Gene, 120 (1992) 277–279

GENE 06734

Structure of the rainbow trout metallothionein *A* gene*

(Recombinant DNA; polymerase chain reaction; metallothionein gene; rainbow trout; fish)

Yunhan Hong** and Manfred Schartl**

Gene Center, Max-Planck-Institute for Biochemistry, 8033 Martinsried, Germany

Received by H.G. Zachau: 2 June 1992; Accepted: 30 June 1992; Received at publishers: 14 July 1992

SUMMARY

To investigate the regulation of metallothionein-encoding genes in fish, we have isolated and sequenced the rainbow trout metallothionein-A-encoding gene (*tMT-A*) by polymerase chain reaction. This gene spans about 1.1 kb, consists of three exons and two introns, and has an A+T-rich 5′-region which contains a TATAAA signal, and two metal responsive elements (*MRE*s). The transcription start point is centered around an A residue 81 nt upstream of the ATG codon.

INTRODUCTION

Metallothioneins (MTs) are a class of low-molecular-weight, Cys-rich, heavy-metal-binding proteins ubiquitously present in eukaryotes (Hammer, 1986). MTs are attractive models for eukaryotic gene expression since they are mainly regulated at the transcriptional level. Although in rainbow trout (*Salmo gairdneri*) two *MT* cDNAs, termed *tMT-A* and *tMT-B*, have been reported (Bonham et al., 1987) and the *tMT-B* gene has been analysed in some detail (Zafarullah et al., 1988; Hong et al., 1992), little is known about the regulation of the *tMT-A* gene. In order to investigate the transcriptional regulation of the *A* gene and to use its promoter to express novel genes in transgenic fish, we have isolated and sequenced this gene.

EXPERIMENTAL AND DISCUSSION

(a) MT genes

Using two oligos (Fig. 1) designed according to the published, functionally uncharacterized 5′-region of the putative *tMT-A* gene (Murphy et al., 1990) and the *tMT-A* cDNA (Bonham et al., 1987), a fragment of about 1.1 kb was amplified from trout genomic DNA by PCR, cloned into pBluescriptII KS+ (Stratagene, La Jolla, CA) and sequenced.

The *tMT-A* gene (Fig. 1) is 1094 bp long and has a tripartite structure common to all vertebrate *MT* genes (Hammer, 1986). The introns are 118 bp and 171 bp in size, respectively, as compared with 108 bp and 635 bp for the *tMT-B* gene (Zafarullah et al., 1988). The introns interrupt codons 9 and 32 and are, therefore, placed precisely as those in the *tMT-B* gene (codon 9 and 31) when an insertion (Ala^{31}) is ignored. This insertion, however, is intriguing for its absence in the other known piscine *MT* genes (Kille et al., 1991; Zafarullah et al., 1988) and all higher vertebrate *MT* genes (Hammer, 1986). Further, both introns share at their 5′ ends a common junction 5′-AAG-TAAGT sequence also found in the *tMT-B* gene.

Correspondence to: Dr. M. Schartl at his present address: Department of Physiological Chemistry I, Biocenter, University Würzburg, Am Hubland, W-8700 Würzburg, Germany.
Tel. (0931)-888-4149; Fax (0931)-888-4150.

* On request, the authors will supply detailed experimental evidence for the conclusions reached in this Short Communication.

** Present address: Department of Physiological Chemistry I, Biocenter, University Würzburg, Am Hubland, W-8700 Würzburg, Germany.

