MOLECULAR AND CELLULAR BIOLOGY, Dec. 1989, p. 5738–5741
0270-7306/89/125738-04$02.00/0

Vol. 9, No. 12

Metal-Specific Posttranscriptional Control of Human Metallothionein Genes

CHANCHAL SADHU†* AND LASHITEW GEDAMU

Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada T2N 1N4

Received 3 April 1989/Accepted 8 September 1989

During the initial 4 h of treatment, copper and zinc similarly activated the rates of transcription and mRNA accumulation from the two human metallothionein (MT) genes, viz., MTI-G and MTII-A, in the hepatoblastoma cell line HepG2. The levels of copper-induced MT mRNAs remained at a plateau for up to 15 h. In contrast, the levels of zinc-induced MT mRNAs gradually declined after about 4 h, despite substantial transcription. The decrease in the zinc-induced MT mRNA half-life is probably due to a posttranscriptional event(s).

Metallothioneins (MTs) are a group of ubiquitous, low-molecular-weight, cysteine-rich proteins. Almost all of the vertebrate MTs are 61 or 62 amino acids long and have highly conserved cysteine residues (6). MTs present an interesting system for the study of gene regulation, at both the transcriptional and posttranscriptional levels. To date, six functional human MT genes, designated MTII-A (9), MTI-A (13), MTI-B (5), MTI-E (16), MTI-F (16, 17), and MTI-G (17), have been isolated and characterized. All of these MT genes have a tripartite structure consisting of three exons and two introns. The mature MT mRNAs have 5′ and 3′ untranslated regions of about 75 and 142 nucleotides, respectively (4). In most tissues, a basal level of MT mRNA is found. MT mRNA synthesis is also induced by various stimuli, including heavy-metal ions and steroid hormones (1, 3, 4). Furthermore, it has been suggested that interferon modulates human MT gene expression at the posttranscriptional level (2).

Patterns of total MT mRNA accumulation in copper- and zinc-induced HepG2 cells. To study the patterns of MT mRNA induction by copper and zinc, semiconfluent cultures of HepG2 cells were treated with one of the inducers for various times (17), and total RNA was isolated (7, 14). Five micrograms of RNA from each time point was attached to the nitrocellulose membrane with a slot-blot apparatus and was probed with the MTII-processed gene probe (9). This probe hybridizes to all of the human MT mRNAs and, hence, gives a general picture of MT gene expression. MT mRNA levels were elevated in response to both inducers within 1 h of induction and continued to increase for up to 6 h (Fig. 1). However, after this time, levels of MT mRNA in zinc-induced cells began to decline, while those in copper-induced cells remained steady. No such difference was observed when the same RNA samples were probed with a human γ-actin probe (Fig. 1), indicating that the difference in MT mRNA accumulation observed in copper- or zinc-induced cells is specific to MTs.

Transcript accumulation and rate of transcription from individual human MT genes. We have subcloned the 3′ untranslated regions of MTI-G and MTII-A into the polylinker region of the RNA probe vector pGEM and synthesized [^{32}P]UTP-labeled antisense RNA (15). A large excess of the antisense RNA was hybridized in solution to increasing amounts of total RNA from HepG2 cells (14). After hybridization, the remaining probe was digested with RNases A and T_1, and the RNase-resistant hybrids were assayed by trichloroacetic acid precipitation and scintillation counting. RNase-resistant counts per minute were plotted against increasing amounts of total cellular RNA, and the absolute number of MT mRNA molecules was calculated from the slopes of these titration data (10).

