Metallothioneins are small, cysteine-rich proteins that function in metal detoxification and homeostasis. Metallothionein transcription is controlled by cell-specific factors, as well as developmentally modulated and metal-responsive pathways. By using the nematode Caenorhabditis elegans as a model system, the mechanism that controls cell-specific metallothionein transcription in vivo was investigated. The inducible expression of the C. elegans metallothionein genes, mtl-1 and mtl-2, occurs exclusively in intestinal cells. Sequence comparisons of these genes with other C. elegans intestinal cell-specific genes identified multiple repeats of GATA transcription factor-binding sites (i.e. GATA elements). In vivo deletion and site-directed mutation analyses confirm that one GATA element in mtl-1 and two in mtl-2 are required for transcription. Electrophoretic mobility shift assays show that the C. elegans GATA transcription factor ELT-2 specifically binds to these elements. Ectopic expression of ELT-2 in non-intestinal cells of C. elegans activates mtl-2 transcription in these cells. Likewise, mtl-2 is not expressed in nematodes in which elt-2 has been disrupted. These results indicate that cell-specific transcription of the C. elegans metallothionein genes is regulated by the binding of ELT-2 to GATA elements in these promoters. Furthermore, a model is proposed where ELT-2 constitutively activates metallothionein expression; however, a second metal-responsive factor prevents transcription in the absence of metals.

Metallothioneins (MT)

1 are a family of structurally related, low molecular weight, cysteine-rich proteins (1). The precise physiological role of MT has not been elucidated. However, evolutionary conservation across many phyla suggests that it plays a role in maintaining the homeostasis of essential trace metals; (b) sequestration of toxic metals, such as cadmium and mercury; (c) acting as a reservoir of essential metals that can be donated to other metalloproteins; and (d) protection against intracellular oxidative damage (2).

Exposure of cultured cells or whole organisms to transition metals, ionizing radiation, heat-shock, or oxidative stress induces MT transcription (1, 3–5). Metallothioneins typically occur in multigene families. The mammalian MT family consists of four members designated MT-I to MT-IV (6). It has been commented that all organisms express MT or MT-like proteins. However, not all tissues within an organism will express all MT isofoms. Numerous studies indicate that individual MT family members display specific cellular patterns of expression. Typically, MT-I and MT-II genes are coordinately expressed. It has been shown, however, that MT-2A mRNA levels do not increase in response to cadmium exposure in human proximal tubule cells (7). Expression of the human MT-1, MT-2, MT-3, and MT-4 genes varies in a cell line-specific manner in response to transition metals (8–12). Mouse MT-1 is weakly expressed in testes (13). In addition, MT-III is expressed primarily in neurons, although its mRNA is detected in testes and prostate (14–16). MT-IV is exclusively expressed in differentiating stratified squamous epithelia (17).

Metallothioneins from invertebrates also show highly restricted patterns of constitutive or metal-inducible expression. For example, sea urchin spMTA and spMTB gene expression is limited to aboral ectoderm and embryonic gut and oral ectoderm, respectively (18, 19). The Drosophila Min and Mto genes are primarily expressed in the midgut (20, 21). In addition, inducible transcription of the two MT genes of the nematode Caenorhabditis elegans occurs exclusively in the intestinal cells of larval and adult nematodes (22).

Upstream regulatory elements and transcription factors that control metal-inducible MT transcription have been identified in a variety of species (2, 23). Several models have been proposed describing the regulation of metal-inducible MT expression (24–26). However, the mechanism(s) that regulate cell-specific MT expression have not been extensively examined. Cell-specific expression has been explored in the context of DNA methylation, by using hypomethylating agents such as 5-azacytidine. Correlation between hypomethylation and the stimulation of MT gene expression has been observed for rainbow trout, mouse, and human MT genes (8, 11, 27). Differences in the levels of cadmium-inducible transcription between human MT-I and MT-I genes in liver-derived cell lines are correlated with single nucleotide changes in the TATA boxes in each promoter (28). Suppression of mouse MT-III gene expression in organs other than the brain has been attributed to CTG triplet repeats within the promoter. However, the binding of a nuclear protein to the CTG sequence has not been demonstrated (29).

This paper is available on line at http://www.jbc.org

The abbreviations used are: MT, metallothionein; bp, base pair(s); EMSA, electrophoretic mobility shift assay; MRE, metal-responsive element; URK, upstream regulatory element; kbp, kilobase pair.

---

*This work was supported in part by National Institutes of Health Grants CA 61337 (to J. H. F.) and by the Alberta Heritage Foundation for Medical Research (to T. F.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

§ Current address: ISSI Consulting Group, Inc., 999 18th St., Suite 1450, Denver, CO 80202.

** To whom correspondence should be addressed: Box 90326, Duke University, Durham, NC 27708-0326. Tel.: 919-613-8037; Fax: 919-684-8741; E-mail: jonf@duke.edu.

1 The abbreviations used are: MT, metallothionein; bp, base pair(s); EMSA, electrophoretic mobility shift assay; MRE, metal-responsive element; URK, upstream regulatory element; kbp, kilobase pair.

---

(Received for publication, March 12, 1999, and in revised form, July 27, 1999)
The participation of specific transcription factors has been proposed as a potential mechanism for the regulation of cell-specific MT transcription. A protein that binds in a metal-dependent manner to CCAAT-homologous sequences in the mouse MT-I promoter has been isolated from rat liver (30). The amino acid sequence of this protein is homologous to members of the liver-enriched C/EBP protein family. However, the function of this protein has not been explored in the context of cellular or tissue-specific regulation of MT transcription. We now report on the identification of UREs and a transcription factor that binds to these elements, which control intestinal cell-specific transcription of C. elegans MT genes.

C. elegans provides a powerful system for investigating the molecular aspects of cell-specific MT transcription. The developmental and cellular biology of C. elegans is thoroughly understood in exceptional detail (31–33). The adult C. elegans hermaphrodite contains 959 somatic cells that comprise reproductive, muscular, nervous, and digestive systems (31–33). The timing of cell division and differentiation is invariant, and the developmental lineage of each cell can be traced back to the fertilized oocyte (31). High levels of evolutionary conservation between C. elegans and other organisms are observed in many signal transduction, gene regulatory, and developmental pathways (34–37). In addition, stable lines of transgenic nematodes that express reporter transgenes in cell-specific and temporal patterns that mimic endogenous genes can easily be generated (38, 39).

Two MT genes, designated mtl-1 and mtl-2, have been identified and characterized in C. elegans (22). Induction of mtl-1 and mtl-2 transcription, following metal exposure, occurs exclusively in all intestinal cells at post-embryonic stages of development. Furthermore, significant levels of intestinal cell transcription are not observed in the absence of stress (22). These observations suggest that both tissue-specific and metal-regulatory factors control C. elegans MT expression.

Several non-metal inducible C. elegans genes are also expressed exclusively in intestinal cells. These include the six vitellogenin genes, vit-1 to vit-6, the cysteine protease cpr-1, and the gut esterase ges-1 (40–42). Multiple copies of heptameric elements, which have the consensus sequence CT-GATAA, are present in the promoters of each of these genes, as well as the two C. elegans MT genes (22, 41, 43, 44). Originally identified in the upstream regulatory regions of the C. elegans vitellogenin genes, these elements are believed to be responsible for controlling the transcription of intestinal cell-specific genes (44, 45). Deletion or mutation of the heptameric elements in reporter transgenes containing vit-2, ges-1, or cpr-1 promoters, causes either a loss of intestinal cell reporter gene expression or expression in non-intestinal cells (41, 43, 44, 46).

The nucleotide sequence of the heptameric element is identical to the consensus-binding site for the GATA family of transcription factors (GATA factors). GATA factors constitute a family of structurally related transcription factors that interact with the (A/T)GATA(A/G) consensus sequence. GATA factors are expressed in distinct developmental and tissue-specific patterns, and their involvement in the regulation of cell-specific gene transcription is well established (reviewed in Simon (47) and Evans (48)).

