# The major late transcription factor binds to and activates the mouse metallothionein I promoter

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**Human (HeLa) cells contain a protein, MLTF, which specifically binds to a DNA sequence in the adenovirus 2 major late promoter and activates transcription of that promoter. The presence of MLTF in uninfected cells suggests that this factor contributes to the transcription of some cellular genes. We find that MLTF binds in a sequence-specific manner to the 5′-flanking region of the mouse metallothionein I (mMTI) gene. Binding was localized between −101 and −94 (relative to the initiation site at +1) by DNA-binding gel electrophoresis assay and DNA methylation interference analysis. As in adenovirus, binding occurred in a region containing the sequence CPuCGTGAC. Deletion of this sequence both eliminated the binding of MLTF and produced a fourfold reduction in transcriptional efficiency in vitro. In contrast to the intact promoter, transcription from the deletion mutant promoter was not stimulated by addition of purified MLTF to an in vitro reconstituted reaction. These results suggest that MLTF contributes to the transcription of cellular genes.**

Transcription of genes by RNA polymerase II is regulated by distinct DNA sequences or elements in the vicinity of the transcription initiation site. These sequences are recognized by protein factors that influence the rate of initiation by interacting with the transcription apparatus. One such protein factor is the adenovirus major late transcription factor (MLTF or USF), which specifically activates transcription from the adenovirus 2 major late (Ad-ML) promoter (Carthew et al. 1985; Sawadogo and Roeder 1985). As isolated from human (HeLa) cells, MLTF is a 46,000-dalton protein that binds to a site in the major late promoter with high affinity (Carthew et al. 1985; Miyamoto et al. 1985; Sawadogo and Roeder 1985; Chodosh et al. 1986).

The presence of MLTF in uninfected cells suggests that this factor is active in the transcription of cellular genes. Such genes should have MLTF-binding sites upstream of their initiation sites. A computer search of promoter sequences revealed that the 5′-flanking sequence of the mouse metallothionein I (mMTI) gene contains a 12-bp region exhibiting strong homology to the MLTF-binding site in the Ad-ML promoter (Glanville et al. 1981). Metallothioneins are a class of heavy-metal-binding proteins important for heavy-metal detoxification and zinc homeostasis (Durnam and Palmiter 1984; Hamer 1986). Metallothioneins are usually iso-

lated as two major isoforms, MTI and MTII, which are encoded by distinct genes (Hamer 1986). The metallothionein genes are transcriptionally induced by heavy metals, glucocorticoids, α-interferon, and interleukin-1 (Durnam and Palmiter 1981; Mayo and Palmiter 1981; Hager and Palmiter 1982; Hamer and Walling 1982; Karin et al. 1984a,b; Friedman and Stark 1985). The mMTI gene is regulated in a manner similar to the other characterized metallothionein genes, and mMTI RNA can be detected in many murine tissues and cell lines in the absence of inducers (Durnam and Palmiter 1981; Mayo and Palmiter 1981).

Extensive genetic analysis of the mMTI promoter has identified sequences important for promoter function (Carter et al. 1984; Stuart et al. 1984, 1985). In addition to the four metal regulatory elements (MREs) dispersed throughout the promoter (Stuart et al. 1985), another region was identified that affects basal expression of the gene without affecting heavy-metal induction (Stuart et al. 1984). Linker scanning mutations and internal deletions localized this region to sequences between −103 and −85, and disruption of these sequences had a fivefold effect on transcription in vivo (Stuart et al. 1984). Furthermore, a synthetic copy of the sequence −105 to −90 has been shown to confer a fivefold activation of basal expression on a 5′ deletion mutant of the mMTI

promoter (P. Searle, pers. comm.). This sequence, −105 to −90, contains the 12-bp sequence homologous to the MLTF- binding site in adenovirus (Fig. 1).

We report here that purified MLTF binds to the sequence in the mMTI promoter and specifically stimulates transcription from the mMTI promoter in vitro.

## Results

### Purified MLTF binds to the mMTI promoter

Figure 1 shows the sequence arrangement of the various recombinant plasmids used in the present study. DNA fragments derived from these plasmids were tested for their ability to bind HeLa cell proteins using a gel electrophoresis DNA-binding assay (Fried and Crothers 1981; Garner and Revzin 1981). When a nearly homogeneous MLTF preparation (80% purity) was incubated with an end-labeled fragment containing the complete mMTI promoter, a discrete complex of slower mobility was apparent in the DNA-binding assay (Fig. 2a). The pattern and mobility of this complex closely resembled the MLTF–DNA complex formed with Ad-ML promoter DNA. The same complex was observed when mMTI DNA was incubated with partially purified MLTF or with HeLa cell extract (Fig. 2a). Additional specific complexes that formed upon incubation of Ad-ML DNA with HeLa extract were not apparent when the same extract was incubated with mMTI DNA (Fig. 2a). The additional complexes result from binding of distinct proteins to the Ad-ML promoter (Carthew et al. 1985; L. Chodosh, unpubl.).

