Proc. Natl. Acad. Sci. USA
Vol. 77, No. 11, pp. 6511–6515, November 1980
Biochemistry

Isolation and characterization of the mouse metallothionein-I gene

(recombinant DNA/DNA sequence/heteroduplex mapping/cadmium)

DIANE M. DURNAM*, FABIENNE PERRIN†, FRANK GANNON†, AND RICHARD D. PALMITER*

*Howard Hughes Medical Institute Laboratory, Department of Biochemistry, University of Washington, Seattle, Washington 98195; and †Laboratoire de Génétique Moléculaire des Eucaryotes du Centre National de la Recherche Scientifique, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'Institut National de la Santé et de la Recherche Médicale, Institut de Chimie Biologique, Faculté de Médecine, Strasbourg 67085, France

Communicated by Earl W. Davie, August 8, 1980

ABSTRACT **Double-stranded cDNA was synthesized from a mouse liver mRNA fraction enriched for metallothionein mRNA activity, ligated to restriction site linkers, inserted into pBR322, and used to transform *Escherichia coli* χ1776. The sequence of the largest plasmid containing DNA that hybridized to metallothionein mRNA was determined and shown to contain a 380-base-pair insert that includes the entire coding region and 3′ untranslated region of metallothionein-I. The metallothionein-I insert was nick-translated and used to screen both a mouse myeloma and a mouse embryo DNA library in bacteriophage λ. A metallothionein-I genomic clone containing 13–15 kilobase pairs of mouse DNA was isolated from each library. Both contain a 3.8-kilobase-pair *Eco*RI fragment that hybridizes to the metallothionein-I probe. The location, size, and orientation of the metallothionein-I gene within the 3.8-kilobase-pair fragment were determined by heteroduplex and restriction mapping. The gene spans 1.1 kilobase pairs and contains at least two introns.**

The quest for a cadmium-binding protein by Margoshes and Vallee (1) led to their discovery of a small metal-binding protein (M_r 6000–7000), later called metallothionein (MT), which is characterized by having a high cysteine content and no aromatic amino acids or histidine. It binds several heavy metals, including Cd, Zn, Hg, and Cu, and has been postulated to be involved in zinc metabolism and heavy metal detoxification. Most vertebrate tissues contain two forms of MT that differ in amino acid sequence; in the mouse, the two forms are designated MT-I and MT-II (for a recent symposium, see ref. 2).

We became interested in studying MT gene regulation because the amount of translatable MT mRNA increases rapidly in response to heavy metals or glucocorticoids (3, 4). MT synthesis occurs predominantly in liver and kidney *in vivo*, but nearly all cell types are responsive in culture (unpublished observations; refs. 5 and 6). In addition, cell cultures can be made resistant to Cd, and these lines overproduce MT (7, 8). The molecular mechanisms by which heavy metals and steroids regulate MT mRNA production are unknown. Because an understanding of gene regulation depends upon specific probes for RNA and DNA sequences, we have initiated our investigation by isolating a MT-I cDNA clone and using it to select genomic clones containing the MT-I gene.

MATERIALS AND METHODS

Preparation of RNA. Swiss Webster mice (25 g) were injected subcutaneously with 1.5 μmol of $CdSO_4$ and 0.5 μmol of $ZnSO_4$. Six hours later, a 5% (wt/vol) liver homogenate was prepared in $NaDodSO_4$ and proteinase K, and total nucleic acids were extracted. RNA was precipitated with 2 M LiCl; poly(A)-RNA was selected by oligo(dT)-cellulose chromatography and sedimented for 20 hr at 280,000 × *g* on 12-ml, 5–20% sucrose gradients in 10 mM Hepes (pH 7.5) (9). Fractions (0.5 ml) were collected and assayed directly by translation; those containing MT mRNA were pooled and sedimented on a second, identical gradient. Fractions were assayed for MT mRNA activity with a nuclease-treated rabbit reticulocyte lysate essentially as described (10) except that no exogenous amino acids other than [^{35}S]cysteine (400 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels) were added and the reaction mixtures contained 5 mM dithiothreitol. After translation, samples (5 μl) were incubated with 20 μl of 100 mM iodoacetate in 0.5 M Tris·HCl (pH 8.8) for 1 hr at 37°C, electrophoresed on a $NaDodSO_4$/polyacrylamide gel for 14 hr at 10 mA, and analyzed by fluorography. Purified mouse MT (11) was included as a standard.

