

The genes for U6 small nuclear RNA in *Tetrahymena thermophila* are repeated in tandem

Tokio Tani and Yasumi Ohshima

Department of Biology, Faculty of Science, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812, Japan

Submitted March 25, 1991

EMBL accession no. X53790

U6 RNA is the most highly conserved spliceosomal RNA involved in pre-mRNA splicing (1). The U6 RNA gene of fission yeast *Schizosaccharomyces pombe* was found to have an mRNA-type intron (2). A consideration of the origin of the U6 intron led to interesting possibility of a catalytic role of U6 RNA in pre-mRNA splicing (3, 4). To investigate whether there are U6 RNA genes which have an intron at the same site as the *S. pombe* U6 RNA gene does, we analyzed U6 RNA genes of 52 organisms by the polymerase chain reaction using primers corresponding to highly conserved regions of U6 RNA (4). In the course of this analysis, we observed two amplified products from the DNAs of *Tetrahymena thermophila*, horseshoe crab and *Drosophila melanogaster*. The size of the smaller amplified product (52 bp) detected in each DNA was identical to that of the amplified product from the U6 RNA gene with no intron (4).

Here, we report the complete nucleotide sequence of the larger amplified product (418 bp) observed in *Tetrahymena* DNA. The larger product contains two U6 RNA genes 264 bp apart from each other, showing that this amplified product was produced due to the tandem repetition of the genes. In a spacer region between two *Tetrahymena* U6 RNA genes, a TATA box-like sequence is found 22 bp upstream of the downstream U6 gene.

ACKNOWLEDGMENTS

We thank Dr. Y. Watanabe, University of Tsukuba, for providing the genomic DNA of *Tetrahymena thermophila*. This research was supported by grants from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1. Brow, D.A. and Guthrie, C. (1988) *Nature* **334**, 213–218.
2. Tani, T. and Ohshima, Y. (1989) *Nature* **337**, 87–90.
3. Brow, D.A. and Guthrie, C. (1989) *Nature* **337**, 14–15.
4. Tani, T. and Ohshima, Y. (1991) *Genes Dev.* in press.
5. Szukálek, A., Kiss, T. and Solymosy, F. (1990) *Nucl. Acids Res.* **18**, 1295.

```

      10      20      30      40      50      60
AAATTGAAAC GATACAGAGA AGATTAGCAT GGCCCTGCA CAAGGATGAC ACGCTCAAAG
***** ** ***** ***** ***** ***** **
AAAUUGGAAC GAUACAGAGA AGAUUAGCAU GGCCCCUGCG CAAGGAUGAC ACGCACAAU
      70      80      90      100     110     120
CAGAAGGATT CCCCATTTT TTTTCTAATT TTTTATAATT AGAATAAAAA AGAAATTAC
* * * * *
CGAGAAUUGG UCCAAUUUU

      130     140     150     160     170     180
ATTTAAATAA AAATGATTAA AATAATTTTA AAGTTAAATT AAACATTAAC TATTAATAAA

      190     200     210     220     230     240
GAATATAAAA TAAATATAA ATAAGTTTC AGTTAATTTT TTCTAAATA ACAAAAATA

      250     260     270     280     290     300
AATAGATACA CACTTGTAG AATAAAATG ATAATCTTTA AGACCCATA AAAAAATA

      310     320     330     340     350     360
AGATAATTA ACTTCTTAT AAGCTTTAG AATTACATA GATTGGACAC CCGAAGCTAT
* * * * *
      GUCCU UCGGGGACAU

      370     380     390     400     410
CCGTTAAAT TGGAACGATA CAGACAAGAT TAGCATGGCC CCTGCACAAG GATGACAC
*** ***** ***** ***** ***** **
CCGAUAAAU UGGAACGAU CAGAGAAGAU UAGCAUGGCC CCUGCGCAAG GAUGACAC
      U6 RNA gene 2

```

The longer amplified product was subcloned into M13mp18 and sequenced by the dideoxy method. The 5' and 3' ends of the *Tetrahymena* U6 RNA genes were tentatively assigned by a comparison with the tomato U6 RNA sequence (5). The upper line shows the non-coding strand sequence of the *Tetrahymena* U6 RNA gene. The lower line shows the sequence of the tomato U6 RNA. The TATA box-like sequence is boxed. The vertical arrowheads indicate the position of the intron present in the *S. pombe* U6 RNA gene. The regions corresponding to the primers used for PCR are represented by solid and dotted arrows over the sequence. The directions of the arrows coincide with that of primers (5' to 3'). The smaller product was amplified with primers represented by the solid and dotted arrows. The larger product was amplified with primers indicated by the solid arrows.