Several GATA factors have been isolated from C. elegans: ELT-1, ELT-2, ELT-3, and END-1 (49–52). The expression of ELT-2 is restricted to intestinal cells in embryonic and post-embryonic stages of C. elegans development. Immunofluorescence, mutagenesis, and ectopic expression experiments suggest that it is a regulator of intestinal cell-specific gene transcription. Furthermore, the intestine is not properly formed in C. elegans in which the elt-2 gene has been disrupted (45). The cell-specific pattern of post-embryonic ELT-2 expression is identical to those of both C. elegans MT genes. Thus, ELT-2 is a potential regulator of mtl-1 and mtl-2 transcription.

Analysis of the 5′-regulatory regions of the C. elegans MT genes reveals the presence of two GATA-like elements in the mtl-1 promoter and five in mtl-2 (Table I). In this report, the contribution of each of these elements in the regulation of C. elegans MT transcription is examined. In addition, the ability of the ELT-2 transcription factor to bind to the GATA elements in the C. elegans MT promoters (a) and effect MT transcription in vivo (b) is investigated. GATA elements and ELT-2 are required for mtl-1 and mtl-2 transcription. In addition, ectopic expression experiments indicate that ETL-2, alone, in the absence of added metal or stress, is sufficient to induce C. elegans MT transcription. Here we report on the roles of GATA elements and the associated transcription factor in controlling cell-specific MT transcription. Data suggest that other factors (i.e. metal sensor) contribute to the metal-inducible expression of the C. elegans MT gene. It has not previously been reported that GATA elements or transcription factors regulate MT transcription. Thus, the results presented in this report provide novel insights into the complex mechanisms that govern the expression of MT genes.

**EXPERIMENTAL PROCEDURES**

**Growth and Culture of C. elegans—**C. elegans were routinely maintained at 20 °C on NGM agar (1.7% agar, 25 mM potassium phosphate; pH 6.0, 50 mM NaCl, 2.5 μg/ml peptone, 5 μg/ml cholesterol, 1 mM MgCl2, 1 mM CaCl2) plates seeded with Escherichia coli strain OP50 as a food source (53). C. elegans used for inoculation of liquid cultures were grown on 100-mm NGM plates and then removed by washing with M9 buffer (22 mM KH2PO4, 42 mM Na2HPO4, 85 mM NaCl, 1 mM MgSO4) (54). Nematodes were then added to complete S medium (0.1 mM NaCl, 50 mM potassium phosphate, pH 6.0, 5 μg/ml cholesterol, 10 mM potassium citrate, 3 mM CaCl2, 3 mM MgSO4, 50 μM EDTA, 25 μM MnCl2, 10 μM FeSO4, 10 μM MnCl2, 10 μM ZnSO4, 1 μM CuSO4), which contained E. coli, and grown at 20 °C with constant agitation (53).

**Deletion Analysis—**Deletion analysis was used to determine the minimal DNA sequence 5′ of the mtl-1 and mtl-2 transcription start sites necessary to confer cadmium-inducible, cell-specific, and development-regulated transcription. This analysis was accomplished by creating a series of reporter transgenes in which successively larger regions of the mtl-1 and mtl-2 promoters were deleted. The reporter transgenes consisted of MT promoter fragments that were inserted into C. elegans expression vectors. These vectors express β-galactosidase (lacZ) that is fused to the nuclear targeting sequence of the SV40 large T antigen. The mtl promoter transgenes were subsequently used to generate transgenic C. elegans (22, 38, 55). Loss of cells and cell-specific patterns of cadmium-inducible β-galactosidase expression were compared between transgenic nematodes that contain reporter transgenes regulated by promoter fragments of varying lengths.

To prepare mtl-1lacZ reporter transgenes, promoter fragments were excised from the mtl-1lacZ fusion construct pMT1.1 (22). pMT1.1 contains an ~1.7-kbp of mtl-1 that is immediately upstream of the initiator (ATG). Digestion of pMT1.1 with SacI and PmlI released an ~1.3-kbp DNA fragment from the 5′-end of the upstream regulatory region. The 3′-overhang generated following the SacI digestion was removed following incubation with T4 DNA polymerase (56). After the blunt ends were joined, a reporter transgene regulated by 366 bp of mtl-1 promoter DNA was produced. This construct was designated p366mtl-1lacZ. A second construct was prepared by incubating pMT1.1 with SacII and BsaAI, removing the 3′-overhang, and then ligating the blunt ends. This generated a 5′-deletion that removed an additional 46 bp to yield p320mtl-1lacZ. A third transgene was prepared that contained 253 bp of the mtl-1 promoter, p253mtl-1lacZ. This construct was produced by first incubating pMT1.1 with XcoI and then isolating a 3289-bp DNA fragment, which contained 253 bp of the mtl-1 promoter attached to an ~3-kbp fragment of the lacZ reporter gene. This fragment was then joined to a 4188-bp DNA fragment that included the pUC19 backbone vector, the 3′ unc-54 region, and the remainder of the lacZ gene (55). The 4188-bp fragment was isolated following the sequential digestion of pMT1.1 with SacII and treating with T4 DNA polymerase to generate blunt ends and then digesting with EcoRV and XcoI.

A reporter transgene consisting of a mtl-2 promoter deletion was
prepared by incubating the mtl-2/lacZ fusion construct pMT2.1 with PetI. pMT2.1 contains 1.23 kb of the DNA adjacent to the 5'-end of mtl-2 attached to a lacZ reporter gene (22). The PetI digestion generated an ~2-kbp fragment from the 5'-end of the upstream regulatory region. Following ligation of the remaining plasmid, the resulting expression vector was transformed into the mtl-2 gene in C. elegans containing lacZ. This reporter transgene was designated p324mtl-2/lacZ. Next, a series of promoter deletions were prepared by removing short regions of DNA from the 5'-end of the upstream regulatory region in p324mtl-2/ lacZ by exonuclease III digestion (56). In these reporter transgenes, designated p282mtl-2/lacZ, p286mtl-2/lacZ, p191mtl-2/lacZ, p183mtl-1/ lacZ, p174mtl-1/ lacZ, and p150mtl-1/ lacZ, the lacZ promoter expression is regulated by 292, 269, 191, 183, and 160 bp of the mtl-2 promoter, respectively.

C. elegans were transformed with the reporter transgenes that contained promoter deletions by microinjecting young adult N2 nematodes with recombinant plasmid DNA and the plasmid pRF4, which encodes the dominant selectable marker rol-6(sa1006), as described previously (22, 38, 57). Transgenic C. elegans were selected and the reporter transgenes maintained as extrachromosomal arrays, as described previously (22).

Mutational Analysis of GATA Elements—To assess the contribution of each GATA element in the regulation of mtl-1 and mtl-2 transcription in vivo, individual GATA elements were modified. Promoter fragments that contained modified GATA elements were inserted into the oligonucleotide-directed mutagenesis (56, 58). To produce uracil-containing, single-stranded DNA templates, genomic DNA fragments of mtl-1 and mtl-2 were first cloned into pGEM plasmids (Promega). A 1566-bp mtl-1 fragment was isolated from pMT1.1 following a BamHI digestion and inserted into pGEM3zf (+), which was cut with the same restriction enzyme. This construct, designated p35mt1, contains 1529 bp of the region in mtl-1 that is upstream from the transcription start site. An ~1.2-kbp mtl-2 DNA fragment was isolated following the digestion of a genomic mtl-2 clone (22) with PetI and BstXI and then cloned into pGEM5zf (--), which was cut with the same enzymes. This construct, designated p3mt2, includes 324 bp of the 5'-regulatory region. The remainder of the insert consists of the coding region of mtl-2 (22). E. coli strain CJ236 (Δ GATA) was transformed with the pGEM plasmids and uracil-containing, single-stranded DNA templates produced by infecting the bacteria with M13K07 helper phage (56).