Preparation of Double-Stranded (ds) cDNA, Insertion into pBR322, and Transformation of χ1776. Double-stranded cDNA was prepared (12) and ligated to a 1:1 mixture of *Hin*dIII and *Eco*RI linkers (Collaborative Research, Waltham, MA) prior to being inserted into *Eco*RI/*Hin*dIII-digested pBR322 as described (13). Transformation of *E. coli* χ1776 was performed as described (14) with the following modifications: (*i*) the 100 mM $CaCl_2$ buffer was replaced by 70 mM $MnCl_2$/30 mM $CaCl_2$/40 mM NaOAc, pH 5.6; (*ii*) the cells were transformed with plasmid at a concentration of 3 μg/ml; and (*iii*) the transformed cells were incubated at 37°C in L broth for 3 hr before being spread on ampicillin plates. All recombinant DNA procedures were carried out in a P2 facility in accordance with the National Institutes of Health guidelines.

Identification of MT Clones. Initial screening for a MT clone was performed by measuring the size of plasmids isolated from 10-ml cultures of the transformants (15) on 0.7% agarose gels. Plasmids containing inserted DNA were immobilized on nitrocellulose filters and hybridized with MT mRNA; RNA that hybridized was eluted and assayed by translation (16).

Screening Genomic Libraries. Bacteriophage λ libraries containing mouse myeloma and mouse embryo DNA were generously provided by Davis (17) and Maki (18), respectively. The libraries were screened (19) with the nick-translated (20) *Eco*/*Hin*d fragment from the cDNA clone (m_1pEH.4) as a hybridization probe. The stringency of the hybridization was increased by including an additional wash step in 5 mM Tris·HCl, pH 7.4/2.5 mM EDTA/0.5% $NaDodSO_4$ at 68°C prior to the formamide washes.

Restriction Mapping. Restriction enzymes were obtained from Bethesda Research Laboratories (Rockville, MD) or Boehringer Mannheim and used according to the protocols supplied. Products of the reactions were analyzed on agarose gels. DNA in agarose gels was denatured and transferred onto Sartorius nitrocellulose filters (21) and hybridized to nick-translated probes as described (22).

Heteroduplex Analysis. Heteroduplexes were formed in 70% (vol/vol) deionized formamide/300 mM NaCl/10 mM Tris·HCl, pH 8.5/1 mM EDTA with both DNAs at 1–3 μg/ml. The

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

Abbreviations: MT, metallothionein; ds, double-stranded; bp, base pair(s); kb, kilobase pair(s).

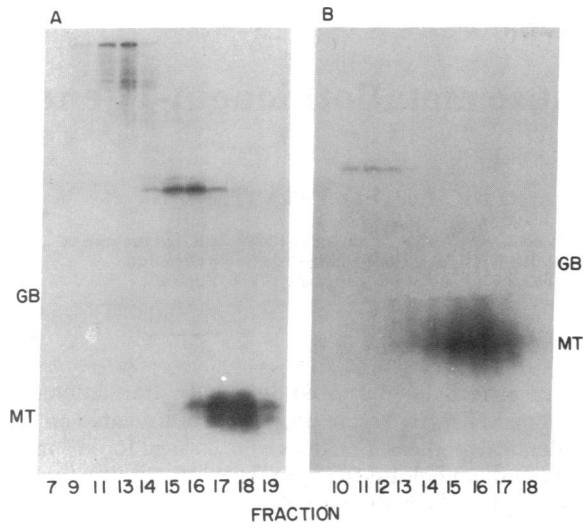

FIG. 1. Fractionation of mouse liver mRNA on sucrose gradients. Aliquots of the gradient fractions were translated in the presence of [^{35}S]cysteine and the products were analyzed by electrophoresis (15–20% gel) and fluorography. The positions of unlabeled MT and globin (GB) standard detected by staining are indicated. (*A*) Partial separation of MT mRNA from other translatable messages. Fractions 16–19 were pooled and further purified on a second identical gradient, the results of which are shown in *B*. Fractions 14–18 of the second gradient were pooled and used in all subsequent procedures.