As observed in the case of the total MTs, the level of MTI-G transcript increased steadily after copper induction for up to 6 h and then reached a plateau. At that time, approximately 1,200 molecules of MTI-G transcript were present per cell (Fig. 2a). The initial pattern of zinc-induced MTI-G transcript accumulation was more or less similar to that of copper; however, after 4 h, the level started to decline. By 15 h of zinc induction, only about 800 molecules per cell were present (Fig. 2b). To analyze whether the patterns of MTI-G mRNA accumulation reflect the patterns of transcription from this gene, we pulse-labeled the HepG2 cells with [^{3}H]uridine for 20 min at various times of induction by copper or zinc (8) and measured the relative amounts of label present in the MTI-G transcripts. To measure the relative rate of transcription of the MTI-G gene, total RNA from the treated HepG2 cells was isolated and hybridized to a 793-base-pair *Hha*I-*Hha*I fragment of the MTI-G gene clone containing the first exon, first intron, and 18 base pairs of the second exon attached to the nitrocellulose membrane (11). For the measurement of MTII-A gene transcription, a 2.3-kilobase *Hin*dIII-*Nde*I fragment of the genomic clone containing all three exons and both introns was used. After hybridization, the filters were washed in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% sodium dodecyl sulfate at 58°C and treated with RNases A and T_1. The radioactivity present on the filters was measured by liquid scintillation counting. The efficiency of hybridization under these conditions using a ^{32}P-labeled RNA probe, was determined to be approximately 30%. Control filters containing only pUC13 DNA were also included in each experiment to determine nonspecific hybridization. As with transcript accumulation, the patterns of transcription of MTI-G in response to copper and zinc were similar during the initial time period (Fig. 2a and b). In both cases, the

* Corresponding author.
† Present address: Department of Molecular Biology, Research Institute of Scripps Clinic, 10666 North Torrey Pines Rd., La Jolla, CA 92037.

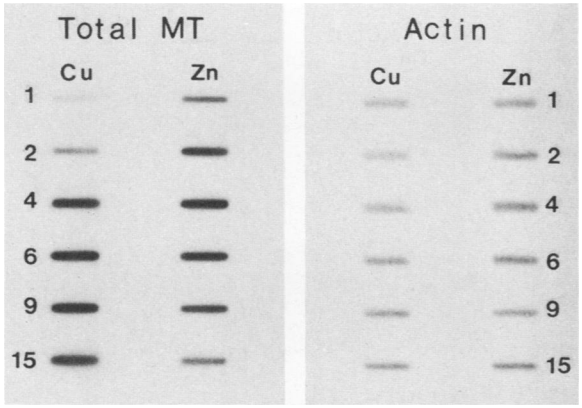

FIG. 1. Patterns of MT gene expression in HepG2 cells induced with copper and zinc. Total RNA was isolated from the HepG2 cells at different times during induction, and 5 μg was applied to each of the slots on the nitrocellulose membrane in the presence of 5× SSC and 1% formaldehyde with a Schleicher & Schuell slot-blot apparatus. The filter was hybridized to the [^{32}P]UTP-labeled antisense RNA probe derived from the MT-II processed gene (left) or the human γ-actin gene (right). After hybridization, the filter was washed and autoradiographed. Hours of induction (1 to 15) are also noted.

transcription rate reached a maximum by about 4 h. After this time, the rate of transcription from the MTI-G gene in copper-induced cultures dropped rapidly. The rate of transcription in zinc-induced cultures showed some decline; however, it was still two to three times higher than that induced with copper.

We also studied the pattern of expression of the MTII-A gene in response to heavy-metal ions. In comparison with the response of the MTI-G gene, the response of the MTII-A gene was much higher. At maximum induction, about 7,800 MTII-A transcript molecules accumulated per cell, while the maximum level of MTI-G transcripts was only about 1,200 molecules per cell. Even though the level of MTII-A transcripts was higher, the overall pattern of induction of MTII-A was quite similar to that of MTI-G in response to both copper and zinc (Fig. 2c and d). The copper induction curve reached a plateau by 4 to 6 h, while in the case of zinc induction, the MTII-A transcript level showed a steady decline after 4 h. By 15 h of induction with zinc, only about one-half the MTII-A mRNA (less than 4,000 molecules per cell) was present, compared with its maximal level (Fig. 2d).