To generate site-directed mutations in the individual GATA elements, antisense oligonucleotides containing 6–10-bp mismatches, which also encoded unique endonuclease restriction sites, were used to prime complementary strand DNA synthesis. These oligonucleotides contained the 6–10-bp mismatch of the 5'-end of each oligonucleotide and 13–18 correct nucleotides at the 3'-end of the mutant sequence (Tables II and III). The sequences of the GATA elements were changed to those previously reported to inhibit GATA-mediated transcription of vit-3 (44). Following DNA synthesis, E. coli DH5α (Δ GATA) were transfected and the double-stranded pGEM plasmids that contained the modified promoters were recovered (56, 58). Mutations were confirmed by restriction endonuclease digestion and nucleotide sequencing.

Mutated mtl-1 promoter fragments were excised from p3mt1 vectors following incubation with PmlI and HindIII. A 432-bp mtl-1 DNA fragment, which includes 366 bp of the mtl-1 promoter, was inserted into the C. elegans expression vector pPD16.51 (55). To generate mtl-2–containing lacZ expression vectors, p3mt2 was first cut with BstXI and NcoI and an ~0.9-kbp fragment isolated. This fragment was sequen tally cut with HpaII, treated with Klenow DNA polymerase, which filled-in the 5'-overhang, and then cut with PetI. The resulting 450-bp PetI/blunt mtl-2 fragment contains 324 bp of the mtl-2 promoter and 150 bp of the structural region of the gene. The structural region includes the 5’-untranslated region, the first exon, and the intron, and 33 bp of the second exon. This DNA fragment was inserted into pPD16.51 that was cut with PetI and SmalI. This construct expressed a fusion protein consisting of the N-terminal 16 amino acids of MTL-2 and a 7-amino acid linker fused to nuclear targeted β-galactosidase. mtl-1 and mtl-2 control expression vectors, p1Control and p2Control, respectively, were prepared by excising non-mutated promoter fragments from the corresponding pGEM vectors using the cloning schemes that are outlined above. These mtl-1 and mtl-2 promoters were transformed by microinjecting young adult N2 nematodes with recombinant plasmid DNA and the rol-6 selectable marker, as described above.

Ectopic Expression of C. elegans GATA-binding Transcription Factors—To examine the effects of ELT-2 expression on MT gene transcription, a line of transgenic C. elegans was generated that contained two independent transgenes, hsp-16l5l-1-1 and mtl-2lacZ. In addition, a second line of transgenic nematodes was developed that contained both the hsp-16l5l-1 and mtl-2lacZ transgenes. In these lines of transgenic C. elegans, heat-shock will induce the ectopic expression of the GATA factors in most of the somatic cells (45, 59). If either ELT-1 or ELT-2 can activate MT transcription in vivo, then heat-shock will induce β-galac tosidase expression in non-intestinal cells and embryos. Similar meth ods were used to study the ability of these GATA factors to control ges-1 transcription in Drosophila and mouse (45, 60, 61).

Transgenic C. elegans containing the mtl-2/lacZ transgene were prepared by first injecting unc-119(ed4) mutant nematodes with p324mtl- 2/lacZ and the selectable marker pPDMM16D. The plasmid pRF4 was used to fill-in the 5'-end of one of the oligonucleotide strands. These artificially generated gapped-stranded DNA templates, genomic DNA fragments of mtl-1 and mtl-2 were first cloned into pGEM plasmids (Promega). A 1566-bp mtl-1 fragment was isolated from pMT1.1 following a BamHI digestion and inserted into pGEM3zf (+), which was cut with the same restriction enzyme. This construct, designated p35mt1, contains 1529 bp of the region in mtl-1 that is upstream from the transcription start site. An ~1.2-kbp mtl-2 DNA fragment was isolated following the digestion of a genomic mtl-2 clone (22) with PetI and BstXI and then cloned into pGEM5zf (--), which was cut with the same enzymes. This construct, designated p3mt2, includes 324 bp of the 5'-regulatory region. The remainder of the insert consists of the coding region of mtl-2 (22). E. coli strain CJ236 (Δ GATA) was transformed with the pGEM plasmids and uracil-containing, single-stranded DNA templates produced by infecting the bacteria with M13K07 helper phage (56).

To generate site-directed mutations in the individual GATA elements, antisense oligonucleotides containing 6–10-bp mismatches, which also encoded unique endonuclease restriction sites, were used to prime complementary strand DNA synthesis. These oligonucleotides contained the 5'-end of each mutant sequence and 13–18 correct nucleotides at the 3'-end of the mutant sequence (Tables II and III). The sequences of the GATA elements were changed to those previously reported to inhibit GATA-mediated transcription of vit-3 (44). Following DNA synthesis, E. coli DH5α (Δ GATA) were transfected and the double-stranded pGEM plasmids that contained the modified promoters were recovered (56, 58). Mutations were confirmed by restriction endonuclease digestion and nucleotide sequencing.

Mutated mtl-1 promoter fragments were excised from p3mt1 vectors following incubation with PmlI and HindIII. A 432-bp mtl-1 DNA fragment, which includes 366 bp of the mtl-1 promoter, was inserted into the C. elegans expression vector pPD16.51 (55). To generate mtl-2–containing lacZ expression vectors, p3mt2 was first cut with BstXI and NcoI and an ~0.9-kbp fragment isolated. This fragment was sequen tally cut with HpaII, treated with Klenow DNA polymerase, which filled-in the 5'-overhang, and then cut with PetI. The resulting 450-bp PetI/blunt mtl-2 fragment contains 324 bp of the mtl-2 promoter and 150 bp of the structural region of the gene. The structural region includes the 5’-untranslated region, the first exon, and the intron, and 33 bp of the second exon. This DNA fragment was inserted into pPD16.51 that was cut with PetI and SmalI. This construct expressed a fusion protein consisting of the N-terminal 16 amino acids of MTL-2 and a 7-amino acid linker fused to nuclear targeted β-galactosidase. mtl-1 and mtl-2 control expression vectors, p1Control and p2Control, respectively, were prepared by excising non-mutated promoter fragments from the corre sponding pGEM vectors using the cloning schemes that are outlined above. These mtl-1 and mtl-2 promoters were transformed by microinjecting young adult N2 nematodes with recombinant plasmid DNA and the rol-6 selectable marker, as described above.

Ectopic Expression of C. elegans GATA-binding Transcription Factors—To examine the effects of ELT-2 expression on MT gene transcription, a line of transgenic C. elegans was generated that contained two independent transgenes, hsp-16l5l-1-1 and mtl-2lacZ. In addition, a
Cell-specific Regulation of Metallothionein Gene Transcription

[$\alpha^{-32}$P]dCTP. For filling-in reactions, 10–20 pmol of annealed oligonucleotide were combined with 33 $\mu$M each of dATP, dGTP, dTTP, 50 $\mu$Ci of [$\alpha^{-32}$P]dCTP; and 20 units of Klenow fragment in reaction buffer (10 mM Tris-HCl, pH 7.5, 5 mM MgCl$_2$, 7.5 mM dithiothreitol) and then incubated for 15 min at room temperature. Reactions were terminated by the addition of 10 mM EDTA (final concentration) and incubation at 75 °C for 10 min. Unincorporated nucleotides were separated from the labeled products by Sephadex G-25 spin column chromatography.

DNA-protein binding reactions using *C. elegans* total protein extracts were performed by incubating 5–10 $\mu$g of protein extract with 25–50 fmol of labeled oligonucleotide (3 × 10$^6$ cpm), 1–5 $\mu$g of poly(dI-dC), and unlabeled competitor oligonucleotide in extract assay buffer (10 mM HEPES, pH 7.6, containing 10% glycerol, 50 mM KCl, 2 mM MgCl$_2$). Protein and competitor oligonucleotides were incubated for 15 min on ice, prior to the addition of labeled probe. After probe addition, reactions were incubated for 30 min at 25 °C and then placed in ice prior to electrophoresis.