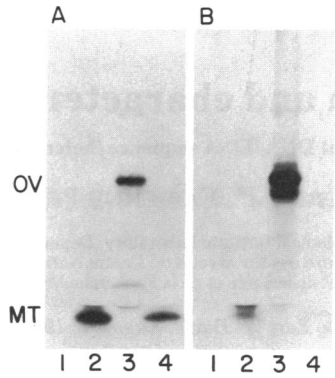

FIG. 2. Hybrid-selection of MT mRNA by m_1pEH.4. RNA isolated by filter hybridization was translated and the products were analyzed by electrophoresis (7.5–20% gel) followed by fluorography. (*A*) Translation products labeled with [^{35}S]cysteine. (*B*) Translation products labeled with [^{35}S]methionine. The positions of ovalbumin (OV) and unlabeled MT standard detected by staining are indicated. Lanes 1, no exogenous RNA added; lanes 2, mRNA enriched for MT mRNA (5.5 μg/ml); lanes 3, total oviduct RNA (64 μg/ml); lanes 4, RNA selected by m_1pEH.4 from a mixture of total oviduct RNA (2.9 mg/ml) and MT mRNA (82 μg/ml).

DNAs were denatured at 75°C for 5 min, incubated at 25°C for 30 min, and mounted for microscopy as described (23). Hybrids between the DNA and MT mRNA were formed in the same buffer by denaturing the DNA at 75°C for 5 min and incubating it with 3 μg of gradient-purified MT mRNA per ml at 54°C for 4 hr before being mounted for microscopy.

RESULTS AND DISCUSSION

Isolation of a MT-I cDNA Clone. Liver from mice treated with $CdSO_4$ and $ZnSO_4$ for 6 hr was chosen as the starting material for isolating MT mRNA because the rate of hepatic MT synthesis reaches a maximum 4–6 hr after administration of heavy metals (unpublished observations; ref. 24). Total mRNA was isolated and fractionated on 5–20% sucrose gradients. mRNA activity was assayed by translation and the products were separated by $NaDodSO_4$/polyacrylamide electrophoresis. Fig. 1*A* shows the distribution of translation products across a typical gradient. MT mRNA was located primarily in fractions 17 and 18. Separation of MT mRNA from other translatable mRNAs was achieved by sedimenting the peak MT mRNA fractions on a second sucrose gradient (Fig. 1*B*). Although MT was the only apparent translation product, subsequent hybridization studies using a MT-I-specific cDNA have shown that MT mRNA was enriched 550-fold relative to total RNA but constituted only 1.4% of the RNA in the purified fraction.

The enriched MT mRNA was used as a template for the synthesis of ds cDNA. Sequential reverse transcriptase and DNA polymerase I reactions followed by the removal of the hairpin loop by nuclease S1 digestion resulted in ds cDNA 250–600 base pairs (bp) long, as judged by electrophoresis on a 2% agarose gel. To maximize the number of blunt ends, we treated the ds cDNA with DNA polymerase I (Klenow fraction) before it was ligated to a combination of *Eco*RI and *Hin*dIII linkers. This should result in 50% of the ds cDNA molecules acquiring heterologous ends. Compatible heterologous ends were generated in pBR322 by treating it with *Eco*RI, *Hin*dIII, and alkaline phosphatase. The resulting 31-bp fragment of plasmid DNA was separated from the large fragment of pBR322 by chromatography on Sephadex G-75. After ligation to the linker-flanked ds cDNA, the reconstructed plasmid was used to transform χ1776. The transformation yielded 4500 ampicillin-resistant colonies per μg of plasmid DNA. This yield is 25-fold higher than that recovered from a transformation by plasmid vector ligated in the absence of ds cDNA.

