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# Characterization of metallothionein cDNAs induced by cadmium in the nematode *Caenorhabditis elegans*

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cDNAs of metallothioneins (MTs) in the nematode *Caenorhabditis elegans* were characterized. The MT-II clone encodes 62 amino acid residues and the predicted $M_r$ is 6462. The MT-I clone contains an additional 12 residues at the *C*-terminal end, and the predicted $M_r$ is 7959. There is a considerable similarity between MT-I and MT-II. Both of these proteins are cysteine-rich and, with a few exceptions, show a good alignment of cysteine residues. No obvious sequence relationship in the coding region was discernible between *C. elegans* MTs and mammalian MTs, aside from Cys-Cys, Cys-Xaa-Cys, and Cys-Xaa-Xaa-Xaa-Cys segments. However, 3′-untranslated region of cDNAs of *C. elegans* MT-I and -II have some consensus sequences found in mammalian MT cDNAs, suggesting that these regions may have some roles in the regulation of MT-gene expression.

## INTRODUCTION

Metallothioneins (MTs) are cysteine-rich, low-$M_r$ heavy-metal-binding proteins (Hamer, 1986; Dunn *et al.*, 1987; Kägi & Schäffer, 1988). They exist in a wide range of organisms, including higher and lower eukaryotes, and even some prokaryotes (Higham *et al.*, 1986; Olafson *et al.*, 1988). MTs may be involved in the detoxication of heavy metals, such as cadmium and mercury, and in the homoeostasis of essential metals such as zinc and copper. Recent characterization of cDNAs of mammalian MTs revealed conserved sequences and evolutionary relatedness. However, MT cDNAs in lower organisms have some diversity, and MTs have been grouped into three classes, namely I, II and III (Kägi & Schäffer, 1988). From an evolutionary point of view, the nematode *Caenorhabditis elegans* is one of the most suitable models for studying gene expression and differentiation. It was previously reported that MTs in *C. elegans* were induced by cadmium (Maruyama *et al.*, 1986). In the present study we cloned the cDNAs of MT-I and -II in *C. elegans*. Sequence analyses have revealed that there exists some diversity between MTs in *C. elegans* and those from the various other sources.

## MATERIALS AND METHODS

*C. elegans* N2 strain obtained from Dr. J. Miwa (NEC Corporation, Kanagawa, Japan) and Dr. N. Munakata (National Cancer Center Research Institute, Tokyo, Japan) was cultivated and exposed to cadmium as described previously (Maruyama *et al.*, 1986). mRNA was purified from cadmium-induced worms (Maniatis *et al.*, 1982). The cDNA libraries in $\lambda$ gt11 and pUC18 were made by using a cDNA synthesis kit according to the manufacturer (Pharmacia). MT-I and MT-II in *C. elegans* (CeMT-I and CeMT-II) were purified to homogeneity by the combination of columns of Sephadex G-75, DEAE-Sephadex A-25 and h.p.l.c. *N*-Terminal amino acid sequences (1–24 for MT-I and 1–14 for MT-II) were determined as described previously:

MT-I: *N*-Ala-Cys-Lys-Cys-Asp-Cys-Lys-Asn-Lys-Gln-Cys-Lys-Cys-Gly-Asp-Lys-Cys-Glu-Cys-Ser-Gly-Gly-Lys-Cys-

MT-II: *N*-Val-Cys-Lys-Cys-Asp-Cys-Lys-Asn-Gln-Asn-Cys-Ser-Cys-Asn- (Kondo *et al.*, 1990)

Four kinds of oligonucleotides for non-complementary strand, which corresponded to amino acid sequences in the *N*-terminal portions, were synthesized as follows: A: 5′-TGT/CTTA/GTTT/CTTGCAA/GTCGCAT/CTTGCA-3′ (32 mixture of 26-mer corresponding to amino acids 2–10 in MT-I); B: 5′-GAGCATTCGCACTTGTCGCCGCACTTGCATTGCTTG-TTCTTGCAGTCGCACTTGCA-3′ (56-mer corresponding to amino acids 2–20 in MT-I); C: 5′-CAA/GTTT/CTCA/GTTT/CTTGCAA/GTCITTITTGCA-3′ (32 mixture of 29-mer corresponding to amino acids 2–11 in MT-II); D: 5′-GAGCAGTTTTCGTTCTTGCAGTCCTTCTTGCA-3′ (32-mer corresponding to amino acids 2–12 in MT-II)