DNA-protein binding reactions were also performed using in *vitro* transcribed and translated ELT-2 protein. ELT-2 protein was synthesized from the full-length elt-2 cDNA, contained in pBluescript SK$^-$ (50), using the TNT-coupled transcription-translation system (Promega). Binding reactions were performed as described previously (50). Briefly, 1 $\mu$l of the *in vitro* transcription/translation reaction mixture was combined with ~50 fmol of labeled oligonucleotide (2.5–5 × 10$^6$ cpm), 0.25–0.5 $\mu$g of poly(dI-dC), and unlabeled competitor oligonucleotide in modified Zhang Buffer (25 mM HEPES, pH 7.6, containing 10% glycerol, 50 mM NaCl, 2 mM MgCl$_2$). Protein and competitor oligonucleotides were incubated for 15 min on ice, prior to the addition of labeled probe. After probe addition, reactions were incubated for 30 min at 25 °C and then placed in ice prior to electrophoresis.

RESULTS

Deletion Analysis of the mtl-1 and mtl-2 Promoters—Deletion analysis was used as the initial step in identifying cis regulatory elements that control the intestinal cell-specific transcription of the *C. elegans* MT genes. In addition, it served to delimit the 5’-boundaries of the minimal promoters for mtl-1 and mtl-2. Minimal promoters are defined as the shortest region of DNA, upstream from the transcription start site, that can regulate cadmium-inducible, intestinal cell-specific, and developmentally modulated gene transcription.

Larval and adult *C. elegans* containing the p366mtl-1::lacZ transgene constitutively expressed β-galactosidase in three pharyngeal cells. This pattern of constitutive expression is similar to that previously observed (22). Larval *C. elegans* exhibited high levels of cadmium-inducible β-galactosidase expression, whereas expression was attenuated in adult nematodes. The intestinal cell-specific pattern and levels of inducible reporter gene expression were identical to those previously observed in *C. elegans* that contain the pMT1.1 reporter transgene (Table IV) (22).

When p320mtl-1::lacZ nematodes were treated with cadmium, β-galactosidase expression was induced in the intestinal cells of L1 and L2 larvae. However, mtl-1 promoter activity in L3, L4, and adult intestinal cells was not observed. The results suggest that the region between –366 and –320 bp contains a regulatory element(s) that controls mtl-1 transcription in larval development. Transgenic nematodes containing p253mtl-1::lacZ constitutively expressed lacZ in three pharyngeal cells; however, cadmium exposure did not induce intestinal cell transcription of the reporter transgene. The region between –320 and –253 includes one GATA element, GATA1.1 (Table I), and this result suggests that this element may be involved in the regulation of mtl-1 transcription.

Transgenic nematodes containing the mtl-2 reporter transgene, p324mtl-2::lacZ, were exposed to cadmium or heat shock. The pattern of inducible mtl-2 promoter activity was identical to that previously observed in transgenic nematodes carrying the pMT2.1 transgene (22). β-Galactosidase activity was detected exclusively in the larval and adult intestinal cell nuclei. In p292mtl-2::lacZ, an additional 32 bp of DNA was removed from the 5’-end of the upstream regulatory region, which contains sequences that are homologous to AP-1 and AP-1 UREs (22). Treatment of transgenic *C. elegans* carrying p292mtl-2::lacZ with cadmium induced reporter gene expression in intestinal cells of larvae and adults. However, the level of promoter activity appeared to be reduced by ~50% compared with that of transgenic nematodes containing either pMT2.1 or p324mtl-2::lacZ. This result suggests that these potential regulatory elements may participate in the transcriptional control of mtl-2.

Cadmium-treated and non-treated transgenic *C. elegans* containing p269mtl-2::lacZ did not express β-galactosidase in any cells. This transgene is missing an additional 23 bp from the 5’-end of the upstream regulatory region of mtl-2, which includes the GATA2.1 element (Table I). This result suggests that GATA2.1 may be essential for mtl-2 transcription.

Additional 5’-deletions of the mtl-2 promoter were also examined (Table IV). β-Galactosidase activity was not detected in any cells of transgenic nematodes, in either the presence or absence of cadmium.

Deletion analysis demonstrated that all information necessary to control cadmium-inducible, intestinal cell-specific, and developmentally regulated mtl-1 and mtl-2 transcription is present within 366 and 324 bp upstream of the transcription start sites, respectively. These fragments were defined as the minimal promoters. In addition, the results suggest that GATA elements are involved in the regulation of *C. elegans* MT gene transcription.

Site-direction Mutation Analysis of mtl-1 and mtl-2 GATA Elements—The functional contribution of GATA elements in regulating transcription of the *C. elegans* MT genes was determined by site-directed mutation analysis. The effect of mutating each GATA element on *C. elegans* MT promoter activity was assessed in *vivo* using transgenic nematodes.

*C. elegans* containing a reporter gene in which the GATA1.1 element (~290 to ~284) was modified did not express significant amounts of β-galactosidase following cadmium exposure, relative to levels observed in transgenic nematodes containing the p1 control transgene (Fig. 1). In contrast, mutation of the GATA1.2 element (~71 to ~65) had no detectable effect on either the level or pattern of β-galactosidase expression in cadmium-treated transgenic *C. elegans*. These results suggest that the GATA1.1 element is essential for mtl-1 transcription.

Cadmium treatment of *C. elegans* carrying transgenes consisting of mtl-2 minimal promoters in which the sequence of either the GATA2.1 (~278 to ~272) or GATA2.4 (~65 to ~59) element was modified did not activate gene expression (i.e. β-galactosidase was not detected) (Fig. 1). The levels of cadmium-inducible β-galactosidase expression in *C. elegans* that con-
metallothionein promoters. mtl-1
the
A sequence-specific DNA-protein complex to these sequences. A sequence-specific DNA-protein complex identified several GATA elements that are required for cadmium induction of mtl-1 and mtl-2—In vivo transcription. The formation of this complex was successfully competed by the addition of excess of unlabeled oligonucleotide (Fig. 3, lanes 3–5). Addition of up to a 100-fold molar excess of mutant GATA2.1 oligonucleotide (Tables II and III) did not affect complex formation (Fig. 3, lanes 6–8). Furthermore, a GATA-specific protein-DNA complex was not generated when C. elegans proteins were combined with a labeled oligonucleotide containing the mutant GATA2.1 sequence (Fig. 3, lanes 10–13). Similar results were obtained when oligonucleotides that contained the GATA 2.4 sequences were used (data not shown).

These results indicate that C. elegans proteins specifically bind to mtl-1 and mtl-2 GATA elements. However, no differences in mobility shift patterns were observed in extracts prepared from cadmium-treated versus non-treated C. elegans (data not shown).

Binding of ELT-2 to mtl-1 and mtl-2 GATA Elements—The ability of the C. elegans GATA-binding transcription factor ELT-2 to form complexes with GATA1.1, GATA2.1, or GATA2.4 elements was investigated. Oligonucleotide probes that included these GATA sequences were incubated with in vitro transcribed/translated ELT-2 protein. ELT-2-DNA complexes were formed in reactions that contained any of the three oligonucleotide probes (Fig. 4). Formation of these complexes was successfully competed by the addition of identical, unlabeled oligonucleotides (Fig. 4, lanes 4–6). In contrast, the addition of up to 100-fold molar excesses of unlabeled mutant oligonucleotides, in which GATA sequences were changed from TGATAA to GGAATC, had no effect on complex formation (Fig. 4, lanes 8–10). These results demonstrate that ELT-2 binds in a sequence-specific manner to the mtl-1 and mtl-2 GATA elements that were shown essential for transcription.