Our first step in screening for a MT clone was to purify plasmids and determine their sizes by agarose gel electrophoresis. Fifteen of the first 130 transformants analyzed contained plasmids significantly larger than pBR322. These 15 plasmids were attached to nitrocellulose filters and hybridized to enriched MT mRNA. After the filters were thoroughly washed, the attached RNA was eluted in boiling H_2O and translated. Visualization of the translation products by electrophoresis on

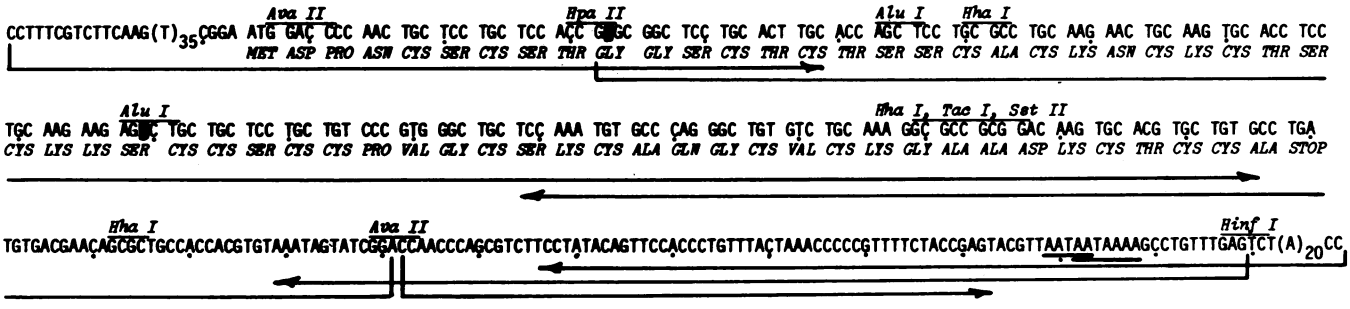

FIG. 3. DNA sequence of the 0.4-kilobase-pair (kb) *Eco/Hin*d fragment of m_1pEH.4. The sequence was determined by the methods of Maxam and Gilbert (26); arrows indicate the direction and extent of sequencing. The MT-I cDNA sequence is bounded by (T)-35 and (A)-20. The corresponding amino acid sequence of MT-I predicted from the DNA sequence is shown. Potential restriction enzyme cleavage sites are indicated. The location of exon junctions are also shown (▮). Two A-A-T-A-A sequences are underlined.

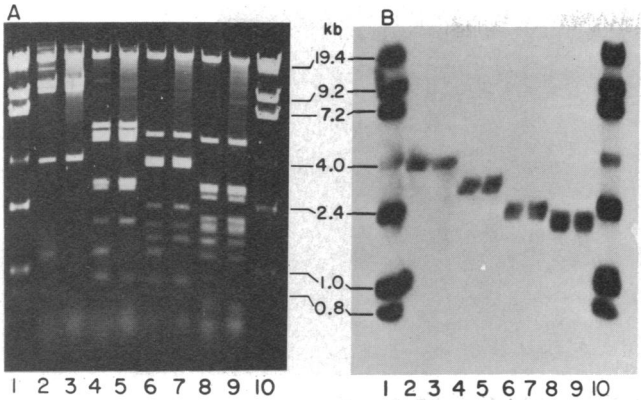

FIG. 4. Restriction analysis of meλ26 and mmλ36. Samples were digested as indicated and analyzed by electrophoresis on a 0.7% agarose gel. (*A*) Lanes 1 and 10 contain *Eco*RI-digested P22 as size markers; the sizes of the fragments are indicated. Lanes 2 and 3 show *Eco*RI digests of meλ26 and mmλ36, respectively. Lanes 4–9 show the results of double digests with *Eco*RI in combination with *Hin*dIII (lanes 4 and 5), *Sst* I (lanes 6 and 7), or *Bgl* II (lanes 8 and 9). The digestion of mmλ26 is shown in lanes 4, 6, and 8; the digestion of meλ36 is shown in lanes 5, 7, and 9. (*B*) Southern blot analysis of the digestions shown in *A*. MT-1-containing fragments were identified by hybridization to ^{32}P-labeled *Eco*/*Hin*d fragment of m_1pEH.4.

