Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7318–7322, December 1984
Biochemistry

A 12-base-pair DNA motif that is repeated several times in metallothionein gene promoters confers metal regulation to a heterologous gene

(cadmium/synthetic DNA/mouse eggs)

GARY W. STUART*, PETER F. SEARLE*, HOWARD Y. CHEN†, RALPH L. BRINSTER†, AND RICHARD D. PALMITER*

*Howard Hughes Medical Institute Laboratory, Department of Biochemistry, University of Washington, Seattle, WA 98195; and †Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104

Communicated by Robert T. Schimke, August 3, 1984

ABSTRACT To define DNA sequences involved in mouse metallothionein-I (MT-I) gene promoter function and metal regulation, we fused the 5′ flanking sequences of the *MT-I* gene to the coding sequences of a viral thymidine kinase (*TK*) gene. A series of 5′ deletion, 3′ deletion, linker-scanning, and internal deletion mutants of the MT-I promoter was constructed and assayed by microinjection into mouse eggs. The results indicate that at least two related promoter elements can confer some metal regulation independently. Those mutations that had the most severe effect on regulation impinge on a 12-base-pair conserved sequence that is repeated several times within the mouse MT-I and other MT promoters. To test the regulatory function of this sequence, it was synthesized as a pair of complementary oligonucleotides and inserted into the promoter of the *TK* gene. A single insertion of this sequence conferred limited metal regulation onto the TK promoter, whereas a construct with two separate inserts was regulated as efficiently as the MT-I promoter.

Metallothionein (*MT*) genes are expressed in most animal tissues and cell lines (1–3). They are transcriptionally regulated by a variety of heavy metals (3, 4), by glucocorticoids (5–7), and by a hormone liberated in response to inflammation (8, 9). Several *MT* genes have now been cloned and shown to retain expression and regulation after transfer into heterologous cells (6, 9–11). The MT promoter and 5′ flanking sequences have been used to confer metal, steroid, and/or inflammatory response to a variety of structural genes that were transferred into cells or animals (12–18). The location of elements involved in these regulatory functions is being actively pursued by the reverse genetic approach of *in vitro* mutagenesis and gene transfer. Short DNA sequences thought to be involved in both metal (13, 16, 18) and glucocorticoid (16) regulation have been proposed based on deletion mutagenesis; however, precise sequence requirements for efficient interaction with regulatory proteins have not been defined. In this study, we show that at least two independent promoter elements mediate the metal inducibility of the mouse *MT-I* gene and that a synthetic copy of one of these elements confers metal regulation to a heterologous gene.

MATERIALS AND METHODS

Plasmid Constructions. Deletions of the MT-I promoter region were constructed by BAL 31 exonuclease digestion from the *Kpn* I site at −650 and the *Xba* I site at +222 of a subclone containing the *MT-I* gene (19). *Bam*HI linkers were inserted and, in the case of the 5′ deletions, MT sequences up to the *Eco*RI site of the plasmid vector were excised. The endpoints of the 3′ deletions were characterized by an initial round of cloning, then joined by their *Bam*HI linkers to appropriately matched 5′ deletions of the *MT-I* gene and to appropriately matched 5′ deletions of the thymidine kinase (*TK*) gene [provided by S. L. McKnight (20); the gene was from herpes simplex virus, type I] to generate linker scanning and internal deletion mutants and linker fusion mutants, respectively. Ultimately, all of the mutants were placed in pBX322, a high copy number variant of pBR322 with an *Xho* I linker near position 3000 (see Fig. 1). The structures of the mutants were confirmed by restriction enzyme mapping and by DNA sequencing.

Linker scanning mutants of the *TK* gene were obtained from S. L. McKnight (21). The *Pvu* II/*Hin*dIII fragment spanning the *TK* gene and including 200 base pairs (bp) of 5′ flanking DNA was inserted between the *Pvu* II and *Hin*dIII sites of pBX322. To insert synthetic DNA, the plasmids (1 μg) were linearized with *Bam*HI and ligated with 45 pmol of each of the oligonucleotides (lacking 5′ phosphates) 5′ G-A-T-C-C-C-T-T-T-G-C-G-C-C-C-G-A 3′ and 5′ G-A-T-C-T-C-G-G-G-C-G-C-A-A-A-G 3′ (provided by J. Habener). Linear molecules were purified by electrophoresis on agarose gels, eluted, and heated to 68°C in 50 mM NaCl. The 13 nucleotide "sticky ends" were annealed at 20°C overnight before transformation and cloning. The oligonucleotide insertion regenerated the *Bam*HI site at only one end, which allows the orientation of the insert to be determined.