In Vivo Activation of mtl-2 Transcription by ELT-2—Several strains of C. elegans containing double transgenes were prepared to define further the interaction between the regulation of C. elegans MT transcription and GATA factors in vivo. When C. elegans strain JF5(hsp-16/elt-2, mtl-2/lacZ) was heat-shocked, β-galactosidase expression was detected in hypodermal, muscle, nerve, and pharyngeal cells of L3-L4 larvae and adult nematodes (Fig. 5, A and B). The mtl-2 gene is not normally transcribed in these cell types (22). This indicates that ELT-2 alone is sufficient to induce mtl-2 transcription. Interestingly, reporter gene expression was not observed in the intestinal cells of L3-L4 larvae and adult JF5 hsp-16/elt-2, mtl-2/lacZ) nematodes. Heat-shock induced reporter gene transcription in the intestinal cells of JF5(hsp-16/elt-2, mtl-2/lacZ) embryos (2-fold or later) or L1-L2 larva (Fig. 5, C and D). In contrast, heat-shock did not induce mtl-2 transcription in embryos or non-intestinal cells of nematodes that did not ectopically express ELT-2 (i.e. JF4(mtl-2/lacZ)). Furthermore, the frequency and level of reporter gene expression in the intestinal cells of heat-shocked JF4(mtl-2/lacZ) C. elegans was 10-fold lower compared with JF5(hsp-16/elt-2, mtl-2/lacZ). Exposure of the JF4(mtl-2/lacZ) or JF5(hsp-16/elt-2, mtl-2/lacZ) strains to cadmium induced reporter gene transcription exclusively in the intestinal cells of post-embryonic nematodes (Fig. 6, A and B). Activation of mtl-2 transcription did not occur.

tained reporter transgenes in which the sequence of the remaining three GATA elements, 2.2, 2.3 or 2.5, were changed were identical to that of transgenic p2 control C. elegans. This suggests that only GATA2.1 and GATA2.4 are involved in the regulation of mtl-2 transcription. In addition, both of these elements must be present in order for mtl-2 transcription to occur.

None of the mtl-1 or mtl-2 GATA mutations caused either an increase in β-galactosidase expression in the absence of metal or expression in non-intestinal cells, suggesting that these UREs participate in activation rather than repression of transcription.

Binding of C. elegans Extract Proteins to GATA Elements in mtl-1 and mtl-2—In vivo site-directed mutagenesis analysis identified several GATA elements that are required for cadmium-inducible transcription of mtl-1 and mtl-2. EMSA were performed to determine if C. elegans proteins specifically bind to these sequences. A sequence-specific DNA-protein complex was formed when a 32P-labeled, double-stranded oligonucleotide probe, which included the GATA1.1 sequence (Tables II and III), was incubated with C. elegans protein extracts (Fig. 2). Formation of this complex was successfully competed by the addition of a 50-fold molar excess of unlabeled GATA1.1 oligonucleotide (Fig. 2, lane 3). The addition of a 50-fold molar excess of an unlabeled oligonucleotide in which the sequence of the GATA1.1 element was changed from CTGATAA to CGGATCC (i.e. mutant GATA) did not compete with the binding (Fig. 2, lane 4). This modification is identical to that used above in the site-directed mutagenesis analysis in which cadmium-inducible reporter gene expression did not occur. An oligonucleotide, in which a sequence adjacent to the GATA1.1 element was modified, did compete with complex formation (Fig. 2, lane 5). In addition, protein-DNA complexes were not produced when nematode extract proteins were incubated with a 32P-labeled oligonucleotide that contained the mutant GATA1.1 sequence (Fig. 2, lanes 7–9).

EMSA were also used to examine protein interactions with the GATA2.1 element from mtl-2. A DNA-protein complex was formed between C. elegans extract proteins and a labeled oligonucleotide that included the GATA2.1 sequence (Fig. 3, lane 2). The formation of this complex was successfully competed by the addition of excess of unlabeled oligonucleotide (Fig. 3, lanes 3–5). Addition of up to a 100-fold molar excess of mutant GATA2.1 oligonucleotide (Tables II and III) did not affect complex formation (Fig. 3, lanes 6–8). Furthermore, a GATA-specific protein-DNA complex was not generated when C. elegans proteins were combined with a labeled oligonucleotide containing the mutant GATA2.1 sequence (Fig. 3, lanes 10–13). Similar results were obtained when oligonucleotides that contained the GATA 2.4 sequences were used (data not shown).

These results indicate that C. elegans proteins specifically bind to mtl-1 and mtl-2 GATA elements. However, no differences in mobility shift patterns were observed in extracts prepared from cadmium-treated versus non-treated C. elegans (data not shown).
in either strain in the absence of metal or heat-shock (Fig. 6, C and D).

Heat-shock treatment of *C. elegans* strain JF6(*hsp-16/elt-1* and *mtl-2/lacZ*), which ectopically expresses ELT-1, predominantly activated *mtl-2* transcription in intestinal cells. The level of intestinal cell expression was comparable to that of heat-shocked JF4(*mtl-2/lacZ*) nematodes. Ectopic expression of ELT-1 did not activate *mtl-2* transcription in embryonic intestinal cells. Reporter gene expression in non-intestinal cells (i.e. body wall and pharynx muscle and hypodermal cells) was observed in 1–2% of the β-galactosidase-expressing JF6(*hsp-16/elt-1*, *mtl-2/lacZ*) *C. elegans*. However, the levels and frequency of expression were considerably lower than those observed in the JF5(*hsp-16/elt-2*, *mtl-2/lacZ*) strain and are not considered significant. These results suggest that ELT-1 does not contribute to the regulation of *C. elegans* MT transcription.

An additional strain of *C. elegans*, JF7(*mtl-2/lacZ*, *elt-2*/*elt-2*), was created through a genetic cross of JF4(*mtl-2/lacZ*) with the homozygous null mutant *elt-2/ca15*) (45). *C. elegans* with *elt-2*/*elt-2* genotype was identified by polymerase chain reaction of genomic DNA isolated from individual nematodes using primers that flank *elt-2* (45). Single JF7(*mtl-2/lacZ*, *elt-2*/*elt-2*) nematodes were isolated and allowed to grow for ~3 days. The progeny were stained for β-galactosidase activity. Of the 357 nematodes examined, 165 (46%) expressed the reporter gene in the intestinal cells. These *C. elegans* were either *elt-2*/*elt-2* or *elt-2*/*elt-2*, as determined by the proper development of the intestinal lumen (45) (Fig. 7B). Fifty-seven nematodes were *elt-2*/*elt-2*, as determined by the improperly formed intestine. The majority of these nematodes did not express β-galactosidase (Fig. 7A). Five of the *elt-2*/*elt-2* *C. elegans* showed reporter gene expression in one or two intestinal cells. However, the level of β-galactosidase expression was substantially lower than that observed in *elt-2*/*elt-2* or *elt-2*/*elt-2* *C. elegans* and is not significant. When the JF4(*mtl-2/lacZ*) strain was grown on cadmium-containing NGM plates, only ~55% of the progeny expressed the transgene in the intestinal cells. The ability of ectopically expressed ELT-2 to activate *mtl-2* transcription (a) and the lack of *mtl-2* transcription in *elt-2*/*elt-2* *C. elegans* (b) indicate that this GATA factor regulates *C. elegans* MT transcription in vivo.

**DISCUSSION**

The ability of transition metals, as well as other physiologic stressors, to induce MT expression has been established for decades (1). Two models have been proposed that describe how metals induce MT transcription (25, 26). However, metal exposure alone is not sufficient to activate expression. For example, cadmium accumulates in the prostate of metal-exposed rats, but MT expression is not induced in this tissue (66). Thus, cell-specific processes are also involved in regulating MT gene expression.