We further compared MTII-A transcript accumulation with MTII-A rate of transcription. During the early stage of induction, there was a parallel increase in MTII-A gene transcription and mRNA accumulation when the cells were treated with either copper or zinc. After about 4 h of induction, there was a slow decline of the zinc-induced MTII-A transcription rate (Fig. 2d), but the decline was much more rapid in the case of copper induction (Fig. 2c). For example, the rate of MTII-A gene transcription was 21 ppm/100 base pairs, and the number of its transcript was 7,500 molecules per cell after 14 h of induction by copper. In contrast, after 14 h of zinc treatment, the rate of MTII-A transcription and the number of mRNA molecules were 36 ppm/100 base pairs and 4,000 molecules per cell, respectively. These data show that the observed patterns of MTI-G and MTII-A mRNA accumulation in HepG2 cells after 4 to 6 h of treatment with copper and zinc cannot be explained by changes in corresponding transcription rates.

Effects of dactinomycin and cycloheximide on the turnover of heavy-metal-induced MT mRNA. To determine whether ongoing mRNA and protein synthesis is essential for the observed MT mRNA turnover, we induced MT synthesis in HepG2 cells by copper or zinc and then blocked further transcription and translation with dactinomycin and cycloheximide treatments, respectively. Total RNA was isolated from those cells at various times during treatment with the inhibitor(s), and the levels of MTI-G and MTII-A transcripts

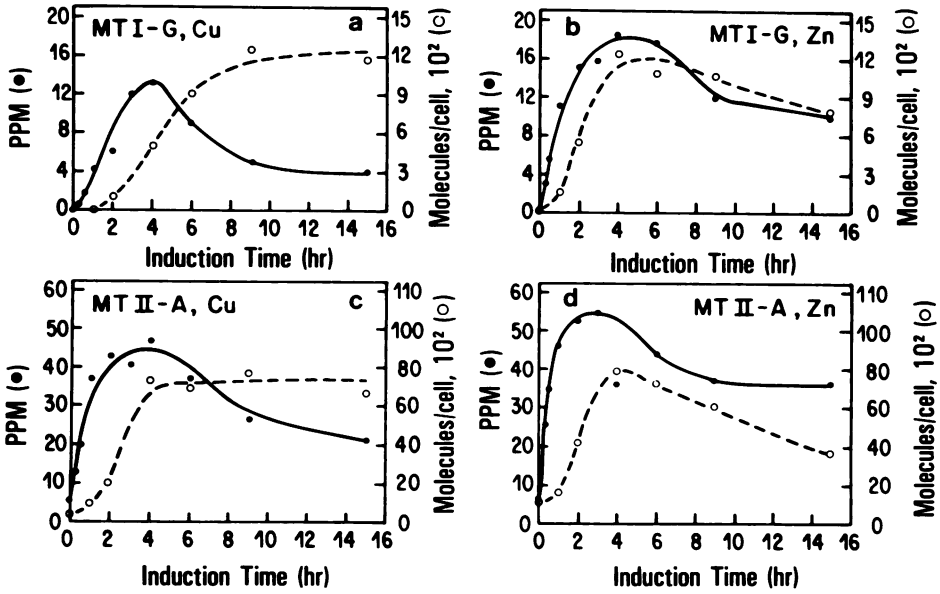

FIG. 2. Time courses of MTI-G (a and b) and MTII-A (c and d) gene transcription and mRNA accumulation. The relative transcription rates (parts per million) were obtained by dividing the observed counts per minute by the input counts per minute and correcting for the efficiency of hybridization. Hybridization to the pUC13 DNA-containing filters was always less than 1 ppm. The data were normalized to parts per million per 100 base pairs for comparison with the rate of transcription from the MTII-A gene. Each value plotted is the average of two independent experiments. The number of MT mRNA molecules was determined by the solution hybridization technique (10, 14).