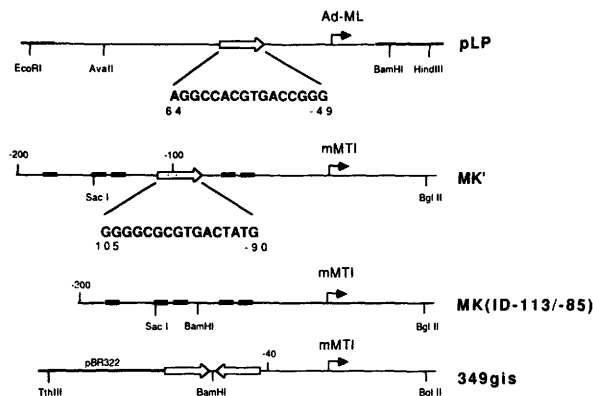MLTF was specifically involved with mMTI DNA in the formation of the complex, as shown when the

binding assay was conducted in the presence of several competitor DNAs (Fig. 2b). An unlabeled mMTI fragment competed for protein bound to the radioactive mMTI promoter DNA, as did an unlabeled fragment containing the MLTF-binding site in the Ad-ML promoter. This suggested that the same protein bound both mMTI and Ad-ML promoter DNAs. Competition was also observed using an unlabeled fragment that only included mMTI sequences from −105 to −90. In contrast, a fragment of the Ad-ML promoter that did not contain the MLTF-binding site had no effect on complex formation. Moreover, no competition was observed with an unlabeled mMTI fragment bearing a deletion of sequences −113 to −85. This deletion removes the sequences homologous to the adenovirus MLTF-binding site. Therefore, as defined by competition analysis, the mMTI sequence homologous to the Ad-ML promoter closely corresponds to the binding site for MLTF.

In the reciprocal experiment, shown in Figure 2c, the six unlabeled fragments were added to a binding reaction containing labeled Ad-ML promoter DNA. All of the fragments that contained a potential MLTF-binding site competed with the labeled Ad-ML DNA for MLTF (Fig. 2c). Fragments that lacked the potential binding site did not abolish formation of the complex on the radioactive Ad-ML DNA. Therefore, by competition analysis, MLTF binds to the mMTI promoter at a site that corresponds closely to the MLTF homology. The relative affinity of MLTF for the mMTI promoter was approximately the same as that for the Ad-ML promoter, as determined by titrations of competitor DNAs (data not shown). Interestingly, when the binding reactions were performed in the presence of 5 mM $MgCl_2$, the relative affinity of MLTF for the mMTI promoter was fivefold lower than that for Ad-ML promoter (data not shown). A similar observation has been made with MLTF binding to the rat γ-fibrinogen promoter (Chodosh et al. 1987). Because the association equilibrium constant for MLTF binding to the Ad-ML site was previously estimated to be approximately $10^{10}$ $M^{-1}$ (Chodosh et al. 1986), the affinity for MLTF interaction with the mMTI promoter is likely within an order of magnitude of this value.