A comparison among the nucleotide sequences of *mtl-1* and *mtl-2*, and other *C. elegans* genes whose expression is limited to intestinal cells, reveals the presence of multiple potential binding sites for GATA family transcription factors (Table 1). Because GATA elements are involved in the regulation of cell-specific gene expression in a variety of organisms, they may also participate in the intestine-specific expression of *mtl-1* and

---

**Table I**

| Site | Gene       | Sequencea |
|------|------------|-----------|
| GATA 1.1 | mtl-1 | −300 TGTCAATAAGCGATCCCGAATCGAGTCAGTA −279 |
| GATA 1.2 | mtl-1 | −81 CCCACCTCTCGAGATCCATATATGAGTGTCGTGTTG −51 |
| GATA 2.1 | mtl-1 | −288 ACACAGCTACAGCAGGCGCGCTTCAGTCATAC −259 |
| GATA 2.2 | mtl-2 | −238 AACACCGCGGCGGGCGTAGGATCGCCTCAC −241 |
| GATA 2.3 | mtl-2 | −109 CAAAGCTGCTCGAGTCTGACCTATCTG −78 |
| GATA 2.4 | mtl-2 | −75 GTGACGCGGCGGCGGGCCTGCGGTAATG −46 |
| GATA 2.5 | mtl-2 | −91 CTGATACGGGC GGCGCGGTGTATTGACGCGACG −54 |

---

**Table II**

| Site Gene Sequence | Site Gene Sequence |
|-------------------|-------------------|
| GATA 2.4 mut      | GATA 2.1 mut      |
| mtl-1             | mtl-1             |
| −91 CTGATACGGGC   | −75 GTGACGCGGCGG |
| aGGCGCGGTGTATTGACGCGACG | aGGCGCGGTGTATTGACGCGACG |
| GATA 2.3 mut      | GATA 2.5 mut      |
| mtl-2             | mtl-2             |
| −75 GTGACGCGGCGG |
| aGGCGCGGTGTATTGACGCGACG | aGGCGCGGTGTATTGACGCGACG |
| GATA 2.2 mut      | GATA 1.1 WT       |
| mtl-1             | mtl-1             |
| −287 CACACCGAGA   | −299 TGTCAATAAATCGATAAAATCGAGAAA −275 |
| aCGGCGCGGTGTATTGACGCGACG | aCGGCGCGGTGTATTGACGCGACG |
| GATA 2.1 WT       | GATA 1.1 mut      |
| mtl-1             | mtl-1             |
| −287 CACACCGAGA   | −299 TGTCAATAAATCGATAAAATCGAGAAA −275 |
| aCGGCGCGGTGTATTGACGCGACG | aCGGCGCGGTGTATTGACGCGACG |

---

**Table III**

| Site Gene Sequence | Site Gene Sequence |
|-------------------|-------------------|
| GATA 2.4 mut      | GATA 2.1 WT       |
| mtl-1             | mtl-1             |
| −75 GTGACGCGGCGG |
| aGGCGCGGTGTATTGACGCGACG | aGGCGCGGTGTATTGACGCGACG |

---

a Nucleotides that have been changed from the original gene sequence are indicated in bold. The locations of the homologous sequences in the genes are shown relative to the transcription start site.
Cell-specific Regulation of Metallothionein Gene Transcription

mtl-2. In vivo deletion and site-directed mutagenesis analyses confirm that one of these elements in mtl-1 and two in mtl-2 are required for cadmium-inducible MT transcription (Table IV and Fig. 1).

Six families of vertebrate GATA-binding transcription factors, GATA-1 through GATA-6, have been identified. Each has distinct but overlapping tissue-specific patterns of expression. Members of the vertebrate GATA-4/5/6 family are expressed in intestinal cells. The factors may be involved in gastrointestinal development (67–74) and may regulate intestinal cell-specific gene expression. The DNA binding domain of ELT-2 is most closely related to the vertebrate GATA-5 factor (50).

Four GATA factors have been isolated from C. elegans: ELT-1, ELT-2, ELT-3, and END-1. The expression of ELT-1 and ELT-3 is limited to embryonic epidermal and hypodermal cells, respectively (51, 52). The GATA factor END-1 is involved in the development of the nematode intestine. C. elegans intestinal cells arise from a single progenitor cell, E cell, which is formed at the 8-cell stage of embryogenesis (75). END-1 is only detected between the 1E and 4E cell stages of development and functions during early intestine formation (49). The pattern of end-1 expression does not overlap those of C. elegans MT genes. Thus, ELT-1, ELT-3, and END-1 are not likely to be regulators of C. elegans MT transcription.

In contrast, Elt-2 protein and mRNA are first detected at the 2E-cell stage and are present in all of the intestinal cells. They are continuously expressed throughout development and in the adult nematode (50). The post-embryonic pattern of elt-2 expression is identical to those of mtl-1 and mtl-2 (i.e. exclusively...
in intestinal cells), which suggests that it may be a regulator of MT gene expression.

Electrophoretic mobility shift assays, using C. elegans protein extracts, confirms that nematode protein(s) bind to the mtl-1 and mtl-2 GATA elements, which were shown by in vivo mutagenesis analysis to be required for MT transcription (Figs. 2 and 3). In vitro expressed ELT-2 forms a sequence-specific complex with these GATA elements (Fig. 4). These results confirm that ELT-2 binds to the C. elegans MT GATA elements. However, they do not conclusively demonstrate that ELT-2 regulates MT transcription.

Ectopic expression of ELT-2 activates mtl-2 transcription in the absence of metal exposure (Fig. 5, A–D). This result indicates that ELT-2 regulates C. elegans MT transcription. Similar results were obtained in studies of the gut esterase gene, ges-1. Embryonic expression of GES-1 is limited to the E cell lineage (42, 46). When ELT-2 is ectopically expressed, however, GES-1 is observed in most of the cells in the embryo (45).

Heat-shock of JF5(hsp-16/elt-2, mtl-2/lacZ) nematodes activates mtl-2 transcription in intestinal cells of embryos at later stages of development (Fig. 5C) (a); intestinal cells of L1–L2 larvae (Fig. 5D) (b); and hypodermal, pharynx, muscle, and nerve cells of L3-L4 larvae and adults (Fig. 5, A and B) (c). The pattern, level, and frequency of mtl-2 transcription in nematodes that express ELT-2 in all cell types are significantly different than those observed in cadmium-treated animals. In addition, heat-shock of the JF4(mtl-2/lacZ) strain does not induce significant levels of mtl-2 transcription. These results indicate that ELT-2 alone is sufficient to activate MT transcription, i.e. elevated concentrations of metal are not necessary to induce transcription.

Some characteristics of GATA elements and ELT-2 resemble those of MREs and MTF-1. For example, deletion or modification of specific GATA-sequences in mtl-1 or mtl-2 prevents metal-inducible transcription in vivo (Fig. 1). Likewise, deletion or modification of specific MREs will prevent metal-inducible transcription of mammalian MTs (76). However, data indicate that GATA elements and ELT-2 do not control metal responsiveness. GATA elements and ELT-2 are responsible for determining the intestinal cell specificity of MT transcription. GATA elements are found in the upstream regulatory regions of C. elegans intestinal cell-specific genes including vitellogenins, gut esterase, P-glycoprotein, and cysteine protease (41, 44, 46, 77). Metal-inducible transcription of these genes has not been reported, suggesting that GATA sequences do not function as C. elegans MREs. In addition, cadmium exposure does not induce the transcription of a GATA element containing intestinal cell-specific aspartic protease. ELT-2 contains a single zinc finger domain; however, mouse and human MTF-1 contain multiple zinc fingers (25, 50). A characteristic of MTF-1, and other mammalian metal-responsive transcription factors, is the ability of low concentrations of EDTA to inhibit MTF-1-DNA complex formation (25, 78–80). The binding of ELT-2 to GATA elements is significantly less sensitive to EDTA than MTF-1 binding to MREs.