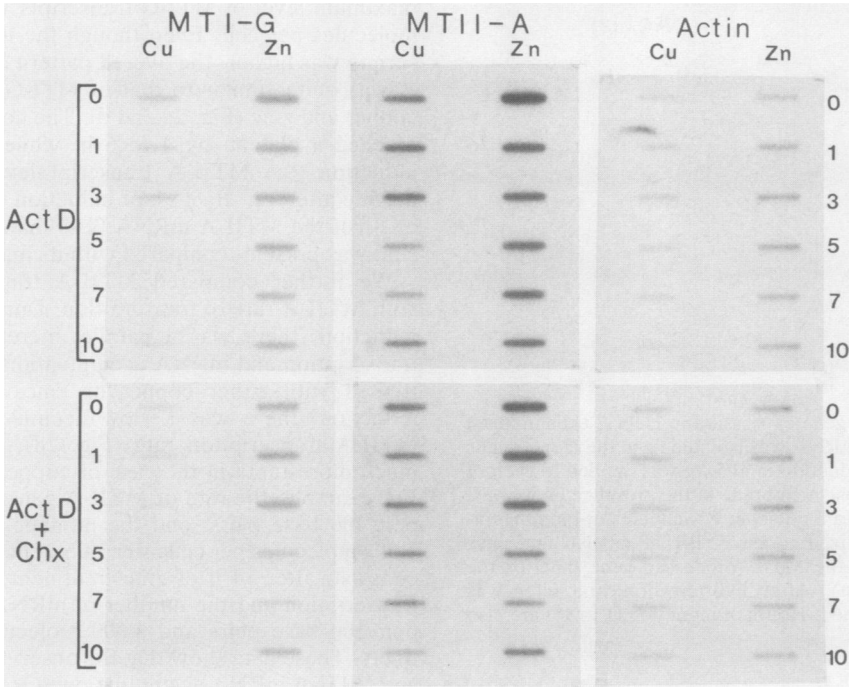

FIG. 3. Effects of dactinomycin (ActD) and cycloheximide (Chx) on the turnover of heavy-metal-induced MT mRNA. MTs were induced by treating the HepG2 cells with either copper or zinc for 3 h. Then the transcription and translation were stopped by the addition of dactinomycin (5 μg/ml) and cycloheximide (10 μg/ml), respectively (12). At different times, cells were harvested and total RNA was isolated. Five micrograms of RNA from each sample were applied in duplicate to a nitrocellulose filter with a Schleicher & Schuell slot-blot apparatus, and the filters were hybridized with either MTI-G- or MTII-A-specific 3′ probes. Left, MTI-G probe; center, MTII-A probe; right, human γ-actin probe. Hours of treatment with dactinomycin alone or in combination with cycloheximide are noted at the sides.

were analyzed by slot-blot hybridization (Fig. 3). In the zinc-induced cells, there was no significant change in MTI-G or MTII-A mRNA levels after treatment with dactinomycin alone or in combination with cycloheximide. A similar pattern was observed in the copper-induced MTII-A and MTI-G transcript levels, though the autoradiographic signals in the MTI-G lanes were generally low. As a control, an identical filter was hybridized to the ^{32}P-labeled γ-actin probe. No appreciable change in the levels of actin mRNA was observed under these conditions. These data show that blockage of cellular transcription leads to the stabilization of previously synthesized MT mRNAs in zinc-treated HepG2 cells.

The major observation of the present study is the metal-ion-specific posttranscriptional control of MT mRNA levels in the human hepatoblastoma cell line HepG2. During the initial 4 h of treatment, the responses of the two MT genes studied to either copper or zinc were found to be similar. However, during the later hours of induction, levels of zinc-induced MT mRNAs declined steadily, while the levels in the copper-induced culture remained constant. This decline in zinc-induced MT mRNA levels was not due to any blockage of transcription from the MT genes. In fact, the rates of transcription in both the MTI-G and MTII-A genes in the zinc-induced culture were observed to be higher than those in copper-induced cells. One of the possible explanations of these observations is the activation of a MT mRNA turnover pathway by zinc. The putative factors involved in the pathway are probably transient, since zinc-induced MT mRNAs seem to remain stable in the absence of transcription and translation. The differential effects of copper and zinc on the expression of the MT gene at the posttranscriptional level may have implications for heavy-metal homeostasis by MT.

