Human metallothionein gene *MT1L* mRNA is present in several human tissues but is unlikely to produce a metallothionein protein

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Abstract A human MT gene from the functional locus on chromosome 16, *MT1L*, is characterised and shown to produce mRNA in at least four human tissues. This gene is unlikely to produce a metallothionein protein because it contains a termination codon at position 26, by analogy to other human MT1 genes. *MT1L* cDNA is almost identical to another metallothionein cDNA clone reported recently, $MT1_R$, suggesting that either there are unmapped human metallothionein genes, or that *MT1L* is polymorphic.

Key words: Metallothionein; Gene; Functional locus; Nucleotide sequence

1. Introduction

Metallothioneins are small cysteine-rich proteins which in humans are encoded by a multigene family on chromosome 16 [1]. The *MT2A* gene, and 13 MT1 isogenes have been linked to one cluster [2], whilst evidence suggests that the *MT3* and *MT4* genes are located nearby [3]. Much work has been devoted to establishing which of these genes are functional, and the proteins encoded by *MT2A*, *MT3*, and the MT1 isogenes *MT1E*, *MT1H* (also known as MTO, [4]) and *MT1X* have been isolated from human tissues [5,6], although additionally, specific mRNA transcripts have been identified in human tissues from *MT1F* and *MT1G* ([7], unpublished data). Up to six isoforms of MT have been isolated from human liver by HPLC [5], and it is likely that additional separation techniques such as capillary electrophoresis [8] will allow a further estimate of how many isoforms are actually present at the protein level. The physiological need for multiple MT1 or 2 isoforms in the human, and in most other mammals studied, is not yet known.

Recently, a PCR product of the cDNA from a novel MT1 isogene was cloned from human reticulocytes using a PCR approach [9] and determined to be unique on the basis of its nucleotide sequence. We show here that this cDNA, called $MT1_R$, is almost identical to the product of the *MT1L* gene on chromosome 16 suggesting that either this gene is polymorphic, or that there are further uncharacterised MT1 genes in the genome. *MT1L* is expressed at the mRNA level in a number of human tissues, but contains an in-frame termination codon at amino acid position 26 which would prevent production of a typical MT protein[2].

2. Materials and methods

2.1. Hybridisation analysis

RNA was isolated and subjected to northern analysis as described previously [11]. Probes were 5′ radiolabelled with [γ-^{32}P]ATP and hybridised to filters in 20% formamide buffer at 42°C for 16 h. Filters were washed up to a stringency of 0.2×SSC, 1% SDS at 45°C and then exposed to autoradiography film (Amersham). The following oligonucleotides were used as probes:

MT1L 5′-AAATGACGGGAGAGGCGA-3′:
MT2A 5′-ATCCAGGTTTGTGGAAGTCG-3′:
MT1G 5′-GGTCACTCTATTTGTACTTGG-3′:
MT1H 5′-CGTGTCATTCTGTTTTCATCTGAC-3′:
MT1X 5′-GCTCTATTTACATCTGAGAGCACA-3′:
β-actin 5′-CACACTTCATGATGGAGTTGA-3′

The MT oligonucleotides were demonstrated to be gene specific using a cosmid blot containing cosmid clones which covered the MT1/2 locus on chromosome 16 as described previously [10]. The β-actin oligonucleotide was used to validate the amount of mRNA loaded in each lane of the northern blot.

2.2. RT-PCR

RT-PCR was performed by a modification of the method outlined previously [12]. Briefly, RNA (1 μg) was reverse transcribed in 20 μl reaction buffer containing: 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.5 mM of each dNTP, 10 μg/ml oligodT_{16}, 20 units RNasin, 200 units M-MLV reverse transcriptase for 1 h at 37°C and then diluted to 200 μl with sterile water. PCR was in a 50 μl reaction containing: 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 250 μM of each dNTP, 0.4 μM of each primer, 2.5 units Taq polymerase (Boehringer-Mannheim) and 4 μl of the reverse transcription reaction or 10 ng of plasmid DNA. PCR was for 35 cycles with a cycle sequence of 95°C for 1 min, 60°C for 1 min, 72°C for 1 min. The following PCR primers were used: *MT1L* sense (5′-TCGCCTCTCCCGTCATTT-3′) and antisense (5′-AGCAGGGCTGTCCCCA-3′).