### Methylation interference patterns of MLTF–DNA complexes

DNA methylation interference experiments were carried out to define further the MLTF-binding site in the mMTI promoter. Labeled mMTI DNA was partially methylated with dimethylsulfate (DMS), which attaches a methyl group to the N-7 position of individual guanines (Maxam and Gilbert 1980). The DNA was incubated with partially purified MLTF, and the protein–DNA complexes were resolved by gel electrophoresis. DNA from the complexes and unbound DNA were recovered, cleaved with piperidine, and electrophoresed on a denaturing polyacrylamide gel. Fragments containing methylated guanines that interfere with protein binding should not be present in DNA recovered from the complexes. Comparison of the cleavage pattern of bound and unbound DNA revealed that the binding site of the
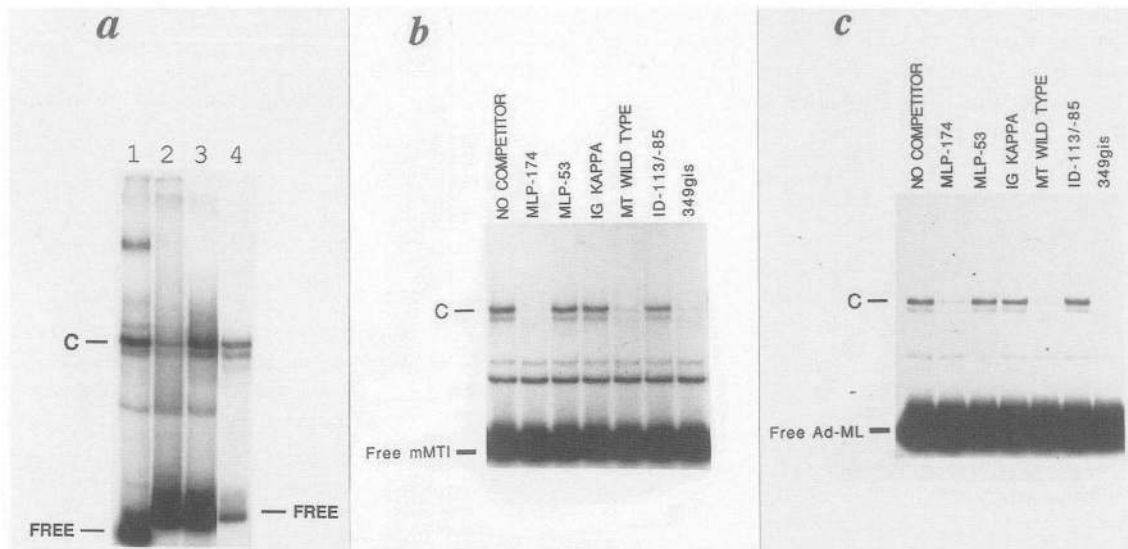


**Figure 1.** Sequence arrangement of mMTI and Ad-ML plasmids. Structures of the plasmid constructs pLP (Chodosh et al. 1986), pMK′ (Stuart et al. 1984), pMK(ID−113/−85) (Stuart et al. 1984), and p349gis (kindly supplied by P. Searle and R. Palmiter) were used in the binding and transcription reactions. p349gis contains two copies of mMTI sequences −105 to −90, inserted behind the proximal 42 bp of 5′-flanking mMTI DNA, as described by Stuart et al. (1984). The MLTF-binding site is shown as an open arrow. Thick lines represent parental plasmid sequences; thin lines represent mammalian and viral sequences. The maps indicate the positions of the major mRNA initiation sites, with numbering relative to the initiation site at +1.

**Figure 2.** (a) Binding of DNA fragments with different preparations of MLTF. Labeled DNA containing the Ad-ML promoter (lane 1) or the mMTI promoter (lanes 2–4) was incubated with carrier DNA and either 8 μg HeLa whole-cell extract protein (lanes 1 and 2), 40 μg of a DEAE-Sephacel flow-through protein fraction containing MLTF (Carthew et al. 1985) (lane 3), or 90 pg (2 fmole) of affinity-purified MLTF monomer (Chodosh et al. 1986) (lane 4). The labeled mMTI DNA fragment was derived from a BglII–SacI digest of pMK′ and was labeled at the BglII end. The labeled Ad-ML DNA fragment was derived from an EcoRI–HindIII digest of pLP. The reaction with purified MLTF monomer did not contain carrier DNA. Protein–DNA complexes and unbound DNA were resolved in a polyacrylamide gel. The predominant protein–DNA complex visualized is labeled C. (b) Binding of MLTF to mMTI in the presence of competitor DNA. The labeled mMTI DNA fragment (4 fmole) was incubated with a 240-fmole competitor fragment, as indicated above each lane, and 4 μl of a DEAE-Sephacel flow-through fraction containing MLTF. The products were resolved in a polyacrylamide gel. Bands that are not labeled C and that electrophorese more slowly than the free DNA are due to nonspecific complexes with HeLa proteins (data not shown). Six competitor fragments were prepared: (MLP-174) a 256-bp fragment extending from −174 to +33 of the Ad-ML promoter (Chodosh et al. 1986); (MLP-53) a 133-bp fragment extending from −53 to +33 of the Ad-ML promoter (Chodosh et al. 1986); (Ig-κ) a 350-bp fragment including the MOPC-41 κ-immunoglobulin promoter (Singh et al. 1986); (MT wild type) a 219-bp fragment derived from a BglII–SacI digest of plasmid pMK′; (ID−113/−85) a 201-bp fragment derived from BglII–SacI digest of plasmid pMK(ID−113/−85); (349gis) a 178-bp fragment derived from a TthIII–BamHI digest of plasmid p349gis. (c) Binding of MLTF to Ad-ML promoter in the presence of competitor DNA. Incubation conditions and competitor fragments used are as in b.