As a reference gene to control for variable efficiencies of introducing mutants into mouse eggs, we used the plasmid pMT-βgal, which was constructed from pGA350 by cloning the *Bam*HI/*Hin*dIII fragment containing the *MT–lacZ* fusion gene (22) into a pBR322 derivative.

Mouse Egg Microinjection Assay. Analysis of pMK′ mutants was performed essentially as described (13). Approximately 2500 copies of the test plasmid together with 500 copies of the reference plasmid, pMT-βgal, were injected (in ≈2 pl) into the male pronuclei of ≈20 fertilized mouse eggs. The eggs were then divided into two groups and incubated for 22 hr in the presence or absence of 50 μM $CdSO_4$. Aphidicolin (2 μM), an inhibitor of DNA synthesis, was included in the medium to arrest the eggs at the one-cell stage; this treatment improved expression of the injected plasmids ≈10-fold. After incubation, the eggs were lysed and the extract was assayed for TK activity as described (13), except that the assay buffer also contained 100 μM 4-methylumbelliferyl β-D-galactoside as a substrate for β-galactosidase. β-galactosidase activity was determined by removing 2 μl of the assay

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MT, metallothionein; TK, thymidine kinase; MRE, metal regulatory element; bp, base pair(s).

mixture after 1 hr, placing it in 1 ml of 0.5 mM NaOH, and measuring the fluorescence at 445 nm (excitation at 365 nm). This measurement provided a control for variables in the injection procedure. The results of different experiments were combined using a general mixed model analysis of variance with the aid of computer program BMDP3V (23).

RESULTS

Assay of MT Promoter Mutants. In plasmid pMK′, ≈1800 bp of mouse MT-I 5′ flanking sequences are fused to the *TK* structural gene as shown in Fig. 1. To identify sequences necessary for regulated expression of the fusion gene (*MK*), a series of mutants of the MT-I promoter region was prepared. The structure of these mutants is shown in the left panel of Fig. 2. The efficiency of *MK* gene expression from these mutant promoters was assayed by microinjecting them along with a reference gene (MT-β-galactosidase) into fertilized mouse eggs, incubating the eggs with or without cadmium, and then assaying for TK and β-galactosidase activities. These enzymatic assays are sensitive enough to detect expression from a single mouse egg microinjected with these plasmids and incubated as described in *Materials and Methods*.

Analysis of 5′ Deletions. As shown in Fig. 2, mutant 5′Δ-217 has essentially the same activity as the wild-type *MK* gene; thus, it appears that DNA sequences upstream of −217 contribute little to the efficiency of the promoter. Deletion to −126 reduced both uninduced and induced expression by a factor of ≈2. In 5′Δ-72, the uninduced expression was reduced to a low level; however, metal regulation was still maintained. Although there was a progressive loss in the extent of induction with further deletion, mutant 5′Δ-59 still showed some regulation by metals. Mutant 5′Δ-49 was uninducible. These data suggest that the 5′ boundary of a minimal metal-regulatory element (MRE) lies between −59 and −49, although expression and induction are clearly enhanced by the presence of MT-I sequences further upstream.