$NaDodSO_4$/polyacrylamide gels followed by fluorography revealed that two of the plasmids had hybridized MT mRNA. To check the specificity of the hybridization, we hybridized a filter containing the larger of the two positive plasmids to a mixture of RNA containing MT mRNA and oviduct RNA. Fig. 2 shows that the MT plasmid specifically hybridizes MT mRNA. Fig. 2*A* shows the results obtained when [^{35}S]cysteine, which constitutes 20 out of 61 amino acids in MT, was used in the translation. Identical results were obtained with [^{35}S]methionine, represented only once in MT (Fig. 2*B*).

Characterization of the MT cDNA Clone. In an attempt to determine the size of the insert of the MT cDNA clone, we discovered that the plasmid was cut by *Hin*dIII but was resistant to digestion by *Eco*RI. Because the cDNA insert was not cleaved by *Hae* III, we circumvented this problem by isolating the *Hin*dIII/*Hae* III fragment containing the MT cDNA, attaching an *Eco*RI linker to the blunt end generated by the *Hae* III digestion, and repeating the transformation procedure. Cleavage of these transformants with *Hin*dIII and *Eco*RI yielded a fragment ≈400 bp long which included the MT cDNA and 16 bp of plasmid DNA acquired during the subcloning. This subclone, m_1pEH.4, was used in all subsequent procedures.

To ascertain whether m_1pEH.4 contained MT-I or MT-II sequences, we determined the sequence of the 400-bp *Eco*/*Hin*d insert and compared it to the DNA sequences postulated from the amino acid sequences of MT-I and MT-II (2, 25). The sequence shown in Fig. 3 indicates that we isolated a MT-I clone; it confirms the MT-I protein sequence except for the presence of asparagine at position 23 instead of aspartic acid. The DNA sequence also reveals the following features. (*i*) m_1pEH.4 contains a 132-bp 3′ untranslated region and a 3′ poly(A) tail in addition to the entire coding region of MT-I. (*ii*) The A-A-U-A-A-A sequence located near the 3′ end of many mRNAs (27) is also present in the MT-I mRNA. (*iii*) The choice of codons is not random. Of the codons that could have a C in the third position, 89% terminate in C, including all of the serine codons and 80% of the cysteine codons. With a random selection, 33% of the serine codons and 50% of the cysteine codons would have C in the third position. (*iv*) The original clone had retained only 1 bp of the *Eco*RI site which was ligated to 35 bp of poly(dA)·poly(dT) located at the 5′ end of the MT-I sequence. Determination of the sequence of the genomic DNA indicates that this poly(dA)·poly(dT) sequence is not present at the 5′ end of the MT-I gene, suggesting that it is an artifact that originated during cDNA cloning.

Isolation of Genomic MT Clones. Genomic DNA libraries were prepared by inserting 10- to 20-kb fragments, resulting from partial *Eco*RI digestion of mouse myeloma or mouse embryo DNA, into bacteriophage λ, Charon 4A (18, 19). Approximately 10^6 plaques of the mouse myeloma stock were screened by using a nick-translated *Eco*/*Hin*d fragment from m_1pEH.4 as a probe. Initially, the screening conditions used were identical to those used in the isolation of a number of egg white genes (19, 28). We were surprised on this first screening to obtain 212 positive clones. Thirty of the clones were purified by two sequential screenings, and the DNA from 14 of these was digested with *Eco*RI and analyzed on a 0.7% agarose gel. Each of these clones had a different *Eco*RI fragment that hybridized with the cDNA probe, suggesting that the cDNA probe has