Abbreviations: aa, amino acid(s); bp, base pair(s); cDNA, DNA complementary to mRNA; kb, kilobase pairs or 1000 bp; *MRE*, metal responsive element; MT, metallothionein; *MT*, gene coding for MT; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; PCR, polymerase chain reaction; t, rainbow trout; *tMT-A*, gene (DNA) coding for tMT-A isoform; *tMT-B*, gene (DNA) coding for tMT-B isoform; *tsp*, transcription start point(s).

```
-429 ggatCCTACT AGTCCTAGAT TAGATATGTC TATAGGCTAT AACACTTTAA
   cc----------PCR primer AL1---------->
-379 ATCATCATGA ATTTGAACAA GACATTTAGT GTGTCTGTAA AATAACAATA
                              +         ∇
-329 TCTGGTAACT AAACGTTAAC AAAAGTCCAG ACCCAGTGGG TTATTTGAAG
                          + +   *       T
-279 GCATTCATAA ATAAATAAAA ATACTAATAC ATGTTTGCAT ATTAACATAA
                              T
-229 TGGGTAGCCA TATTTGAATG AATCAATTGT CAACATGGTA ATTAGGCTAT
-179 GTAGGCTATT TAAACAATAG GCTACTATTC CCTTGATGGG CATGTTTTCG
                    ← MREb
-129 CTCTAGATAA TAAACCGTGT GCAAGCAAGC CTATTAATGA GCTGTCTGCG
                          MREa →
 -79 TGAACGCGCG ACTCTGTTCT GCACACGGCA CCTGTCTGCC CCGGACGATA
                                +1
 -29 TAAATTCGAA GTCTCGCTAG TTAGAAATTA AACGCTGACA ACACACCACT
                <----------PCR primer AR2----------ctaggcc
  22 GACACCCAGA CAAACTACTA CGATCCATTC GGATAAAGAA GGTAGCTCAA
                <----extension primer----
                   M  D  P   C  E  C  S   K
  72 AAACTGGAAA ATGGATCCTT GTGAATGCTC CAAAAgtaag ttatgtatat
 122 cgatcaattt atcaagacta tgggctatgg atacgcctta agcgtatttc
 172 taaaatctta acaggctaat taccaattac ctaactcaac tgtttattta
       T  G  S   C  N  C   G  G  S   C  K  C  S   N  C  A
 222 tagCTGGATC TTGCAACTGC GGTGGATCCT GCAAGTGCTC CAACTGCGCA
     C  T  S   C  K  K  A
 272 TGCACCAGTT GTAAGAAAGC AAgtaagtgg ttcatattct aaatccaact
 322 agtgttatta ggccaatagt ctttttctta tcgctggtgg cactttaaag
 372 ccccatgtta gcctaattaa cctcaattgc ctcattaggt tgcaacgaaa
                                                S  C  C
 422 tagtctttgg tcatcactaa tcgattattt ttcggcccat cagGTTGCTG
       D  C  C   P  S  G   C  S  K  C   A  S  G   C  V  C
 472 CGACTGCTGT CCCTCCGGCT GCAGCAAGTG TGCCTCAGGC TGCGTGTGCA
     K  G  K  T   C  D  T   S  C  C   Q  ***
 522 AGGGCAAGAC TTGTGATACC AGCTGTTGTC AGTGAGGCCT GTGTATGACA
 572 TCACAATGCA GTCCATTCCC TATGACTATG AAGTTGTACC ATCTTGAGCA
 622 TAGCTTTTGT ACCTTGTCAA ATGTAAGGAA ATAAATTGCA TGTAACTT
                <----------PCR primer AR1----------cttaagcc
```

Fig. 1. The nt sequence and deduced aa sequence of the *tMT-A* gene. Numbering begins with the *tsp* (+1). Coding and flanking sequences are shown by capitals and introns by lower case letters. Putative TATAAA box, AATAAA polyadenylation signal and GT-AG splicing junctions are in bold letters. *MRE* sequences are underlined with arrows depicting their orientations relative to the *tsp*. PCR and extension primers are underlined with broken arrows indicating their extension directions. Encoded aa residues are represented by one-letter codes aligned with the second letter of codons. Three asterisks mark the stop codon. Positions at which the nt are different from that published (Murphy et al., 1990) are marked: + indicates addition, ∇ deletion and * substitution. The different nt are shown below the sequences. The GenBank accession number is M81800.