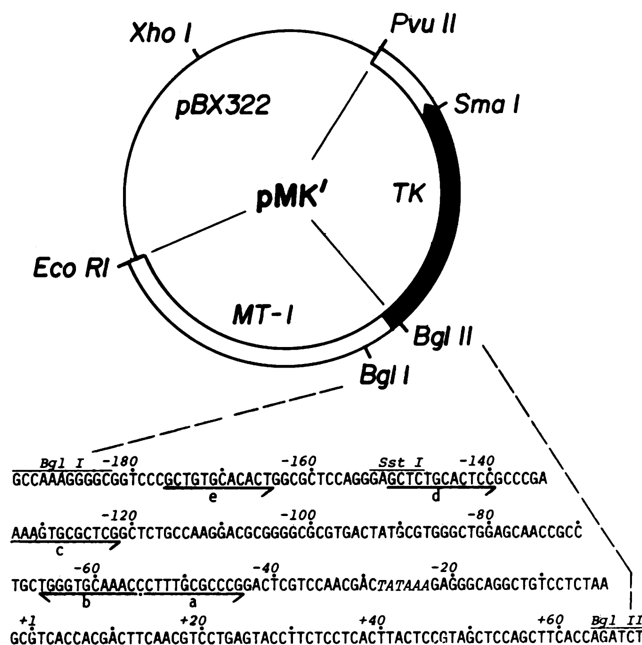


FIG. 1. Representation of plasmid pMK′. Plasmid pMK′ (5.9 kbp) contains ≈1700 bp of MT-I 5′ flanking DNA and 66 bp of exon 1 joined to the *TK* structural gene at the *Bgl* II site and inserted between *Eco*RI and *Pvu* II sites of pBX322. The resulting MT-I:TK fusion mRNA (MK) is shown as a solid arrow. DNA sequence of the MT-I promoter region is shown with cap site indicated as +1. The T-A-T-A-A-A box is in italics, and five potential metal regulatory regions are labeled a–e. See Fig. 3 for a comparison of these MRE sequences.

Analysis of Linker Fusion Mutants. To define the 3′ boundary of regulatory elements, a series of 3′ deletions in the MT-I promoter region was constructed. To maintain the appropriate spacing between MT upstream sequences and the transcription unit, we joined the 3′ MT-I deletions to suitably matched 5′ deletions of the TK promoter region. Three of these linker fusion (LF) mutants were joined downstream of the TATA box and their expression is indistinguishable from that of pMK′ (Fig. 2), suggesting that the cap sites and 5′ ends of MK and TK mRNA are functionally equivalent. Expression of LF-46/-37, in which the TATA box region of MT-I has been replaced by that of TK, also resembled that of the wild-type *MK* gene. Although the induction of the neighboring LF-44/-33 is less than that of LF-46/-37, we will argue later that both of these mutants lie close to the 3′ boundary of a MRE and that the changes introduced by LF-46/-37 are less disruptive than those produced by LF-44/-33. Removal of another 14 nucleotides of MT-I sequence in LF-60/-49 caused a marked drop in promoter efficiency. This result suggests that the 3′ boundary of an important promoter element lies between −46 and −60; this element may be identical to the minimal MRE defined by 5′ deletions because the induction of LF-60/-49 was also diminished. The observation that this mutant still responds to cadmium implies that MT-I sequences upstream of −60 (as in LF-60/-49) and downstream of −59 (as in 5′Δ-59) are able to independently confer significant metal inducibility.

Analysis of Linker Scanning and Internal Deletion Mutants. In the linker scanning (LS) mutants, short regions (≈10 bp) of the MT-I promoter region are replaced by *Bam*HI linker DNA; in the internal deletion (ID) plasmids, larger regions of MT-I sequence are missing (Fig. 2). The activity of LS-23/-16 was essentially the same as wild type; however, induction of LS-60/-49 was very much reduced relative to pMK′, consistent with the location of a MRE overlapping these nucleotides. As with LF-60/-49, the residual induction was significant and implies the existence of other sequences capable of mediating induction. To test the alternative possibility that the residual induction might be due to incomplete disruption of a unique MRE that may allow weak binding of a regulatory protein, we also tested a mutant lacking MT-I sequence from −67 to −42 (ID-67/-42). This mutant exhibited a limited but significant induction, suggesting that additional MREs are present in the MT-I promoter.

Three linker scanning mutations between −103 and −59 (see LS-95/-85, LS-82/-72, and LS-67/-59) appear to affect basal promoter function without affecting regulation by cadmium (Fig. 2), while expression of the most distal mutant, LS-113/-103, was similar to wild type. Deletion of sequences in this region (ID-113/-85) also decreased basal promoter activity without affecting induction. Thus, sequences between −103 and −59 appear to be required for optimal promoter efficiency. ID-113/-85 removes the sequence G-G-G-C-G-C-G-T-G that is conserved in other *MT* genes (9). This internal deletion and LS-95/-85 also remove a stretch of alternating purines and pyrimidines, T-A-T-G-C-G-T-G, that may be involved in basal promoter function (16).

The remaining internal deletion mutants indicate that there are additional MREs between −113 and −152 and possibly between −152 and −191; thus, ID-191/-42 showed no induction by cadmium, whereas ID-152/-42 was slightly inducible, and ID-113/-42 was induced ≈6-fold (Fig. 2).