The oligonucleotides assigned to residues 4 and 9 of CeMT-II correspond not to cystine and glutamine, but to lysine and glutamic acid respectively; an earlier determination of the protein sequence was in error. Oligonucleotides A and C were synthesized as mixtures of the possible complementary sequences, and B and D were synthesized as a 'guessmer' according to the codon usage. In oligonucleotide C, two deoxyinosines were used (Takahashi *et al.*, 1985), since AAA and AAG were used almost equally for lysine in other genes in *C. elegans*. By using the mixture of four oligonucleotides for the first screening, a pUC18 library yielded 12 positive clones and a $\lambda$ gt11 library yielded 11. Second and third screenings were performed by using each oligonucleotide separately. The cDNA of MT-II (pCeMT-II) was obtained from the pUC18 library, and that of MT-I (pCeMT-I) was selected from a $\lambda$ gt11 library and was subcloned into pUC18. Sequence determination of both strands was performed by the dideoxy method, using denatured plasmid templates (Hattori & Sakaki, 1986). The small sizes of the strands inserted in pUC18 permitted determination of the complete sequences. The sequences were unambiguously confirmed by repeated analyses and by sequence analyses of smaller, independent, clones.

Abbreviations used: MT, metallothionein; CeMT-I and -II, *Caenorhabditis elegans* metallothioneins I and II.

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## RESULTS AND DISCUSSION

In Fig. 1, nucleotide sequences and deduced amino acid sequences of *C. elegans* MT-I and MT-II cDNAs (pCeMT-I and pCeMT-II) are shown. That these clones contained full-length open reading frames was suggested by the following: first, these sequences are consistent with *N*-terminal amino acid sequences obtained from purified CeMTs (1–24 for MT-I and 1–14 for MT-II), with the exception of aspartic acid at position 11 in MT-I, which is replaced with a glycine residue [possibly attributable to an error in protein-sequence determination (Kondo *et al.*, 1990)]; secondly, the $M_r$ values for MT-I and MT-II predicted on the basis of deduced amino acids, are 7959 and 6462 respectively, whereas those of purified MTs were 6000–7000 (Maruyama *et al.*, 1986). MT-II is composed of 62 amino acids, and contained 18 cysteine residues. The positions of the cysteine residues were well conserved in MT-I and MT-II, although MT-I contained the additional 12 amino acid residues in the *C*-terminal portion and MT-II had three amino acid insertions between residues 14 and 19 (Figs. 1 and 2*a*). Amino acids besides cysteine were also well conserved, and maximum matching revealed 66% sequence similarity (Fig. 2*a*).

There was no obvious relationship between the positions of cysteine residues in CeMTs and those in mammalian MTs (Fig. 2*b*). However, some similarities in the coding region between CeMTs and other MTs were found. First, MT molecules in *C. elegans* contained three Cys-Cys segments. Secondly, they have repeated Cys-Xaa-Cys segments. Finally, the central segment reported by Nemer *et al.* (1985) was partially conserved. As Fig. 3(*a*) shows, CeMT-I and CeMT-II, in both vertebrates and non-vertebrates, contained a Cys-Xaa-Xaa-Xaa-Cys-Lys-Cys segment. In CeMT-I and the MT of sea urchin (*Strongylo-*