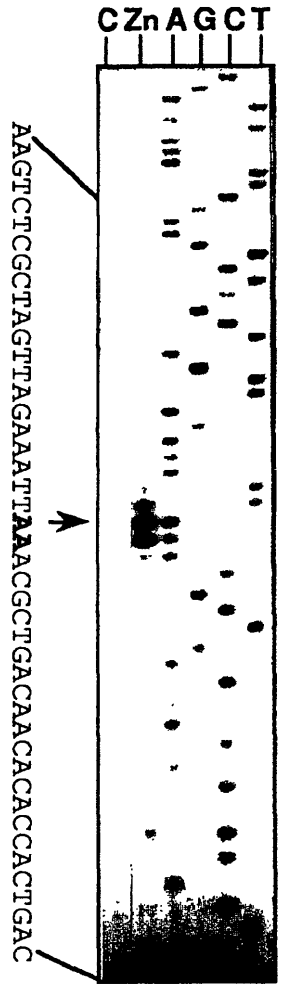

Fig. 2. Mapping of the *tsp* of the *tMT-A* gene by primer extension analysis. A 17-nt oligo (Fig. 1) was 5′-end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP and used to prime reverse transcription of total RNA from control (C) and Zn^{2+}-treated (Zn) rainbow trout hepatoma cells. About 20 μg of total RNA was used for each reaction. The extended products were analysed on a 8% sequencing gel and their sizes determined by comparison with the sequencing products run in adjacent lanes (A, G, C and T) generated using the tMT-A DNA as a template primed with the same oligo. Multiple bands are visible. Two major bands of 45 and 46 nt, respectively, which are faintly visible in the control lane and are evident in the Zn^{2+}-induced RNA sample, are indicated in bold. The larger band is arbitrarily designated as the *tsp* for convenience and shown by an arrow, which corresponds to an A residue 81 nt upstream of the ATG codon.

The *tsp* was mapped by primer extension analysis around an A residue 81 nt upstream of the start codon. However, as in many other vertebrate genes, multiple bands were observed, two of which were equally predominant, suggesting the presence of two major *tsp* (Fig. 2). The *tsp* is located at a position similar to that of the *tMT-B* gene (Zafarullah et al., 1988; Hong et al., 1992). The primer-extended products were only faintly visible in the control sample but became much more significant in the RNA sample from the Zn^{2+}-treated cells, clearly demonstrating the heavy-metal inducibility of transcription of the *tMT-A* gene. The 5′ region is A+T-rich (62%), which is similar to the *tMT-B* promoter (Zafarullah et al., 1988; Hong et al., 1991) but contrasts with the highly G+C-rich mammalian *MT* promoters (Hammer, 1986). The *tMT-A* promoter contains a consensus TATAAA signal (26 bp upstream of the *tsp*). It is characterized by the presence of two *MRE*s. The *tMT-B* promoter also contains only two *MRE*s (Hong et al., 1992; Zafarullah et al., 1988).

(b) Similarities and differences

The *tMT-A* coding region, as presently determined, agrees exactly with the published cDNA sequence (Bonham et al., 1987). In the 5′ region, however, differences at four positions were detected between our sequence and that previously reported (Murphy et al., 1990) (Fig. 1). Whether these discrepancies reflect a polymorphism is unknown.

The *MT-A* gene shares 78.4% similarity with the *MT-B*

gene at the nt level. The homologous nt, however, are unevenly distributed. Higher similarities were found in the translated region (95.1% aa, 91.3% nt), in the 5′ region (73.4% nt) and in the 3′ untranslated region (83.4% nt), while the introns have much lower similarity values (69.2% and 55.6% for introns 1 and 2, respectively). All this demonstrates the distinctness of two independent *MT* genes in rainbow trout, which primarily have been duplicated versions of the same gene in the tetraploid genome of a salmonid fish.

ACKNOWLEDGEMENTS

This work contains a part of Y.H. PH.D thesis and was supported by grants to M.S. provided by G.I.F. (No. I-18-127.3/87) and the Bundesministerium für Forschung und Technologie through 'Schwerpunkt: Grundlagen und Anwendungen der Gentechnologie' (No. 26). Y.H. is a fellow of the Deutsche Akademische Austauschdienst.

REFERENCES

Bonham, K., Zafarullah, M. and Gedamu, L.: The rainbow trout metallothioneins: molecular cloning and characterization of two distinct cDNA sequences. DNA 6 (1987) 519–528.

Hammer, D.H.: Metallothionein. Annu. Rev. Biochem. 55 (1986) 913–951.

Hong, Y., Winkler, C., Brem, G. and Schartl, M.: Development of a heavy metal-inducible fish specific expression vector for gene transfer in vitro and in vivo. Aquaculture (1992) in press.

Kille, P., Stephens, P.E. and Kay, J.: Elucidation of cDNA sequences for metallothioneins from rainbow trout, stone loach and pike liver using the polymerase chain reaction. Biochim. Biophys. Acta 1089 (1991) 407–410.

Murphy, M.F., Colier, J., Koutz, P. and Howard, B.: Nucleotide sequence of the trout metallothionein A gene 5′ regulatory region. Nucleic Acids Res. 18 (1990) 4622.

Zafarullah, M., Bonham, K. and Gedamu, L.: Structure of the rainbow trout metallothionein B gene and characterization of its metal responsive region. Mol. Cell. Biol. 8 (1988) 4469–4476.