Identification of a Consensus Sequence Involved in Metal Regulation. We have presented evidence that a sequence between −59 and −46 is critical for efficient regulation by metals. Fig. 3 compares the wild-type sequence with the sequence of various mutants in this region. The corresponding regions of the human *MT-IA*, and human *MT-IIA*, and mouse

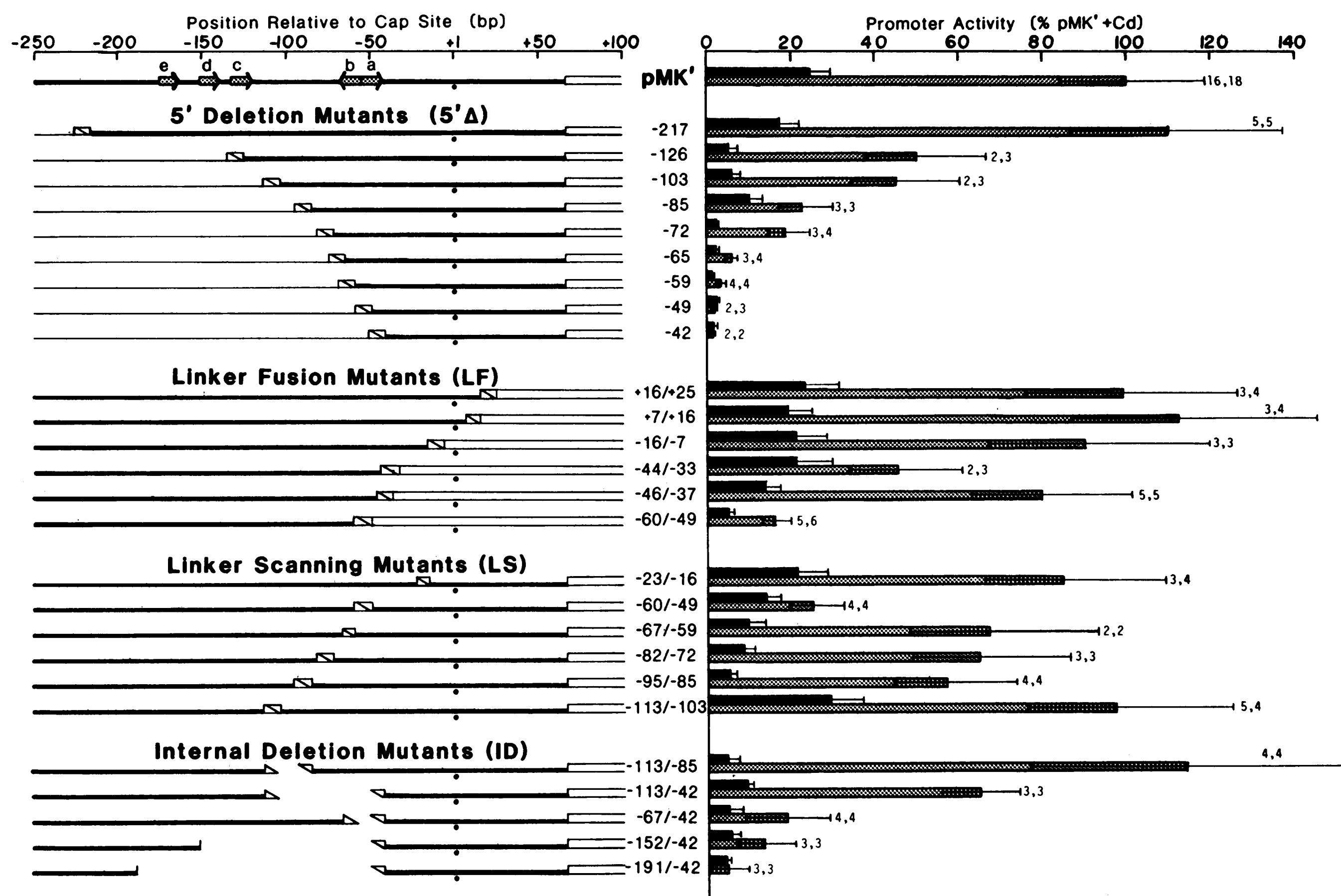
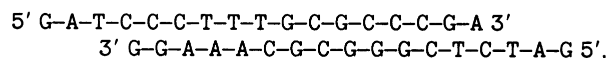