PCR reactions were electrophoresed through 1.4% agarose/1×TBE and visualised by ethidium bromide staining. PCR products were excised from the agarose gels, recovered by centrifugation through glass wool, blunt-ended with mung bean nuclease (Promega) and ligated in pUC19.

2.3. Nucleotide sequencing

Clones were purified from *Escherichia coli* and sequenced using Sequenase version 2 (USB). Resulting fragments labelled by the incorporation of [^{35}S]dATP were electrophoresed through 6% polyacrylamide gradient gels and detected by autoradiography. Alternatively, clones were purified using the QIAGEN Plasmid mini kit and sequenced using the ABI PRISM Dye primer or Dye terminator Cycle

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[2] A preliminary report on the deduced amino acid sequence encoded by *MT1L* has been presented in [10]. The complete nucleotide sequence of *MT1L* has been submitted to GENBANK.

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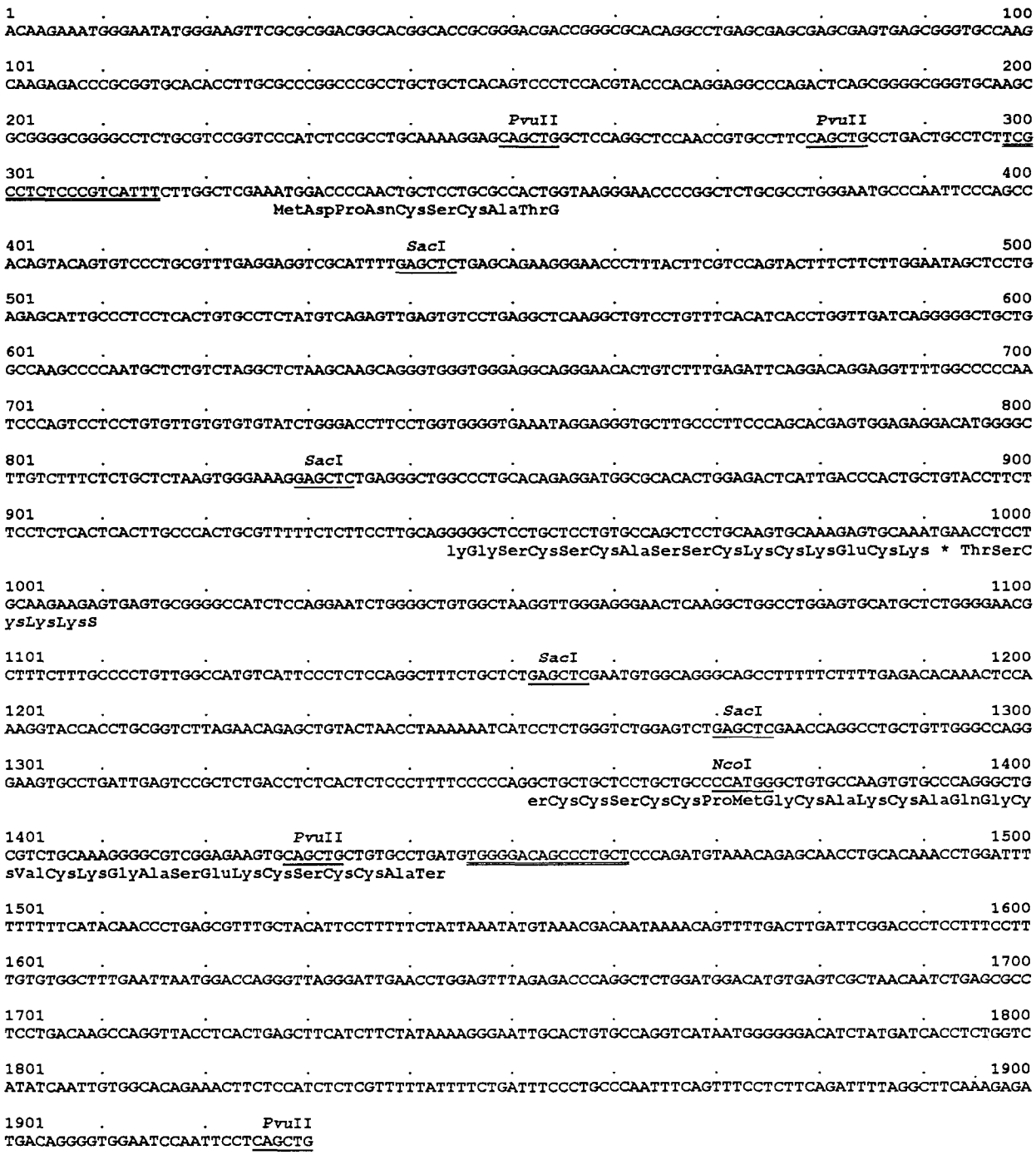