We thank Dean Hamer for the gift of the MTII-A gene and Janet Price-Haughy for technical assistance. We also thank Ruth Stewart for processing of the manuscript.

C.S. was supported by a postdoctoral fellowship from the Alberta Heritage Foundation for Medical Research. This work was supported in part by the Medical Research Council of Canada.

LITERATURE CITED

1. **Durnam, D. M., and R. D. Palmiter.** 1981. Transcriptional regulation of the mouse metallothionein-I gene by heavy metals. J. Biol. Chem. **256**:5712–5716.
2. **Friedman, R. L., S. P. Manly, M. McMahon, I. M. Kerr, and G. Stark.** 1984. Transcriptional and post-transcriptional regulation of interferon-induced gene expression in human cells. Cell **38**:745–755.
3. **Hager, L. J., and R. D. Palmiter.** 1981. Transcriptional regulation of mouse liver metallothionein-I gene by glucocorticoids. Nature (London) **291**:340–342.
4. **Hamer, D. H.** 1986. Metallothionein. Annu. Rev. Biochem. **55**:913–951.
5. **Heguy, A., A. West, R. I. Richards, and M. Karin.** 1986. Structure and tissue-specific expression of the human metallothionein I_B gene. Mol. Cell. Biol. **6**:2149–2157.
6. **Kagi, J. H. R., and M. Nordberg (ed.).** 1979. Metallothionein. Birkhauser Verlag, Basel.
7. **Kaplan, B. B., S. L. Bernstein, and A. E. Gioio.** 1979. An improved method for the rapid isolation of brain ribonucleic acid. Biochem. J. **183**:181–184.
8. **Karin, M., A. Haslinger, H. Holtgreve, G. Cathala, E. Slater, and J. D. Baxter.** 1984. Activation of a heterologous promoter in response to dexamethasone and cadmium by metallothionein gene 5′-flanking DNA. Cell **36**:371–379.

9. **Karin, M., and R. I. Richards.** 1982. Human metallothionein genes—primary structure of the metallothionein II-gene and a related processed gene. Nature (London) **299**:797–802.
10. **Lee, J. J., F. J. Calzone, R. J. Britten, R. C. Angrer, and E. H. Davidson.** 1986. Activation of sea urchin genes during embryogenesis. Measurement of transcript accumulation from five different genes in *Strongylocentrotus purpuratus*. J. Mol. Biol. **188**:173–183.
11. **McKnight, G. S., and R. D. Palmiter.** 1979. Transcriptional regulation of the ovalbumin and conalbumin genes by steroid hormones in chick oviduct. J. Biol. Chem. **254**:9050–9058.
12. **Rahmsdorf, H. J., A. Schonthal, P. Angel, M. Litfin, U. Ruther, and P. Herrlich.** 1987. Posttranscriptional regulation of c-fos mRNA expression. Nucleic Acids Res. **15**:1643–1659.
13. **Richards, R. I., A Heguy, and M. Karin.** 1984. Structural and functional analysis of the human metallothionein I-A gene: differential induction by metal ions and glucocorticoids. Cell **37**:263–272.
14. **Sadhu, C., and L. Gedamu.** 1988. Regulation of human metallothionein genes. Differential expression of MTI-F, MTI-G, and MTII-A genes in the hepatoblastoma cell line (HepG2). J. Biol. Chem. **263**:2679–2684.
15. **Sadhu, C., N. Jahroudi, and L. Gedamu.** 1987. Human metallothionein-specific riboprobes. Nucleic Acids Res. **15**:5491.
16. **Schmidt, C. J., M. F. Jubier, and D. H. Hamer.** 1985. Structure and expression of two human metallothionein-I isoform genes and a related pseudogene. J. Biol. Chem. **260**:7731–7737.
17. **Varshney, U., N. Jahroudi, R. Foster, and L. Gedamu.** 1986. Structure, organization, and regulation of human metallothionein I_F gene: differential and cell-type-specific expression in response to heavy metals and glucocorticoids. Mol. Cell. Biol. **6**:26–37.