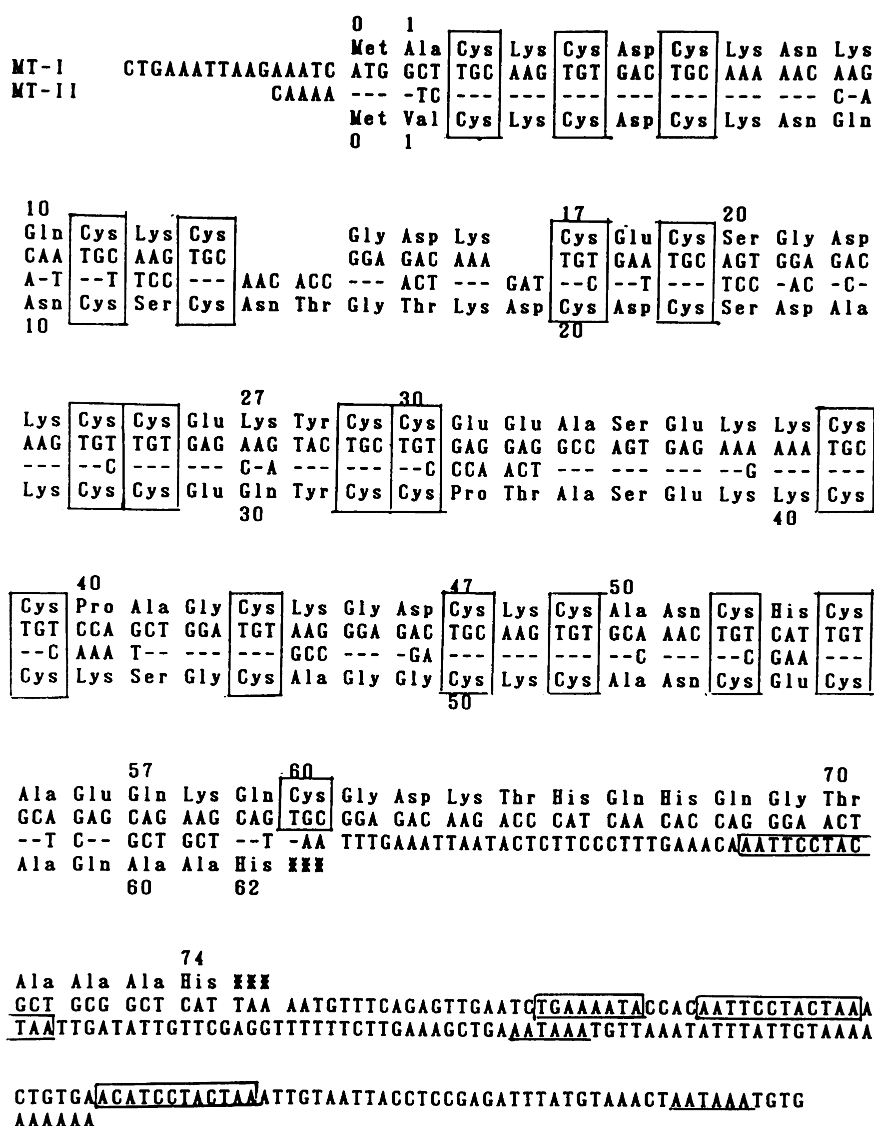


**Fig. 1. Nucleotide sequences and deduced amino acid sequences of *C. elegans* MT-I and MT-II cDNAs (pCeMT-I and pCeMT-II)**

Cysteine residues, and the consensus sequences in the 3′-untranslated region, as described in the text, are enclosed in boxes. The polyadenylation signal, AATAAA, is underlined. Three amino acid insertions were observed between residues 14 and 19 of CeMT-II. For alignment of cysteine residues, the sequence corresponding to *N*-terminal residues 13–17 of CeMT-I is represented by an absence of amino acids and sequences, although the sequence of CeMT-I is shown in full.

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(a)  CeMT-I    1        10                20                30                40                50                60              70   74
               ACKCDCKNKQCKC--GDK-CECSGDKCCEKYCCEEASEKKCCPAGCKGDCKCANCHCAEQKQCGDKTHQHQGTAAAH
                :*:*:**  : :  * * : :*  *::* *::  *****::  *: * :*:**: :*                ***
     CeMT-II   VCKCDCKNQNCSCNTGTKDCDCSDAKCCEQYCCPTASEKKCCKSGCAGGCKCANCECAQ-----------------AAH
               1        10        20        30        40        50                         60

(b)  Human MT-II   MDPNCSCAAGDSCTCAGSCKCKECKCTSCKKSCCSCCPVSCAKCAQGCICKGASDKCSCCA
                   ****:*:*   *: :** :*:* :*:**:***::*::***:**: **:*:* ****:*::*
     Mouse MT-II   MDPNCSCASDGSCSCAGACKCKQCKCTSCKKSCCSCCPVSCAKCSQGCICKEASDKCSCCA
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**Fig. 2. Interspecies comparisons between CeMTs (*a*) and MTs of other eukaryotes (*b*)**

:, Conserved cysteine residues; *, other conserved amino acids; -, absence of an amino acid. Amino acid sequences of human and mouse MTs are those published by Nemer *et al.* (1985).