The transcription of C. elegans MT genes is limited to intestinal cells as a consequence of the presence of GATA elements

2 J. H. Freedman, unpublished observations.
3 J. H. Freedman and L. H. Moilanen, unpublished observations.
in the promoters (a) and ELT-2 being expressed exclusively in intestinal cells (b). Thus, intestinal cell-specific expression is controlled simply by limiting the expression of an essential transcription factor to this specific cell lineage. ELT-2 can activate MT transcription in the absence of metal exposure (Fig. 5), and it is constitutively expressed in intestinal cells in embryonic and post-embryonic stage of development (45, 50). However, mtl-1 and mtl-2 are not usually transcribed unless the nematodes are exposed to metal. This indicates that additional regulatory processes contribute to the control of metal-inducible MT gene expression. Since ELT-2 is not the “metal sensor,” additional factors must act to regulate the metal inducibility. These factors may directly interact with ELT-2. Vertebrate GATA-factors interact with a variety of proteins, including AP-1, SP-1, and YY-1 (81–87). This interaction can result in either the stimulation or repression of transcription. Alternatively, UREs that bind metal-responsive transcription factors independently of GATA factors may be present in the promoters of the C. elegans MT genes.

ELT-2 expression in embryos failed to induce mtl-2 transcription in intestinal cells before the 2-fold stage of development. Previous studies have shown that heat-shocked JM57(hsp-16/elt-2) express ELT-2 protein in most of the cells in early embryos. Ectopically expressed ELT-2 induces the expression of other intestinal cell-specific genes in non-intestinal cell lineages (45). Thus, although ELT-2 alone appears to be sufficient to active mtl-2 transcription, additional processes must participate in determining the correct developmental pattern of expression. Developmentally modulated MT expression has been described in a variety of other species. Transcription of the Drosophila Mtn gene is not detected until the beginning of germ band retraction in endodermal gut primordia (88). In mice, MT-IV is not detected prior to day 7 postpartum (17). Furthermore, MT is not detected in human fetal brain in less than the 35-week-old fetus (89). The mechanisms responsible for developmental regulation of MT transcription have not been elucidated.

Several observations are consistent with a model for the regulation of metal-inducible, intestinal cell-specific C. elegans MT gene expression that incorporates a metal-sensitive repressor protein. First, intestinal cell expression of mtl-2 in heat-shocked L3–L4 and adult JF5(hsp-16/elt-2, mtl-2/lacZ) nematodes is infrequent and weak compared with other cell types and the levels observed in cadmium-treated nematodes. Second, although ELT-2 alone activates MT transcription independent of cell type or metal exposure, and it is constitutively expressed in the intestinal cells, the MT genes are only transcribed following metal exposure. In the model, a repressor protein inhibits the ability of ELT-2 to constitutively activate MT transcription. Since cadmium treatment did not affect the binding of ELT-2 to the GATA elements, the metalloregulatory protein would not inhibit ELT-2 binding, rather it prevents ELT-2-mediated transcriptional activation of the C. elegans MT genes. In the presence of metal, repression is released and then ELT-2 can activate transcription in the intestine.

Repressor-mediated regulation of stress-inducible gene tran-
Cell-specific Regulation of Metallothionein Gene Transcription

The ectopic expression of ELT-2 in late embryos and L1–L2 larvae exclusively activates mtl-2 transcription in intestinal cells. The lack of expression in non-intestinal cells may be due to the presence of developmental stage-specific regulators, which are not present in older larvae and adult *C. elegans*. The intestinal expression may be the result of relatively high levels of ELT-2 produced in the heat-shocked JF5(elt-2, mtl-2/lacZ) nematodes that may overcome or “titrate out” a repressor protein. Further investigations will be necessary to confirm the repressor model and resolve any inconsistencies.

Regulation of gene expression by GATA elements and transcription factors is an evolutionarily conserved process. Since ELT-2, a homologue of vertebrate GATA (50), can bind to mtl-1 and mtl-2 UREs and control MT transcription, a similar process may function in higher eukaryotes. Sequence analysis of the upstream regulatory regions in MT genes, from a variety of species, identified multiple copies of the GATA consensus sequence. The GATA sequences are interspersed among consensual MRE sequences in invertebrates (sea urchin and fly), amphibia (frog), fishes (stone loach, rainbow trout, northern pike, and carp), and mammals (rat, mouse, Chinese hamster, sheep, and human). The functionality of GATA elements and transcription factors in regulating the expression of these MT genes has not been examined. GATA elements are also present in several invertebrate MT genes that show highly restricted patterns of expression, including the *Drosophila Mto* gene (4, 20) and sea urchin *spMTA* and *spMTB* genes (18, 19, 93). In addition, the expression patterns of the *Drosophila* GATA factors “serpent” and *dqGATA-c* overlap those for *Mtn* and *Mto* (71, 94, 95). Thus, GATA elements and factors may be components of an evolutionarily conserved mechanism that controls cell-specific transcription of MT genes.

Acknowledgments—We thank Dr. Jim McGhee, University of Calgary, Alberta, Canada, for the ELT-2 cDNA clone and helpful discussions. Several nematode strains were obtained from the Caenorhabditis Genetic Center, funded by the National Institutes of Health.

REFERENCES

1. Hamer, D. H. (1986) *Annu. Rev. Biochem.* 55, 913–951
2. Palmiter, R. D. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 8429–8430
3. Koppantchijakul, L., Leibbrand, M., and Cherian, M. G. (1989) *Cell 58*, 383–395
4. Bonneton, F., Theodore, L., Mokdad, E., Erraisse, N., Cadic, A., and Wegnez, M. (1990) *J. Biol. Chem.* 265, 5712–5716
5. Uchida, Y., Takio, K., Iriyama, K., Tominaga, M., and Amano, S. (1991) *Neuron* 7, 337–347
6. Fukushige, T., Hawkins, M. G., and McGhee, J. D. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 105, 1934–1939
7. Uchida, Y., Takio, K., Tominaga, M., and Amano, S. (1991) *Neuron* 7, 337–347
8. Shworak, N. W., O’Connor, T., Wong, N. C., and Gedamu, L. (1993) *J. Biol. Chem.* 268, 24460–24466
9. Sukow, M. J., Ishikawa, Y., Shimano, H., Osada, S., and Nishihara, T. (1995) *J. Biol. Chem.* 270, 20988–20990
10. Aniskovitch, L. P., and Jacob, B. T. (1997) *Arch. Biochem. Biophys.* 341, 337–346
11. Kenyon, C. (1988) *Science* 240, 1448–1453
12. Sulston, J. (1988) *in The Nematode Caenorhabditis elegans* (Wood, W. B., ed) pp. 123–155, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
13. Durnam, D. M., and Palmiter, R. D. (1981) *Science* 214, 2149–2157
14. Heuchel, R., Radtke, F., Georgiev, O., Stark, G., Schaffner, W., and Aebi, M. (1998) *EMBO J.* 17, 2870–2875
15. Radtke, F., Heuchel, R., Georgiev, O., Hergersberg, M., Gariglio, M., Dembic, Z., and Schaffner, W. (1995) *EMBO J.* 14, 1355–1362
16. Palmiter, R. D. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 1219–1223
17. Price-Haughey, J., Bonham, K., and Gedamu, L. (1987) *Biochem. Biophys. Acta* 905, 158–168
18. Stern, M. J., Marqerrere, L. E., Daly, R. J., Lowenstein, E. J., Kokel, M., Batzer, A., Olivier, P., Paison, T., and Schlessinger, J. (1993) *Mol. Cell. Biol.* 13, 1175–1188
19. Sharkey, M., Graba, Y., and Scott, M. P. (1997) *Trends Genet.* 13, 145–151
20. Haun, C., Alexander, J., Stainier, D. Y., and Okkema, P. G. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 5072–5075
21. Su, Y. C., Treisman, J. E., and Skolnik, E. Y. (1998) *Genes Dev.* 12, 2371–2380
22. Mello, C., and Fire, A. (1995) *Methods Cell Biol.* 48, 451–482
23. Fire, A. (1996) *EMBO J.* 15, 2673–2680
24. Spiehi, J., and Blumenthal, T. (1985) *Cell Mol. Biol.* 5, 2495–2501
25. Britton, C., McKerrow, J. H., and Johnstone, I. L. (1998) *J. Mol. Biol.* 283, 19–27
26. Kennedy, B. P., Aamodt, E. J., Allen, F. L., Chung, M. A., Heschl, M. F., and McGhee, J. D. (1995) *J. Mol. Biol.* 220, 890–908
27. Kagan, C. B., Chung, M. A., Heschl, M. F., and McGhee, J. D. (1995) *Dev. Biol.* 170, 397–419
28. Hawkins, M. G., McGhee, J. D., and McCaffery, J. M. (1994) *J. Mol. Biol.* 240, 221–233
29. MacMorris, M., Broverman, S., Greenspoon, S., Lea, K., Madej, C., Blumenthal, T., and Spiehi, J. (1992) *J. Mol. Cell. Biol.* 16, 1652–1662
30. Fukushige, T., Hawkins, M. G., and McGhee, J. D. (1998) *Dev. Biol.* 198, 266–302
31. Aamodt, E. J., Chung, M. A., and McGhee, J. D. (1991) *Science* 252, 579–582
32. Simon, M. C. (1995) *Nature* 376, 19–21
33. Evans, T. (1997) *Trends Cardiol. Med.* 7, 75–83
34. Zhu, J., Hill, R. J., Heid, P. J., Fukuyama, M., Sugimoto, A., Priess, J. R., and Rothman, J. H. (1997) *Genes Dev.* 11, 2839–2890
35. Hawkins, M. G., and McGhee, J. D. (1995) *J. Biol. Chem.* 270, 14666–14671
36. Page, B. D., Zhang, W., Steward, K., Blumenthal, T., and Priess, J. R. (1997) *Genes Dev.* 11, 1651–1661
37. Gilvard, J., Sells, Y., Barry, J. D., and McGhee, J. D. (1999) *Dev. Biol.* 220, 265–280
38. Lewis, J. A., and Fleming, J. T. (1995) in *Caenorhabditis elegans: Modern Biological Analysis of an Organism* (Epstein, H. F., and Shakes, D. C., eds) Vol. 8, pp. 3–29, Academic Press, New York
39. Sulston, J., and Hodgkin, D. (1988) in *The Nematode Caenorhabditis elegans* (Wood, W. B., ed) pp. 587–606, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
40. Fire, A., Harrison, S. W., and Duxin, D. (1990) *Genes Dev.* 4, 189–198
41. Ausubel, M. F. (1998) *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York, NY
42. Mello, C. C., Kramer, J. M., Stinchcomb, D., and Ambros, V. (1991) *EMBO J.* 10, 3895–3970
43. Kunkel, T. A., Roberts, J. D., and Zakour, R. A. (1987) *Methods Enzymol.* 154, 221–233
44. Bell, D. M., Leung, K. K., Wheatley, S. C., Ng, L. J., Zhou, S., Ling, K. W.,
Cell-specific Regulation of Metallothionein Gene Transcription