Fig. 1. Nucleotide sequence of the human *MT1L* gene. The putative amino acid sequence is indicated below the coding regions. Recognition sites for the restriction endonucleases *Nco*I, *Sac*I and *Pvu*II are indicated. Binding sites for oligonucleotides designed to amplify the gene or cDNA by PCR are shown (double underlined).

Sequencing Reaction Kit (Perkin Elmer Cetus) and the ABI PRISM 377 DNA Sequencer (Perkin Elmer Cetus).

3. Results

The nucleotide sequence of the human MT gene, *MT1L*, is shown in Fig. 1. This sequence was derived from subclones of two unrelated genomic clones, a bacteriophage lambda clone λ39-2 [13] and a cosmid clone chMT-51 [2]. The sequence as shown was derived from λ39-2, whilst the coding regions of the gene were confirmed from chMT-51. On the basis of the predicted amino acid sequence (by analogy to other mammalian MT proteins), this gene encodes a MT1 isoform, however, it contains an in-frame termination codon at a position equivalent to amino acid 26 (see Fig. 1). On the basis of this sequence, PCR primers were designed which were shown, using cosmid clones of the MT1/2 locus on chromosome 16 [2] to only amplify *MT1L* (data not shown). mRNA isolated from

human pancreas was reverse transcribed and the resultant cDNA used in PCR with the *MT1L* primers. A fragment of 128 nucleotides was cloned into pUC19 and sequenced. This fragment was identical in sequence to the cognate exons of the *MT1L* gene (shown in Fig. 1) and can be concluded to be an authentic cDNA clone from this gene. Note that the smaller size of the fragment compared to the predicted product using these PCR primers (i.e. 230 bp) is due to the exonuclease activity of mung bean nuclease, used to prepare blunt ends on the PCR product in preparation for cloning. Importantly, this independent clone confirmed that the termination codon at position 26 was also present in the mRNA derived from this gene.

The expression of *MT1L* was examined in a panel of human tissues using RT-PCR as described above, and by northern blotting using an oligonucleotide confirmed to hybridise specifically to *MT1L* (Fig. 1). As indicated in Table 1, a PCR product of the correct size (230 bp) was observed using *MT1L* primers in several tissues, including pancreas, thyroid gland, pineal gland and hypothalamus. *MT2A* was detected by RT-PCR using specific primers in all tissues examined.

RNA was isolated from three human thyroid glands and analysed by northern blotting using oligonucleotides specific for human MT genes. *MT2A* mRNA was detected in all three samples, whilst *MT1L* mRNA was detected in two of the three samples (Fig. 2). In addition, *MT1G*, *MT1H* (also known as MTO [4]) and *MT1X* mRNAs were observed in at least one of the samples (Fig. 2) whilst *MT1A*, *MT1B*, *MT1E* and *MT1F* mRNA were not detected (data not shown).

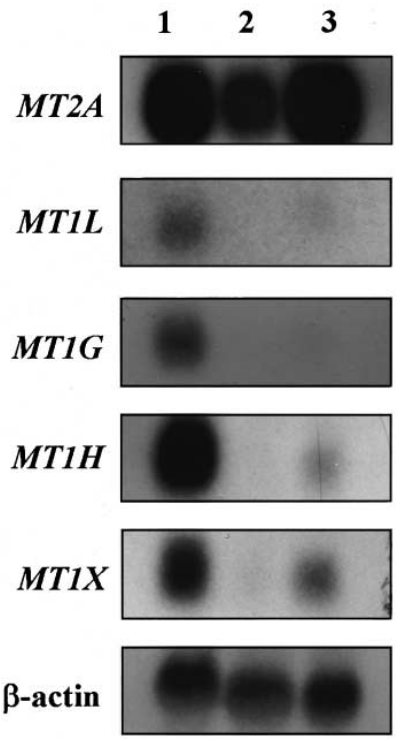

Fig. 2. Expression of MT mRNA in the human thyroid. RNA was isolated from 3 human thyroids (lanes 1–3) and the expression of various human MT-I and -II isogenes was examined by northern analysis. Gene specific oligonucleotides were used to examine the expression of the individual MT isogenes. A human β-actin oligonucleotide was used to confirm that equal amounts of RNA were present in each lane.