factor in the mMTI promoter was region 5′-CGCGTGAC-3′ (−101 to −94) (Fig. 3a). This sequence is part of the dodecanucleotide that is highly homologous to the MLTF-binding site in adenovirus. The analogous methylation interference experiment was carried out with a labeled DNA fragment containing the Ad-ML promoter (Fig. 3b). The binding site of MLTF, as delineated by methylation interference, was in the region 5′-CACGTGAC-3′ (−60 to −53) which lies within the DNase I footprint of MLTF (Carthew et al. 1985; Miyamoto et al. 1985; Sawadogo and Roeder 1985). The nucleotide sequence is almost identical in the Ad-ML- and mMTI-binding regions. Moreover, close contacts between MLTF and the major groove of the DNA helix, as delineated by the methylation interference pattern, followed a common spatial arrangement (Fig. 3c). The quantitative effect of methylation at each of these positions was also similar for the two complexes. However, an additional close contact with the guanine at position −100 on the mMTI-coding strand was detected by methylation interference. The equivalent position in the Ad-ML promoter is not occupied by a guanine residue but an adenine residue. This suggests that MLTF does not interact with unique features of the base pair at this position but does make close contact in the major

groove at this position. Interestingly, the sequence of the MLTF-binding sites in both promoters has imperfect dyad symmetry, and yet the arrangement of positions where methylation of guanine residues interferes with binding is asymmetric with respect to the palindrome. This degree of asymmetry in the pattern of close contacts with guanine residues has not been observed with prokaryotic proteins that bind as oligomers and recognize palindromic sequences (Johnson et al. 1978; Pabo and Sauer 1984). It was previously observed that purified MLTF exists in solution in monomeric form (Chodosh et al. 1986). Possibly, MLTF binds DNA as a monomer and recognizes its symmetric cognate sequence in an asymmetric manner.

