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Cloning and Characterization of a New Multi-Stress Inducible Metallothionein Gene in *Tetrahymena pyriformis*

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A new multi-stress-inducible metallothionein (MT) gene isoform has been cloned and characterized from the ciliate *Tetrahymena pyriformis*. Both the 5′- and 3′-UT regions of the *Tp-MT2* gene are very different from the previously reported Tp-*MT1* isoform in this organism and from other described MT genes in *Tetrahymena pigmentosa* and *Tetrahymena thermophila*. The putative protein sequence of *Tp-MT2* contains cysteine clusters with characteristics of the typical *Tetrahymena* Cd-inducible MT genes. However, the sequence has a special feature of four intragenic tandem repeats within its first half, with a conserved structural pattern $x_{5/8}CCCx_6CCx_6CxCxNCxCCK$. To investigate the transcriptional activities of both *Tp-MT2* and *Tp-MT1* genes toward heavy metals (Cd, Hg, Cu, Zn) and H_2O_2, the mRNA levels of these two isoforms were evaluated by means of real-time quantitative PCR. Results showed that *Tp-MT2* had a higher basal expression level than *Tp-MT1* and both genes were induced by Cd, Hg, Cu, and Zn ions after short exposure (1 h), although to different extents. Cd was the most effective metal inducer of both two isoforms, but the relative expression level of *Tp-MT2* was much lower than that of *Tp-MT1*. Different expression patterns were also shown between the two genes when treated with Cd over a period of 24 h. We suggest that *TpMT-1* plays the role of a multi-inducible stress gene, while *TpMT-2* may have a more specific function in basal metal homeostasis although it may have undergone a functional differentiation process. The putative functional significance and evolutionary mode of the *TpMT-2* isoform are discussed.

Key words: gene expression; intragenic tandem repeats; metallothionein; metal stress; *Tetrahymena pyriformis*.

Introduction

Metallothioneins (MTs) constitute a superfamily of cysteine-rich proteins with low molecular weight and high metal-binding affinity, present in all animal phyla, plants, eukaryotic microorganisms, and cyanobacteria (Binz and Kägi 1999). In higher eukaryotes, especially in mammalian animals, many important biological functions of MTs and the corresponding gene polymorphism have been

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demonstrated, although their primary cellular function still remains unclear (Coyle et al. 2002; Hamer 1986; Palmiter 1998).

Recently, much information on MT gene structure and biochemical properties in invertebrates has accumulated. In many invertebrates, various MT isoforms are also present with a high degree of complexity in the gene organization and expression patterns (Dondero et al. 2005; Gruber et al. 2000; Jenny et al. 2004; Leignel et al. 2005; Tanguy and Moraga 2001). Functional diversities among those isoforms were hypothesized to be a consequence of evolutionary adaptation, driven by fluctuating conditions in their living environments. It has been postulated that MTs in lower eukaryotes may play the role of general stress proteins because of their potent metal-binding and redox capabilities, but such functions depend on the specific requirements of the particular organism (Coyle et al. 2002).

In the unicellular ciliate protozoan *Tetrahymena*, different MT isoforms have been reported in three species, *T. pyriformis*, *T. pigmentosa*, and *T. thermophila*, and placed into two groups mainly according to which metal inducer they preferentially respond to: Cd or Cu (Boldrin et al. 2003; Piccinni et al. 1994, 1999; Santovito et al. 2001; Shang et al. 2002). Those isoforms differ from all MTs reported in other organisms, since the arrangement of Cys residues between them showed only limited similarities (Boldrin et al. 2003). Previous studies indicated that the MTs of *Tetrahymena* not only play a role in heavy metal homeostasis and detoxification, but also respond to a wide variety of stimulants, from chemical to physical and biological ones, at the regulation and expression level (Boldrin et al. 2002; Dondero et al. 2004). However, both the functional significance of the multiple MT isoforms that exist among different *Tetrahymena* species and the genetic mechanisms involved in the formation of the peculiar MT genes have not been fully clarified. Thus, further comparative studies concerned with *Tetrahymena* MT genes may contribute to our understanding of their functional diversities and evolution process.

In this study, we report the cloning and transcription characterization of a new MT-like gene in *T. pyriformis*, named *TpMT-2*. It has the typical cysteine cluster characteristics of *Tetrahymena* Cd-inducible MT genes, but presents the special feature of four intragenic tandem repeats. The mRNA levels of *TpMT-2* and the former reported Cd-MT gene *TpMT-1* (Piccinni et al. 1999) in this organism have been evaluated under exposure to sublethal concentrations of heavy metals (Cd, Hg, Cu, Zn) and H_2O_2, using the real-time quantitative PCR method. Different transcriptional patterns were observed between the two gene isoforms. The putative functional significance and evolutionary mode of *TpMT-2* are discussed.

Results

Full-Length of *TpMT-2* cDNA

PCR amplification of the MT gene from *T. pyriformis* genomic DNA revealed two distinct products (Fig. 1). The one with shorter nucleotide sequence length (about 300 bp) was shown to be 100% identical to the previously described Cd-MT gene (*TpMT-1*). The second product (about 550 bp) appeared to be a new gene also having the sequence characteristics of *Tetrahymena* Cd-inducible MTs. The full-length mRNA sequence of this gene was obtained by reverse-transcription PCR, coupled with Rapid Amplification of cDNA Ends (RACE). The 752-base long cDNA (*TpMT-2*) showed that it contained an open reading frame (ORF) of 546 nt, including 82 and 124 bp of 5′-and 3′-untranslated regions (UTRs) respectively. Both

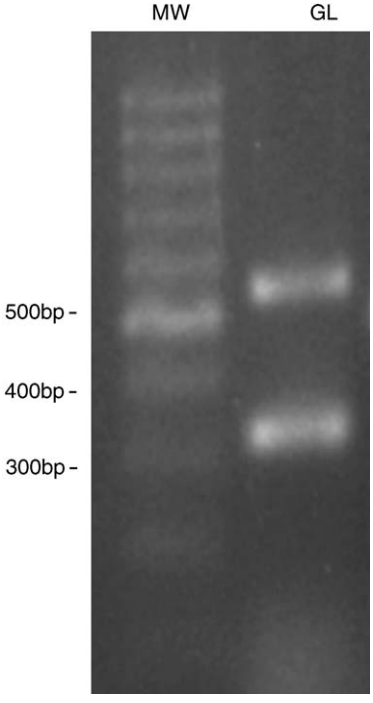

Figure 1. Agarose gel electrophoresis of the PCR products from genomic DNA. MW: DNA markers. GL: *Tetrahymena pyriformis* (strain GL)