAAACCGAGCGGCGCTA....
...CCGCGGCACCGCGTCACCTTCGCGCGTCCCGAGTATTGGGACTACAGGAGCCTAGCTTTGGCTCGCCGCGAT....

e. ...ATAAATTTTTTGAACTGTCCATTAATAATAATGGAAC...
...TATTTAAAAAACTTGACAGGTAATTATTATTACCTTG...

FIGURE 2. Nucleotide sequence of yeast $tRNA_I^{met}$, cloned $tRNA_I^{met}$ gene and flanking regions. The results are depicted in three blocks of DNA sequences coding for a)the region flanking the 5' end, d) the structural gene, and e) that flanking the 3' end of the tRNA gene, respectively. The sequence of yeast $tRNA_I^{met}$ (9) us shown in b) and the anticodon is underlined. The consensus sequence proposed by Koski (19) as intragenic promoter for tRNA genes is shown in c). Vertical arrows indicate the base coincidence.

with a HeLa cell transcription system (18) to test if this applies in the present case. Preliminary data indicate that a 88±1 base precursor is transcribed in this system. At the 3' flanking region a tract of six consecutive T's is present in the non-coding strand which is believed to represent a terminator signal (19, 20).

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