### MLTF activates mMTI transcription in vitro

Mutational analysis has shown that sequences bearing the MLTF-binding site between −105 and −90 of the mMTI promoter contribute fivefold to the basal level of transcription in vitro (Stuart et al. 1984). It was therefore important to show that purified MLTF could stimulate transcription in vitro from the mMTI promoter in a manner that was dependent upon recognition of these same sequences. Transcription of the mMTI gene in
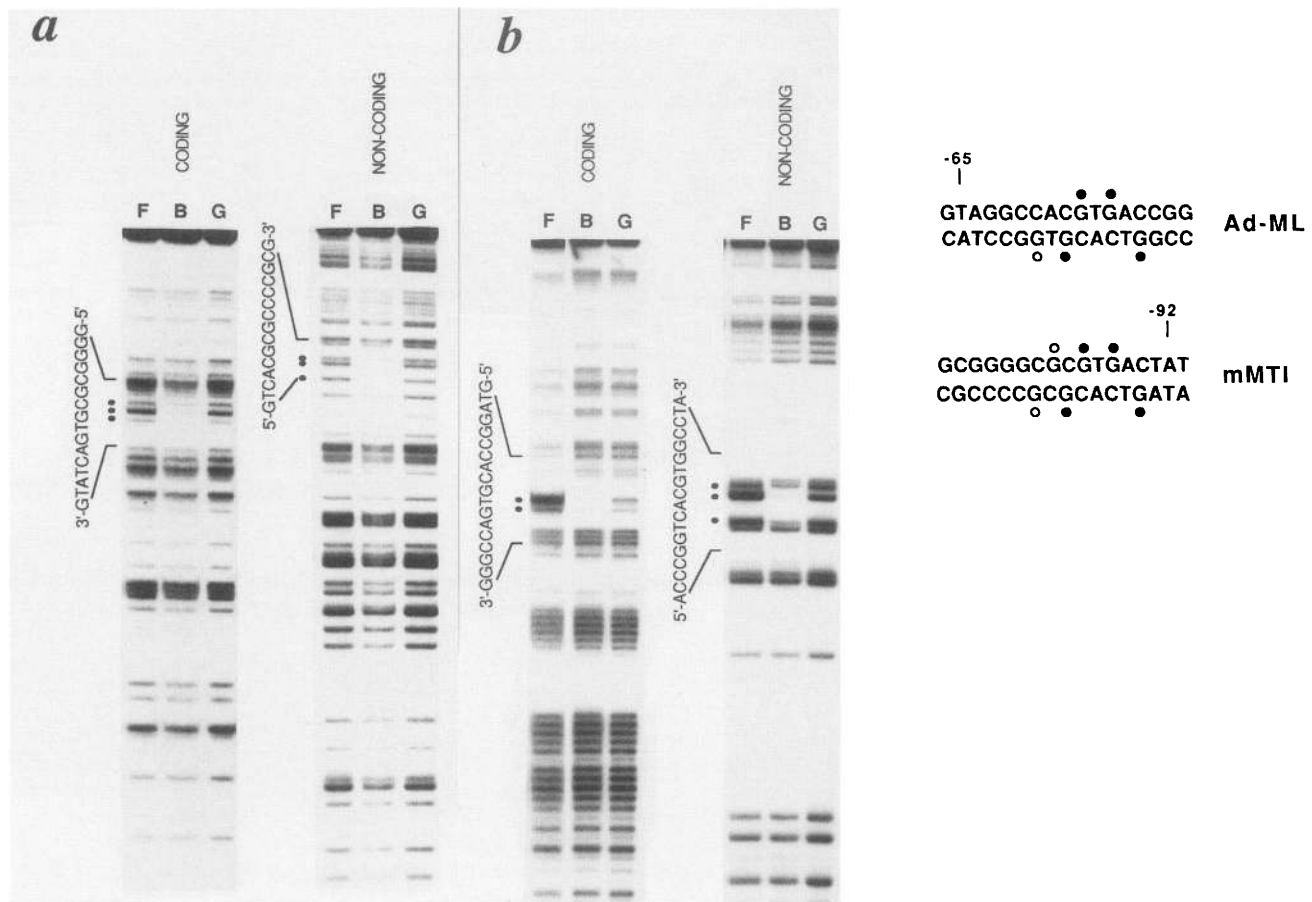
**Figure 3.** Methylation interference analysis of MLTF binding to mMTI and Ad-ML DNAs. (a) Binding to the mMTI promoter. The coding strand was 3′-end-labeled with the large fragment of *E. coli* DNA polymerase I and the noncoding strand was 5′ end-labeled with [γ-³²P]ATP and T4 polynucleotide kinase at the *Bgl*II site of pMK′ located downstream from the initiation site. (F, B) DNAs recovered from the free and specifically bound species, which were produced during the binding reaction and resolved in a polyacrylamide gel; (G) a chemical cleavage ladder at G residues in the labeled DNA. (b) Binding to the Ad-ML promoter. The coding strand was 3′-end-labeled, and the noncoding strand was 5′-end-labeled at the *Bam*HI site of plasmid pLP. The plasmid was recut with *Ava*II, and the 177-bp fragment was isolated. (F, B, and G are described above.) (c) Close contact points between MLTF and the mMTI or the Ad-ML sequences. The sequences of mMTI and Ad-ML DNA containing the MLTF-binding regions are displayed. Contacted guanine residues identified by methylation interference are marked by closed circles for strongly interfering residues and by open circles for weakly interfering residues. The sequence palindrome in the Ad-ML promoter is centered between positions −57 and −58, and extends on one side to −63 and on the other side to −52. The sequence palindrome in the mMTI promoter is centered between −98 and −99, and extends on one side to −103 and on the other side to −94.

vitro with a HeLa whole-cell extract was assayed by S1 nuclease analysis (Fig. 4a). RNA synthesized from this promoter initiated accurately at positions used by in vivo (Glanville et al. 1981). A promoter with an internal deletion from −113 to −85, which deletes the MLTF-binding site (ID −113/−85), was transcribed fourfold less efficiently than wild type (Fig. 4a). This mutant promoter is about fivefold less active than wild type in vivo (Stuart et al. 1984). In addition, a mMTI promoter construct was tested in which two copies of the MLTF-binding site from −105 to −90 had been inserted as an inverted repeat behind position −42 of a truncated mMTI promoter (see Fig. 1). This promoter is as active as the uninduced wild-type promoter in vivo (P. Searle, pers. comm.). In vitro, this promoter construct (349gis) was transcribed at a level twofold higher than wild type

(Fig. 4a). Thus, sequences containing the MLTF-binding site contribute to the transcription activity of the mMTI gene in a HeLa extract.

The S1 nuclease assays also showed evidence of RNAs that were significantly longer than those generated at the mMTI initiation site. Production of these RNAs also appeared dependent on the presence of the MLTF-binding site and addition of MLTF. Because circular DNA templates were used in the transcription reactions, RNAs generated by multiple rounds of transcription of the template would have been detected by S1 nuclease analysis as RNAs with the length observed in Figure 4. Thus, the longer RNAs detected in the above experiments probably were generated by initiation at the mMTI site.