FIG. 2. Relative expression of pMK' mutants in mouse eggs. (*Left*) The wild-type pMK' and derived 5' deletion, linker fusion, linker scanning, and internal deletion mutants are shown diagramatically. Promoter region of the wild-type *MK* gene is shown with the positions of the potential MREs indicated by lettered arrows. *TK* gene sequences are shown as an open box, *MT-I* sequences are indicated by a heavy line, and pBX322 vector sequences are shown by a light line. *Bam*HI linkers are shown as boxes with a diagonal line, and position of cap site (+1) is indicated by a dot. The mutant nomenclature indicates the first base of the *Bam*HI linker on the 5' side and the first base of wild-type sequence on the 3' side of the linker; for example, LS-60/-49 retains wild-type sequence up to and including the base at −61, followed by a *Bam*HI linker, with wild-type sequence commencing again at −49. (*Right*) Relative TK activity (±SD) obtained after microinjecting these plasmids into mouse eggs and incubating eggs for 22 hr with (stippled bars) or without (solid bars) $CdSO_4$. Plasmid MT-βgal was introduced together with each test plasmid to provide an internal standard for comparison of the MT promoter activities within each experiment. Data from different experiments, each typically consisting of induced and uninduced TK and β-galactosidase activities from pMK' and 3–12 mutants, were compiled and analyzed. Numbers next to the error bars indicate number of experimental observations for eggs without and with $CdSO_4$ treatment, respectively.

MT-II genes are also shown. The sequence C-Y-T-T-T-G-C-R-Y-Y-C-G was found in all four wild-type MT promoters 15–17 bp upstream of the TATA box. We suggest this may be the binding site for a protein that mediates transcriptional regulation of *MT* genes. The consensus sequence shown in Fig. 3 extends 3 nucleotides downstream of the 3' boundary established with LF-46/-37; however, the only change introduced to the consensus sequence in LF-46/-37 is C→G at position −45. Furthermore, in LF-44/-33 the last G of this sequence (at −44) is changed to a C, and the regulation of this mutant is impaired (Fig. 2). Thus, it appears that the last G of the consensus sequence is more important for regulation than the preceding C.

The MT-I promoter contains four other sequences related to the consensus (Fig. 3). The positions of these presumptive MREs are indicated in Figs. 1 and 2. One of the sequences, MRE-b, is in an inverse orientation relative to the others, and it forms a palindrome (centered at −55) with MRE-a. However, disruption of MRE-b by LS-67/-59 had little effect on induction (Fig. 2); hence, the palindrome *per se* does not appear to be important for metal regulation. Multiple copies of closely related sequences are also present in other *MT* genes (9, 16, 18).

Oligonucleotides Generating a MRE Confer Metal Regulation on the TK Promoter. To test the validity of the MRE identified in Fig. 3 and to begin to explore its properties in isolation from other MT sequences, we inserted the synthetic DNA fragment,


5' G-A-T-C-C-C-T-T-T-G-C-G-C-C-C-G-A 3'
3' G-G-A-A-A-C-G-C-G-G-G-C-T-C-T-A-G 5',

into the *Bam*HI sites of two different TK linker scanning mutants. The resulting synthetic DNA insertion (SDI) mutants contain a single copy of MRE-a. The positions of these insertions within the TK promoter are shown in Fig. 4. Neither the wild-type *TK* gene nor the TK linker scanning mutants LS-105/-95 and LS-42/-32 was inducible by cadmium when injected into mouse eggs. The level of expression of LS-105/-95 was low, because this mutation disrupts the second distal transcription element identified by McKnight (21), whereas expression of LS-42/-32 was similar to that of wild-type *TK*. Insertion of MRE-a into LS-42/-32, a position similar to its normal position in MT promoters, appeared to allow some induction; however, its insertion into LS-105/-95 conferred greater inducibility (≈3-fold). The inducibility of SDI-105/-95 is comparable to that of mutant MT-I promoters that lack a full complement of MREs.

To test whether two copies of MRE-a might provide more efficient regulation of the *TK* gene, we combined SDI-105/