29665

Sham, M. H., Koopman, P., Tam, P. P., and Cheah, K. S. (1997) Nat. Genet. 16, 174–178
61. Nonchev, S., Vesque, C., Macnachion, M., Seitanidou, T., Ariza-McNaughton, L., Fraim, M., Marshall, H., Sham, M. H., Krumlauf, R., and Charnay, P. (1996) Development 122, 543–554
62. Maduro, M., and Pilgrim, D. (1995) Genetics 141, 977–988
64. Fire, A. (1992) Genet. Anal. Tech. Appl. 9, 151–158
63. Land, M., Islas-Trejo, A., Freedman, J. H., and Rubin, C. S. (1994) J. Biol. Chem. 269, 9234–9244
65. Zhang, R., Min, W., and Sessa, W. C. (1995) J. Biol. Chem. 270, 15320–15326
60.淩, M. H., Koopman, P., Tam, P. P., and Cheah, K. S. (1997) Nat. Genet. 16, 174–178
61. Nonchev, S., Vesque, C., Macnachion, M., Seitanidou, T., Ariza-McNaughton, L., Fraim, M., Marshall, H., Sham, M. H., Krumlauf, R., and Charnay, P. (1996) Development 122, 543–554
62. Maduro, M., and Pilgrim, D. (1995) Genetics 141, 977–988
64. Fire, A. (1992) Genet. Anal. Tech. Appl. 9, 151–158
63. Land, M., Islas-Trejo, A., Freedman, J. H., and Rubin, C. S. (1994) J. Biol. Chem. 269, 9234–9244
65. Zhang, R., Min, W., and Sessa, W. C. (1995) J. Biol. Chem. 270, 15320–15326
66. Ghatak, S., Oliveria, P., Kaplan, P., and Ho, S. M. (1996) Prostate 29, 91–100
70. Laverriere, A. C., MacNeill, C., Mueller, C., Poelmann, R. E., Burch, J. B., and Evans, T. (1994) J. Biol. Chem. 269, 23177–23184
71. Rehorn, K. P., Thelen, H., Michelson, A. M., and Reuter, R. (1996) Development 122, 4023–4031
72. Soudais, C., Bielinska, M., Heikinheimo, M., MacArthur, C. A., Narita, N., Saffitz, J. E., Simon, M. C., Leiden, J. M., and Wilson, D. B. (1995) Mol. Cell. Biol. 15, 2235–2246
73. Tamura, S., Wang, X. H., Maeda, M., and Futai, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10876–10880
74. Yoshida, T., Sato, R., Mahmood, S., Kawasaki, S., Futai, M., and Maeda, M. (1997) FEBS Lett. 414, 333–337
75. Sulston, J. E., Schierenberg, E., White, J. G., and Thomson, J. N. (1983) Dev. Biol. 100, 64–119
76. Searle, P. F., Stuart, G. W., and Palminter, R. D. (1987) EXS 52, 407–414
77. Lincke, C. R., The, I., van, G. M., and Borst, P. (1992) J. Mol. Biol. 228, 701–711
78. Czupryn, M., Brown, W. E., and Vallee, B. L. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10395–10399
79. Koizumi, S., Otsuka, F., and Yamada, H. (1991) Chem. Biol. Interact. 80, 45–157
80. Remondelli, P., Molteo, O., and Leone, A. (1997) FEBS Lett. 416, 54–158
81. Merika, M., and Orkin, S. H. (1995) Mol. Cell. Biol. 100, 2437–2447
82. Herzg, T. C, Jobe, S. M., Ask, H., Molkentin, J. D., Cowley, A. W., Izumo, S., and Markham, B. E. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7543–7548
83. Yamagata, T., Mitani, K., Ueno, H., Kanda, Y., Yazaki, Y., and Hirai, H. (1997) Mol. Cell. Biol. 17, 4272–4281
84. Walters, M., and Martin, D. I. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10444–10448
85. Raich, N., Clegh, C. H., Grofl, J., Romee, P. H., and Stamatoyannopoulos, G. (1995) EMBO J. 14, 801–809
86. Mackay, J. P., and Crossley, M. (1996) Trends Biochem. Sci. 23, 1–4
87. Kawana, M., Lee, M. E., Quertermous, E. E., and Quertermous, T. (1995) Mol. Cell. Biol. 15, 4225–4231
88. Bonneton, F., and Wegnez, M. (1995) Dec. Genet. 16, 253–263
89. Suzuki, K., Nakajima, K., Otaki, N., and Kimura, M. (1994) Biol. Signals 3, 188–192
90. Ghoshal, K., Li, Z., and Jacob, S. T. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 10390–10395
91. Sen, C. K., and Packer, L. (1996) FASEB J. 10, 709–720
92. Satyal, S. H., Chen, D., Fox, S. G., Kramer, J. M., and Morimoto, R. I. (1998) Genes Dev. 12, 1962–1974
93. Nemer, M., Stuebing, E. W., Bai, G., and Parker, H. R. (1995) Mech. Dev. 50, 130–137
94. Abel, T., Michelson, A. M., and Maniatis, T. (1993) Development 119, 623–633
95. Lee, M. H., Huang, L.-H., Yeh, J.-Y., Heheisel, J., Lehrach, H., Sun, Y. H., and Tsai, S.-F. (1995) J. Biol. Chem. 270, 25510–25518