To test whether the dependence of mMTI transcrip-

tion activity on sequences between −85 and −113 was due to promoter activation by MLTF, transcription in vitro was performed with a partially purified transcription system (Samuels et al. 1982), where MLTF had been separated from the general transcription factors by column chromatography (Carthew et al. 1985). In contrast to the results obtained using HeLa extract, all of the mMTI promoters were equally active in the general reconstituted transcription system (Fig. 4b). Addition of partially purified MLTF to the general transcription system produced a threefold activation in transcription from the wild-type mMTI promoter (Fig. 4b). The mutant mMTI promoter ID−113/−85, which had been shown to be unable to bind MLTF, was not transcriptionally stimulated by addition of MLTF. Moreover, the mMTI promoter 349gis, with two copies of the MLTF-binding site inserted behind the minimal promoter, gave a fivefold stimulation of transcription when MLTF was added to the reaction. Even though the MLTF- binding site had been duplicated and moved 54 bp closer to the mMTI initiation site, there was only a minor effect on the ability of the promoter to be transcriptionally stimulated by MLTF. These results indicate that MLTF binds specifically to the mMTI promoter, and this specific protein−DNA interaction contributes directly to promoter strength in vitro.

## Discussion

We have demonstrated that a promoter-specific transcription factor from HeLa cells that is involved in adenovirus gene transcription is also involved in the transcription of cellular genes. This factor MLTF likely activates transcription of a cellular gene, the mMTI gene, through sequence-specific binding to its promoter. This proposed mechanism in which MLTF activates transcription by binding to a promoter was originally suggested by the close correlation between the sequence-specific DNA-binding and transcription activities of MLTF on the Ad-ML promoter (Carthew et al. 1985; Sawadogo and Roeder 1985; Chodosh et al. 1986) and by the finding that a single polypeptide possesses both of these activities (Chodosh et al. 1986). Other transcription factors that possess sequence specific DNA-binding activities are also involved in the transcription of both cellular and viral genes (Dynan et al. 1986; Jones et al. 1986; Morgan et al. 1987). Thus, it appears to be a common strategy for animal viruses to utilize host promoter-specific transcription factors for viral gene expression.

Several lines of evidence support the contention that the mMTI promoter element (−101 to −94) interacts with MLTF. First, this sequence is highly homologous to the MLTF-binding site in the Ad-ML promoter (Carthew
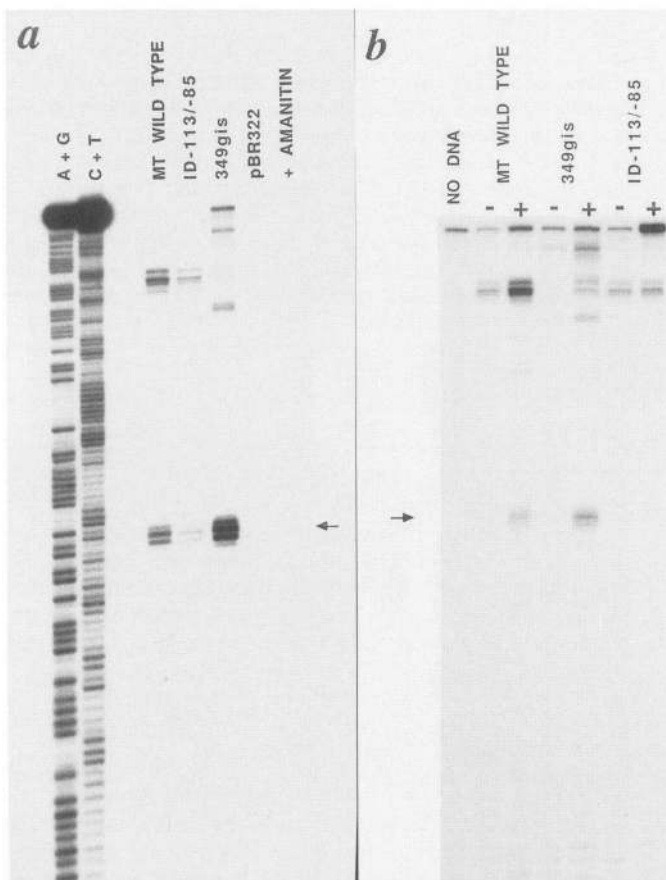