*centrotus purpuratus*) an opposite orientation was observed. Lower organisms, such as yeast and the fungus *Neurospora*, also have similar sequences (Fig. 3*b*). Conserved sequences have also been found in the 3′-untranslated region (Fig. 4). Two consensus sequences, namely (*a*) TTTCTA and (*b*) TGTAAATA, have been reported in mammals (Griffith *et al.*, 1983; Peterson *et al.*, 1984). CeMT-I and -II show the similarity in both sequences, suggesting a role in the regulation of gene expression, e.g. stability of mRNA, translation and mRNA processing.

Differences between *C. elegans* and mammalian MTs were also evident. There was no apparent correlation between the position of the cysteine residues in *C. elegans* and mammalian MTs (Fig. 2). Moreover, *N*-terminal amino acids were alanine and valine in CeMT-I and -II respectively, whereas they were methionine in mammalian MTs. Both CeMT-I and -II contain one tyrosine residue, although this amino acid is not found in the MTs of other species (Hamer, 1986; Dunn *et al.*, 1987; Kägi & Schäffer, 1988).

In conclusion, our results have revealed that sequences of MTs in *C. elegans* show both similarity to, and diversity from, mammalian MTs. Using the classification system of Kägi & Schäffer (1988), we would classify the CeMTs as class II, since all 20 cysteine residues in mammalian MTs in class I are invariant (Kägi & Schäffer, 1988; see also Fig. 2*b*). The amino acid chain of CeMT-I is longer than that of CeMT-II, and there are fewer differences between MT-I and -II in other species (Hamer, 1986; Dunn *et al.*, 1987; Kägi & Schäffer, 1988), suggesting that there are functional differences in the isoforms of MT-I and -II. In fact, when MTs are induced by cadmium in *C. elegans*, the total metal (cadmium + zinc + copper) contents in both MTs was about 6 g-atom/mol of protein, and the content of zinc in MT-II was less than 2% of total metal content, whereas that in MT-I accounted for about 20% (Maruyama *et al.*, 1986). That is, MT-I contains relatively high amounts of zinc compared with MT-II.

(*a*)

| | |
|---|---|
| *C. elegans* MT-I | CkgdCkC |
| *C. elegans* MT-II | CaggCkC |
| Human MT-I | CtgsCkC |
| Human MT-II | CagsCkC |
| Equine MT-IA | CagsCkC |
| Equine MT-IB | CagsCkC |
| Sheep MT | CagsCtC |
| Rabbit MT-II | CatsCkC |
| Hamster MT-I | CsssCgC |
| Hamster MT-II | CagsCkC |
| Monkey MT-I | CadsCkC |
| Monkey MT-II | CagsCkC |
| Mouse MT-I | CtssCaC |
| Mouse MT-II | CagaCkC |
| *Drosophila* MT | CgsgCkC |
| | CgsdCkC |
| Crab MT-I | CkegCqC |
| | CssgCkC |
| Crab MT-II | CktgCkC |
| | CssgCkC |
| Consensus | CxxxCkC |

(*b*)

| | |
|---|---|
| *C. elegans* MT-I | CkCgdkCeCs |
| Sea urchin MT | CkCgsgCsCt |
| *Neurospora* MT | CnCgsgCsCs |
| Yeast MT | CsCptgC |
| Consensus | CkCxxxCxCx |

**Fig. 3. Conserved central segments in CeMTs and other MTs**

Sequences of central segments in other MTs are those published by Nemer *et al.* (1985). (*a*) Normal orientation; (*b*) opposite orientation.

(*a*)

| | |
|---|---|
| *C. elegans* MT-I | AATTCCTACTAA |
| | ACATCCTACTAA |
| *C. elegans* MT-II | AATTCCTACTAA |
| Consensus | AATTCCTACTAA |
| |   :: ::: |
| Consensus in mammals |   TTTCTA |

(*b*)

| | |
|---|---|
| *C. elegans* MT-I | TGAAAATA |
| *C. elegans* MT-II | TGAAATTA |
| Consensus | TGAAAATA |
| | :: ::::: |
| Consensus in mammals | TGTAAATA |

**Fig. 4. Comparison between sequences of 3′-untranslated regions of CeMT-I and CeMT-II cDNAs**

Conserved sequences in mammals are described those published by Griffith *et al.* (1983) and Peterson *et al.* (1984).

The possibility that the genes for MT-I and -II are differently regulated remains to be investigated.

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