			Sequence		Regulation
1	MT-I wild type	-59	aaacCCTTTGCGCCCGgact	-40	(++)
2	LF-44/-33	-59	aaacCCTTTGCGCCCcggga	-40	(+)
3	LF-46/-37	-59	aaacCCTTTGCGCCgGgatc	-40	(++)
4	LS-60/-49	-59	cgggaTcccGCGCCCGgact	-40	(+)
5	LF-60/-49	-59	cgggaTccgGCGCggtccga	-40	(+)
6	5'Δ-49	-59	cgggaTcccGCGCCCGgact	-40	-
7	Synthetic MRE-a in TK LS mutants		ccggatcCCTTTGCGCCCGagatccgg		+
8	CONSENSUS		CYTTTGCRYYCG		
9	Human MT-IIA	-62	ggggCTTTTGCACTCGtccc	-43	
10	Mouse MT-II	-63	ggagCTTTTGCGCTCGaccc	-44	
11	Human MT-IA	-63	gggtCCTTTGCGTCCGgccc	-44	
12	Mouse MT-I (a)	-59	aaacCCTTTGCGCCCGgact	-40	
13	Mouse MT-I (b)	-51	aaagggTTTGCACCCagcag	-70	
14	Mouse MT-I (c)	-137	ccgaaaagTGCGCTCGgctC	-118	
15	Mouse MT-I (d)	-155	gggagCTcTGCACTCcgccc	-136	
16	Mouse MT-I (e)	-180	tcccgCTgTGCACaCtggcg	-161	

FIG. 3. Comparison of MREs. Lines 1–6 show sequences of wild-type *MT-I* gene between −59 and −40 and several mutants that impinge on this region. Nucleotides that are identical to wild type are underlined. Regulation by $CdSO_4$ is summarized as follows: ++, normal regulation; +, impaired regulation; −, no regulation. Values in parentheses are from mutants retaining MREs upstream that could contribute to the observed regulation (see Fig. 2). Line 7 shows sequence of synthetic MRE-a with adjacent *Bam*HI linkers as they occur in the TK linker scanning mutants described in Fig. 4. Line 8 shows a consensus sequence derived by comparison of activities of mutants in the lines above and homologies with the MT sequences shown in the lines below. Lines 9–11 show corresponding regions of other *MT* genes (9, 16, 18); lines 12–16 show the five potential MT-1 MREs lettered as in Figs. 1 and 2. Capital letters signify identity with the consensus sequence.

-95 with SDI-42/-32 by using the *Eco*RI site at −80. This double mutant containing two MREs improved the regulation considerably, giving ≈10-fold induction (Fig. 4).

DISCUSSION

Multiple homologous promoter elements are a common feature of eukaryotic genes. The G+C-rich elements of the TK promoter and simian virus 40 early promoter are repeated twice and six times, respectively (20, 21, 24). A single distal element in the TK promoter provides some function but duplicate elements appear to act cooperatively to provide full activity (21, 29). All six of the simian virus 40 promoter elements are required for full T-antigen expression *in vivo*, and they have been shown to bind a specific transcription factor *in vitro* (24, 25). Multiple homologous promoter elements are also involved in promoter regulation; several glucocorticoid receptor binding sites are found in the promoter of mouse mammary tumor virus (26), two heat shock consensus elements are required for efficient induction of heat shock genes reintroduced into flies (ref. 27; H. Pelham, personal communication), and multiple copies of a short homology unit are associated with induction of genes of the yeast histidine pathway (28). The results presented here indicate that the mouse MT-I promoter contains at least two similar regions, each of which is able to confer some degree of metal regulation independently. The boundaries of the distal region are not closely defined, but analysis of a number of mutants allowed a fairly precise definition of boundaries of the most proximal region. Comparison of the sequence of this region with other *MT* genes revealed the conserved sequence C-Y-T-T-G-C-R-Y-Y-C-G, suggesting that this sequence contributes to a MRE. Three sequences related to this consensus sequence were also found upstream, in a region to which regulatory function was ascribed (between −113 and −191). Another lies adjacent to MRE-a, but in an inverse orientation (Fig. 1).

To obtain direct proof of the regulatory function of the proximal element, we inserted a synthetic DNA fragment containing MRE-a at two positions within the TK promoter, one at a position similar to its normal situation within *MT* genes (in a construction with a fully functional TK promoter) and one where it substituted for the second distal promoter element of the *TK* gene. In the latter case, a moderate level of TK induction (≈3-fold) was observed, whereas SDI-42/-32 was less inducible. It seems likely that in the presence of heavy metals MRE-a binds a regulatory protein that facilitates transcription, perhaps in a manner similar to the distal promoter element of the *TK* gene. The presence of fully functional TK promoter elements in SDI-42/-32 may obscure the contribution of MRE-a in that position. That MRE-a can function when inserted in LS-42/-32 is indicated by the improved regulation observed with the double mutant containing two MREs (Fig. 4).