**Figure 4.** S1 nuclease analysis of RNA synthesized in vitro from the mMTI promoter. (a) Transcription reactions with HeLa whole-cell extract. Reactions contained 6 μl of HeLa whole-cell extract and 200 ng of supercoiled DNA template, as indicated above each lane. (MT wild-type) Contained pMK′ as template; (ID−113/−85) contained pMK(ID−113/−85) as template; (amanitin) contained pMK′ as template and 0.5 μg/ml α-amanitin. (A + G) A chemical cleavage ladder of A and G residues in the probe DNA; (C + T) a chemical cleavage ladder of C and T residues in the probe DNA. → Transcripts initiating at the expected position. (b) MLTF-dependent in vitro transcription. All reactions contained a mixture of RNA polymerase II and fractionated general RNA polymerase II transcription factors prepared from HeLa whole-cell extract, as described previously (Samuels et al. 1982). The DNA template used in each reaction is indicated above each lane. (MT wild-type) Contained pMK′ as template. (Lanes − and +) Reactions that contained 0 or 3 μl of MLTF, respectively. (→) Transcripts initiating at the expected position.

et al. 1985; Miyamoto et al. 1985; Sawadogo and Roeder 1985). Second, a nearly homogeneous MLTF preparation (80% purity) binds with high affinity to this sequence in the mMTI promoter. Third, this sequence effectively competes with Ad-ML DNA for binding of MLTF. Finally, the pattern of close contacts of MLTF on Ad-ML and mMTI DNA, as mapped by methylation interference, is located over homologous regions. The MLTF-binding site in the mMTI promoter contributes to the basal transcriptional efficiency of the promoter both in vivo and in vitro (Stuart et al. 1984; Fig. 4a). Moreover, transcription from the mMTI promoter was stimulated by the addition of MLTF to a partially purified transcription system only if the promoter contained the MLTF site. Stimulation of transcription by MLTF was not highly dependent upon the exact position of the MLTF site, with respect to the mMTI TATA box or initiation site.

A search through the other cloned mammalian metallothionein genes failed to reveal other potential MLTF-binding sites, even though other transcriptional elements are highly conserved among these genes (Carter et al. 1984; Karin et al. 1984; Searle et al. 1984; Stuart et al. 1984). Basal activity of the human MT-IIA promoter is controlled instead by two copies of a transcriptional enhancer, which interact with distinct transcription factors (Lee et al. 1987). Possibly, the basal expression of individual metallothionein genes is controlled by the number and strength of different promoter elements such as the MLTF element, which thereby tailors expression to fit specialized functions of the different genes. Surprisingly, the metallothionein gene of *Neurospora crassa* contains a potential MLTF-binding site in its promoter region, though it is unclear yet if this sequence plays a role in metallothionein expression (Munger et al. 1985).

Although the mMTI gene is active when transfected into HeLa cells (Mayo et al. 1982), it was important to establish that MLTF is present in murine cells. MLTF activity was detected in extracts made from a wide variety of mouse cell lines, including a Friend erythroleukemia cell line, an EC-F9 cell line, a WEHI B cell line, and a BALB/c–3T3 cell line (R. Carthew, L. Chodosh, and A. Baldwin, unpubl.). The correlation between the near ubiquitous expression of the mMTI gene in murine tissues (Durnam and Palmiter 1981; Mayo and Palmiter 1981) and the apparent presence of MLTF in many of these same tissues is consistent with MLTF having a functional role in mMTI expression. Interestingly, there is no detectable transcription of the mMTI gene in murine lymphocytes (Durnam and Palmiter 1981; Mayo and Palmiter 1981), even though levels of MLTF in extracts from lymphocytes are comparable to levels in extracts from cells that express mMTI. Possibly, other determinants such as chromatin structure prevent MLTF from interacting with the mMTI promoter in these cells, or a cell-type transcriptional element represses transcription of the gene.

A search for other potential MLTF-binding sites in the 5'-flanking regions of mammalian genes has turned up several candidates. Genes with potential binding sites include the rat and human growth hormone genes (Barta et al. 1981; Denoto et al. 1981), the rat β- and γ-fibrinogen genes (Fowlkes et al. 1984), the human ε-globin gene (Baralle et al. 1980), and the human kininogen gene (Kitamura et al. 1986). It has recently been shown that the γ-fibrinogen promoter binds MLTF and is transcriptionally stimulated by MLTF in vitro (Chodosh et al. 1987). There is no common pattern of regulation for this diverse group of genes. Thus, it remains unclear whether MLTF binding to promoters merely functions to maintain constitutive promoter strength or functions to regulate promoter activity through complex combinational interactions with other transcription factors.

## Materials and methods

### Plasmids

The plasmid pLP contains 206 bp of Ad-ML promoter DNA (−174 to +33 relative to the start site inserted in pUC13 (Chodosh et al. 1986). The mMTI plasmids pMK' (Stuart et al. 1984), pMK (ID−113/−85) (Stuart et al. 1984), and p349gis were kindly supplied by P. Searle and R. Palmiter. p349gis contains two copies of mMTI sequences, −105 to −90, inserted behind the proximal 42 bp of 5'-flanking mMTI DNA, as described by Stuart et al. (1985).