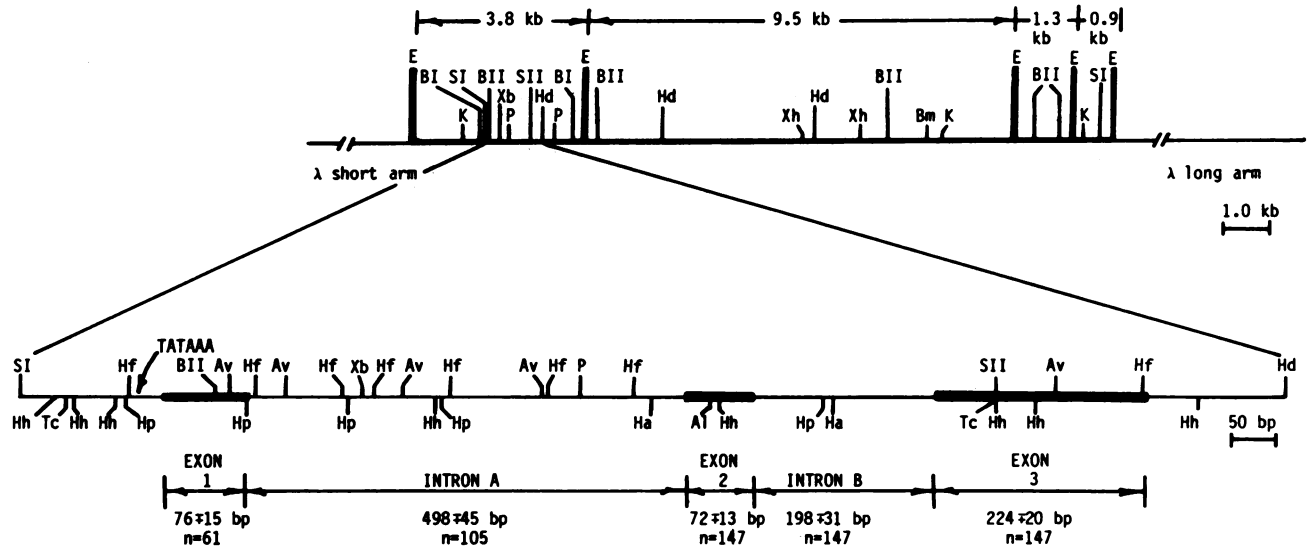

FIG. 5. Restriction map of meλ26. Upper restriction map shows the orientation of the *Eco*RI fragments within the clone. Only the sites used to orient the fragments are shown. Lower restriction map is an expanded map of the MT-I gene region. Only the sites in proximity to exons are indicated. The location (ranges are shown) of the exons (dark bars), as revealed by heteroduplex mapping (Fig. 6), are indicated; n = number of measurements. SI, *Sst* I; SII, *Sst* II; Hh, *Hha* I; Tc, *Tac* I; Hp, *Hpa* II; Hf, *Hin*fI; BI, *Bgl* I; BII, *Bgl* II; Av, *Ava* II; Xb, *Xba* I; Tq, *Taq* I; Hd, *Hin*dIII; P, *Pst* I; Ha, *Hae* III; Al, *Alu* I; Bm, *Bam*HI; Xh, *Xho* I; K, *Kpn* I.