4. Discussion

We describe the complete nucleotide sequence of the human MT1 gene *MT1L*, and show that this gene produces a mRNA which is present in at least four human tissues. However, the predicted amino acid sequence of this gene is interrupted by a termination codon at position 26, which suggests that it is unlikely to produce a functional MT protein. There are however two other formal possibilities. One is that *MT1L* produces a truncated MT protein of 25 amino acids. MTs consist of two functionally separated domains which are capable of binding metals when experimentally separated, and the predicted product of *MT1L* corresponds to an almost complete β-domain. It is possible that such a protein would be capable of binding group II heavy metals, the hallmark of MTs. No such truncated isoform of MT has been identified to date, although if expressed at low levels, it may be difficult to detect using standard methods.

Table 1
Amplification of *MT1L* and *MT2A* cDNA by PCR

Tissue	*MT1L*	*MT2A*
Liver	–	+
Small intestine	–	+
Large intestine	–	+
Pancreas	+	+
Thyroid	+	+
Spleen	–	+
Heart	–	+
Thymus	–	+
Pineal gland	+	+
Hypothalamus	+	+

RNA was isolated from human tissues and subjected to RT-PCR using primers specific for the human *MT1L* and *MT2A* genes. The tissues in which specific products from the two genes were (+) and were not (–) amplified are indicated.

Alternatively, *MT1L* could encode a selenoprotein, since a TGA codon has been shown, in a small class of proteins, to specify a selenocysteine residue (reviewed in [14]). We feel that *MT1L* is not a promising candidate for a selenocysteine-MT, since this gene does not contain many of the consensus features exhibited by some (but not all) selenoproteins. For example, the 3′UTR of the mRNA encoding the selenoproteins glutathione peroxidase and iodothyronine deiodinase contain several conserved 3–4 nucleotide sequences and the 3′UTR is predicted to form a highly conserved stem-loop structure [15–17]. These features appear necessary for the incorporation of a selenocysteine residue and do not appear to be present in the relevant region of *MT1L*. Nonetheless, it is intriguing to speculate on the altered properties that inclusion of a selenocysteine residue would impart on a metallothionein, and it has been observed that relatively low molecular weight, uncharacterised selenoproteins are present in the human thyroid (J. Arthur, personal communication).

Recently, it has been reported that a clone encoding a novel isoform of MT, $MT1_R$ was isolated from human reticulocytes [9]. Fig. 3 shows a comparison of the nucleotide sequences between the predicted cDNA of *MT1L*, and the cDNA of $MT1_R$. It is apparent that these sequences are virtually identical, with the obvious major discrepancy being the TGA termination codon at position 26. We have confirmed the nucleotide sequence in this vicinity in two independent genomic

Fig. 3. Comparison of the *MT1L* and $MT1_R$ nucleotide sequences. The nucleotide sequence of the predicted *MT1L* cDNA is shown with the predicted amino acid sequence (three letter code) above. Mismatches with the $MT1_R$ cDNA nucleotide sequence are shown below. Dots indicate where the $MT1_R$ sequence is identical to the *MT1L* sequence.

clones and one cDNA clone derived from the pancreas. Of the 14 genes in the linked locus on chromosome 16 only the gene designated *MT1L* [2] could have given rise to the sequence reported here, based on PCR using genomic clones spanning the whole locus. However, it is possible that the $MT1_R$ cDNA is derived from a presently uncharacterised gene which is distinct from *MT1L*. In light of the recent discovery of at least two important but hitherto unsuspected isogenes of MT, *MT3* and *MT4* [18,19], this is a plausible explanation, although the apparent similarities between *MT1L* and $MT1_R$ are much greater than between any other pair of human MT genes yet characterised.

Alternatively, given the apparent similarities between the 2 sequences, it is possible that the putative gene encoding $MT1_R$ is a polymorphic form of *MT1L*. We have, however, confirmed the sequence of this gene (or its cDNA) from three individual people. Analysis of the nucleotide sequence of the *MT1L* gene from a larger number of individuals is necessary to resolve this point.

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