### DNA-binding reactions

DNA fragments were isolated and labeled with [α-$^{32}$P]dATP and the large fragment of *Escherichia coli* DNA polymerase, as described previously (Carthew et al. 1985). Whole-cell extract from HeLa cells (Manley et al. 1980) was fractionated as described (Samuels et al. 1982), and MLTF was purified to homogeneity as described (Chodosh et al. 1986). Binding reactions contained 2–4 fmoles of labeled DNA and, if necessary, 2 μg poly(dI-dC)·(dI-dC) as carrier DNA. Binding reactions (10 μl) were performed in 12 mM HEPES NaOH (pH 7.9), 60 mM KCl, 0.6 mM dithiothreitol (DTT), 1 mM EDTA, and 12% glycerol. After incubation at 30°C for 15 min, the complexes were resolved in a nondenaturing 4% polyacrylamide gel, as described previously (Carthew et al. 1985).

### Methylation interference analysis

The labeled DNA (25 fmoles; 10$^5$ cpm) was methylated with DMS for 5 min at 20°C. After two ethanol precipitations, the DNA was incubated with 40 μg of the DEAE-Sephacel flow-through fraction protein in a binding reaction scaled up 10-fold from the analytical reactions. The products were electrophoresed in a 4% polyacrylamide gel and, after autoradiography to visualize the DNA, the DNA was eluted from the regions of the gel containing the free DNA and protein–DNA complexes (Baldwin and Sharp 1987). The DNA was cleaved with 1 M piperidine in 100 μl at 90°C for 30 min. A portion of the original methylated DNA was also piperidine-treated to serve as a marker ladder. After lyophilization, the products were electrophoresed in an 8% polyacrylamide–8 M urea sequencing gel and were visualized by autoradiography.

### Transcription reactions

Reactions with HeLa whole-cell extract (Manley et al. 1980) contained 6 μl of extract and 200 ng of supercoiled DNA tem-

plate in a total volume of 10 μl. Reactions were performed as described elsewhere (Carthew et al. 1985) except that reactions also contained 10 mM ammonium sulfate and 200 μm of each nucleoside triphosphate. RNA derived from reactions was analyzed by S1 nuclease analysis (Berk and Sharp 1977). All reactions were stopped by addition of 1 μg/ml α-amanitin, and template DNA was digested with 1 unit DNase I (Promega) at 30°C for 10 min. RNA was extracted with phenol : chloroform : isoamyl alcohol followed by a chloroform extraction. RNA was then coprecipitated in ethanol with 50 fmoles (300,000 cpm) of radioactive probe DNA. RNA was hybridized to the probe DNA for 3 hr at 45°C in 10 μl of 80% formamide, 400 mM NaCl, 50 mM PIPES (pH 6.4), and 1 mM EDTA; it was subsequently digested with 1200 units S1 nuclease (Boehringer-Mannheim) at 16°C for 60 min in 400 μl mM sodium acetate (pH 4.5), 4 mM ZnSO$_4$, 280 mM NaCl, 10 μg/ml denatured calf thymus DNA, and 5% glycerol. Digestions were stopped with 10 mM EDTA and 20 μg tRNA, and the nuclease-resistant products were ethanol-precipitated and electrophoresed in an 8% polyacrylamide–8 M urea sequencing gel. In all analyses, probe DNA was a BglII–BamHI fragment derived from plasmid p349gis, which was end-labeled with T4 polynucleotide kinase and [γ-$^{32}$P]ATP at the BglII site.

Transcription reactions involving the addition of MLTF to the reaction were performed using a mixture of purified RNA polymerase II and fractionated general RNA polymerase II transcription factors prepared from HeLa whole-cell extract, as described previously (Samuels et al. 1982). Final reaction volumes were 20 μl, including 100 ng of supercoiled DNA template, 100 ng of carrier DNA, and a mixture of chromatographic fractions, as indicated necessary for MLTF activation of the Ad-ML promoter (Carthew et al. 1985). Reaction conditions were as described (Carthew et al. 1985), except that reactions also contained 10 mM ammoniom sulfate but did not contain MgCl$_2$ or nucleoside triphosphates. After incubation at 30°C for 60 min, nucleoside triphosphates and MgCl$_2$ were added to final concentrations of 60 μM and 5 mM, respectively, and the reaction proceeded for an additional 30 min at 30°C. RNA was prepared and analyzed as described above.

## Acknowledgments

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# The major late transcription factor binds to and activates the mouse metallothionein I promoter.

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