The improved regulation of the double MRE insertion is compatible with other results, indicating that there are at least two functional regulatory elements within the mouse MT-I promoter and that they may act cooperatively. While 5′ deletions suggested the presence of upstream MREs, the efficient regulation of ID-113/-42 provides the most compelling argument for their presence. Note, by comparing the effects of ID-113/-42 and ID-67/-42, that the upstream MREs appear to function best when they are moved nearer the TATA box. In the wild-type MT promoter, the upstream MREs presumably enhance the effect of those nearer the TATA box, perhaps in a manner similar to the way the second distal element of the TK promoter potentiates the first distal element (29).

The observation that a 5′ deletion mutant of the human *MT-IIA* gene lacking sequences upstream of −50 was still inducible by cadmium led Karin *et al.* (16) to propose that

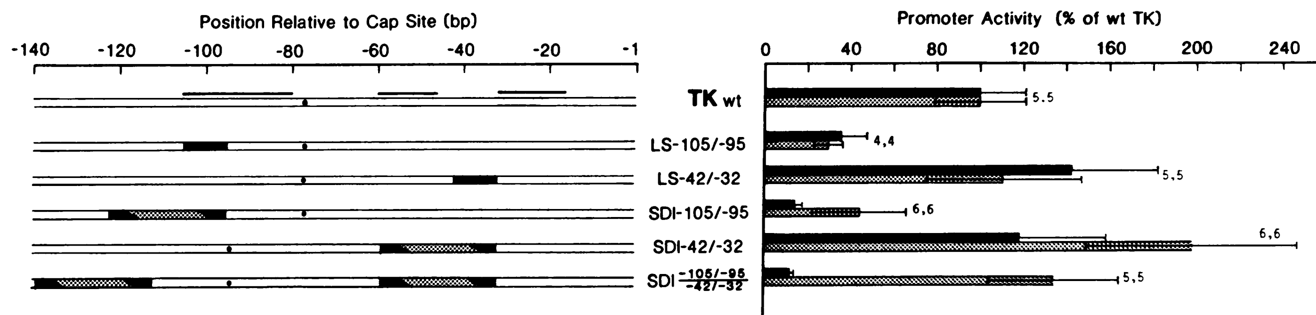


FIG. 4. Metal regulation conferred to the TK promoter by a synthetic MRE. (*Left*) Structure of the wild-type (wt) and mutant TK promoters. Lines above the top figure indicate location of promoter elements defined by McKnight (20, 21). Positions of *Bam*HI linkers in TK linker scanning mutants are shown by solid boxes. Synthetic DNA fragments containing MRE-a were inserted into each LS mutant to generate the SDI mutants shown; sequence and orientation of the SDI inserts is shown in Fig. 3. These two mutants were combined (bottom line) by using the *Eco*RI site (shown by a dot) that lies between them. (*Right*) Corresponding TK activities (±SD) obtained from eggs injected with these plasmids and incubated with (stippled bars) or without (solid bars) $CdSO_4$. Number of determinations is shown by numbers next to the error bars. For comparison with Fig. 2, the pMK′ activities averaged 80% (without Cd) and 430% (with Cd) of wt TK activity.

the short sequence immediately 3′ of this mutation confers metal inducibility. This element was identified by its homology to another element, located within an upstream region, that is capable of conferring regulation by heavy metals independently. The consensus derived, Y-X-C-G- -C-C-C-G-G- -C-T-C, overlaps the sequence proposed here but extends further 3′. The consensus proposed here is more closely conserved between *MT* genes and does not require insertions or deletions to maximize homology. The synthetic sequence that we have shown to confer metal regulation to the *TK* gene lacks the 3′ part of their consensus sequence. Moreover, our 5′Δ-49 retains the complete consensus proposed by Karin *et al.* (16), yet it is uninducible; likewise, the regulation of LS-60/-49 is impaired, although the sequence they identified is intact. An alternative explanation for the inducibility of mutant 5′ Δ-50 of the human *MT-IIA* gene that is consistent with our data is that another MRE downstream of −50 confers regulation. In fact, a sequence, C-G-C-T-G-C-A-C-T-C-C, just upstream of the cap site of the human *MT-IIA* gene shares homology with the MRE consensus proposed here. Despite these arguments, a region larger than either of the proposed consensus sequences may be required for optimal metal regulation.

In conclusion, we have identified a short region of the mouse MT-I promoter sequence that is sufficient to confer inducibility to a heterologous gene. Further studies will be required to determine whether a larger sequence might confer greater inducibility and to elucidate which of the other similar sequences are functional. This study also suggests that multiple MREs contribute to the induction of *MT* genes by metals. It will be important to ascertain the significance of the number, position, and orientation of MREs relative to other promoter elements.