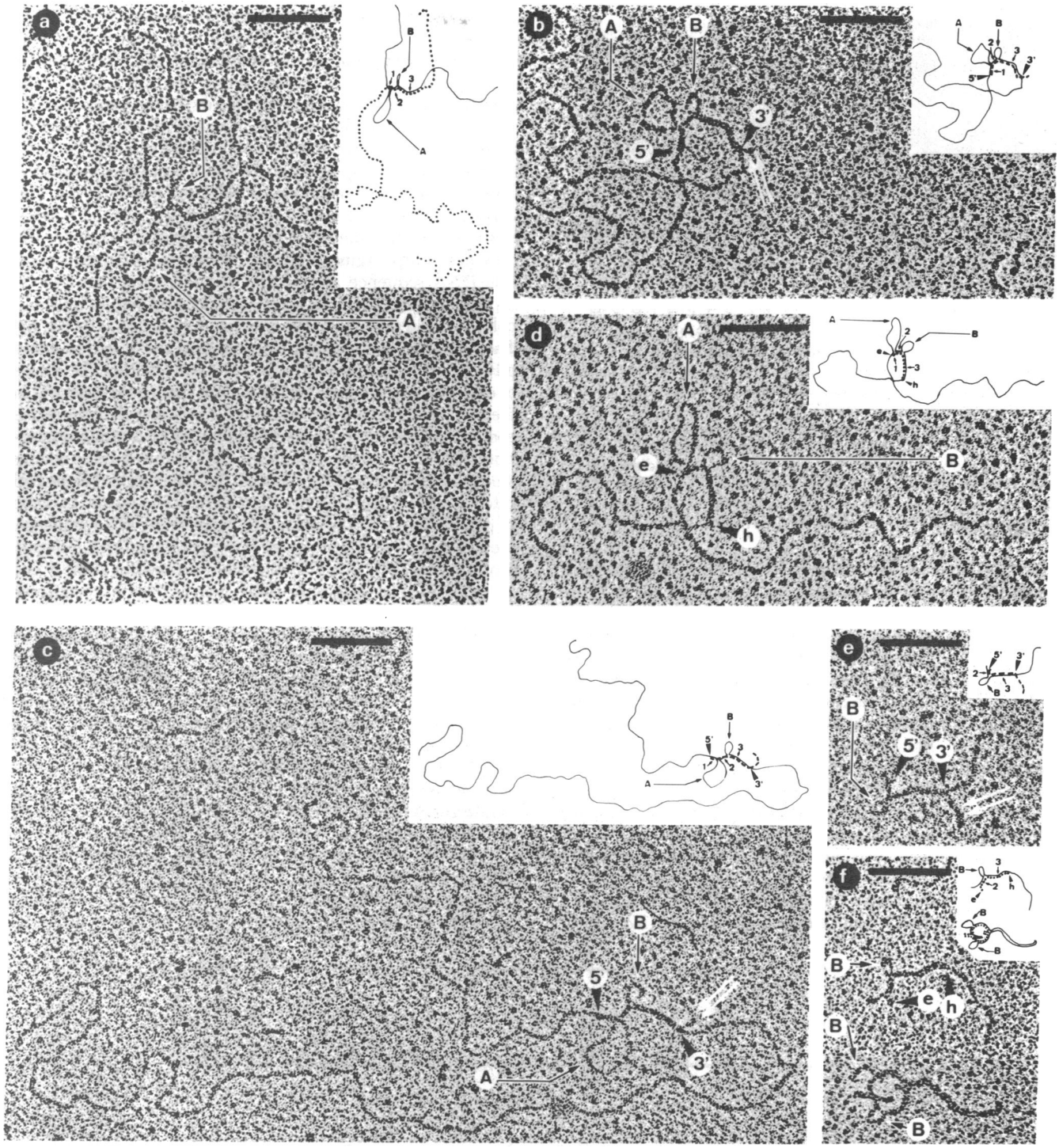

FIG. 6. Heteroduplex analysis of mmλ36 and meλ26. (*Insets*) The solid line represents the cloned genomic DNA (mmλ36 or the subcloned 3.8-kb *Eco*RI fragment of meλ26), the dashed line shows the RNA, and the dotted line illustrates the cloned cDNA (the *Eco/Hin*d fragment or m_1pEH.4 linearized by *Sal* I). 5′ and 3′ arrowheads indicate the ends of the RNA (*b*, *c*, and *e*); the *Eco*RI and *Hin*dIII ends of the ds cDNA are indicated by e and h, respectively, in *d* and *f*. A and B designate the single-stranded introns; 1, 2, and 3 designate the exons. The double white arrows point to the poly(A) track. (*a*) Heteroduplex molecule between mmλ36 and *Sal* I-cut m_1pEH.4. (*b*) Hybrid molecule between mmλ36 and MT mRNA. (*c*) Hybrid molecule between MT mRNA and the subcloned 3.8-kb fragment of meλ26 linearized by *Bam*HI. (*d*) Heteroduplex between the 3.8-kb fragment of meλ26 and the *Eco/Hin*d fragment of m_1pEH.4. (*e*) Hybrid molecule between the 1.0-kb *Pst/Pst* fragment of the genomic DNA and MT mRNA. (*f*) Heteroduplex molecule between the *Pst/Pst* genomic fragment and the *Eco/Hin*d fragment of m_1pEH.4. At the top is seen a typical molecule revealing the hybridization of exons 2 and 3 as well as a short unhybridized DNA tail; at the bottom is seen two such heteroduplex molecules hybridized together. (Bar = 0.1 μm.)