We thank Steve McKnight and Bob Kingsbury for the providing TK mutant plasmids used in the construction of many of the mutants described here, Gyn An for providing pGA350, which contains the MT-βgal fusion gene, Joel Habener for providing the synthetic DNA oligomers, Larry Loeb for the gift of aphidicolin, Myrna Trumbauer for microinjecting the plasmids into mouse eggs, Abby Adams for secretarial assistance, and our colleagues for constructive suggestions during this work. This work was supported by grants from the National Institutes of Health.

1. Kagi, J. H. R. & Nordberg, M. (1979) *Metallothionein* (Birkhauser, Basel, Switzerland).
2. Mayo, K. E. & Palmiter, R. D. (1981) *J. Biol. Chem.* **256,** 2621–2624.
3. Durnam, D. M. & Palmiter, R. D. (1981) *J. Biol. Chem.* **256,** 5712–5716.
4. Durnam, D. M. & Palmiter, R. D. (1984) *Mol. Cell. Biol.* **4,** 484–491.
5. Hager, L. J. & Palmiter, R. D. (1981) *Nature (London)* **291,** 340–342.
6. Mayo, K. E., Warren, R. & Palmiter, R. D. (1982) *Cell* **29,** 99–108.
7. Karin, M. & Herschman, H. R. (1981) *Eur. J. Biochem.* **113,** 267–272.
8. Durnam, D. M., Hoffman, J. S., Quaife, C. J., Benditt, E. P., Chen, H. Y., Brinster, R. L. & Palmiter, R. D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1053–1056.
9. Searle, P. F., Davison, B. L., Stuart, G. W., Wilkie, T. M., Norstedt, G. & Palmiter, R. D. (1984) *Mol. Cell. Biol.* **4,** 1211–1230.
10. Hamer, D. H. & Walling, M. J. (1982) *J. Mol. Appl. Genet.* **1,** 273–288.
11. Karin, M., Cathala, G. & Nguyen-Huu, M. C. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4040–4044.
12. Pavlakis, G. N. & Hamer, D. H. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 397–401.
13. Brinster, R. L., Chen, H. Y., Warren, R., Sarthy, A. & Palmiter, R. D. (1982) *Nature (London)* **296,** 39–42.
14. Palmiter, R. D., Chen, H. Y. & Brinster, R. L. (1982) *Cell* **29,** 701–710.
15. Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E. & Brinster, R. L. (1983) *Science* **222,** 809–814.
16. Karin, M., Haslinger, A., Holtgreve, H., Richards, R. I., Krauter, P., Westphal, H. M. & Beato, M. (1984) *Nature (London)* **308,** 513–519.
17. Karin, M., Haslinger, A., Holtgreve, H., Cathala, G., Slater, E. & Baxter, J. D. (1984) *Cell* **36,** 371–379.
18. Richards, R. I., Heguy, A. & Karin, M. (1984) *Cell* **37,** 263–272.
19. Durnam, D. M., Perrin, F., Gannon, F. & Palmiter, R. D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6511–6515.
20. McKnight, S. L., Gavis, E. R., Kingsbury, R. & Axel, R. (1981) *Cell* **25,** 385–398.
21. McKnight, S. L. & Kingsbury, R. (1982) *Science* **217,** 316–324.
22. An, G., Hidaka, K. & Siminovitch, L. (1982) *Mol. Cell. Biol.* **2,** 1628–1632.
23. Dixon, W. (1981) *BMDP Statistical Software* (University of California Press, Los Angeles).
24. Everett, R. D., Baty, D. & Chambon, P. (1983) *Nucleic Acids Res.* **11,** 2447–2464.
25. Dynan, W. S. & Tjian, R. (1983) *Cell* **35,** 79–87.
26. Payvar, F., DeFranco, D., Firestone, G. L., Edgar, B., Wrange, O., Okret, S., Gustafsson, J. A. & Yamamoto, K. (1983) *Cell* **35,** 381–392.
27. Pelham, H. R. B. (1982) *Cell* **30,** 517–528.
28. Hinnebusch, A. G. & Fink, G. R. (1983) *J. Biol. Chem.* **258,** 5238–5247.
29. McKnight, G. S., Kingbury, R. C., Spence, A. & Smith, M. (1984) *Cell* **37,** 253–262.