sequences that are homologous to a large number of regions in the genomic DNA. To overcome this problem, we incorporated a more stringent wash step (see ***Materials and Methods***); m_1pEH.4 hybrids were stable under these conditions whereas all 30 λ hybrids were unstable. The remaining 182 clones from the original screening were pooled and screened under the more stringent hybridization conditions to ultimately yield one clone, designated mmλ36. *Eco*RI digestion of mmλ36 yielded fragments of 3.8 and 9.5 kb in addition to the long and short arms of λ. Under the stringent hybridization conditions, one clone

(meλ26) was also isolated from the mouse embryo library; it contained the 9.5- and 3.8-kb *Eco*RI fragments of mmλ36 and two additional fragments of 1.3 and 0.9 kb. Only the 3.8-kb *Eco*RI fragment of both clones hybridized to the cDNA probe (Fig. 4*B*).

Characterization of mmλ36 and meλ26. Direct comparison of mmλ36 and meλ26 shows that, aside from the additional 0.9- and 1.3-kb fragments of meλ26, the two clones gave identical restriction patterns when they were digested with *Eco*RI alone or in combination with *Hin*dIII, *Sst* I, or *Bgl* II (Fig. 4*A*). When the DNA fragments from these digestions were blotted onto nitrocellulose, the cDNA probe hybridized to fragments of the same size in both clones (Fig. 4*B*), suggesting that the two clones share identical DNA sequences.

To facilitate the mapping of the MT-I gene region, we subcloned each *Eco*RI fragment of meλ26 into the *Eco*RI site of pBR322. By comparing the restriction maps of the subcloned *Eco*RI fragments with that of meλ26, we established the orientation of the *Eco*RI fragments within the genomic DNA (Fig. 5). Cleavage with enzymes having known restriction sites in arms of λ allowed the orientation of the inserted DNA within λ. Comparison of the restriction map of meλ26 with that of mmλ36 indicates that the 9.5- and 3.8-kb *Eco*RI fragments occupy identical positions relative to each other but that they are inserted into λ in opposite orientations. The similarity of the MT-I gene regions of the myeloma and embryo DNA suggests that no gross rearrangement of the DNA occurred in the myeloma cell line.

Heteroduplex Analysis of mmλ36 and meλ26. Heteroduplex mapping was used to derive additional information about the structure, orientation, and location of the MT-I gene (Fig. 6). Hybridization of MT-I mRNA to either mmλ36 (Fig. 6*b*) or the 3.8-kb *Eco*RI fragment of meλ26 (Fig. 6*c*) shows that the gene contains at least two introns. The sizes of the introns and exons are indicated in Fig. 5. A poly(A) tail of about 100 bases is visible and establishes the 5′-3′ orientation (Fig. 6 *b*, *c*, and *e*). The heteroduplex shown in Fig. 6*a* verifies the 5′-3′ orientation and also shows that ≈40 bases of the 76-base exon 1 hybridize to the cDNA sequence, suggesting that the cDNA clone is missing ≈36 bases of the 5′ leader region of MT-I mRNA. Fig. 6 *c* and *d* shows the location of the exons within the 3.8-kb fragment; Fig. 6 *e* and *f* shows the location of exons 2 and 3 within the 1.0-kb *Pst*/*Pst* fragment, which contains the majority of the MT-I gene sequence (Fig. 5).

The structural map of the MT-I gene derived from heteroduplex measurements correlates well with the restriction map of the gene. Fig. 5 shows the structural map superimposed on an expanded restriction map of the 3.8-kb fragment of meλ26. With the exception of one *Alu* I site, all of the restriction sites predicted from the cDNA sequence (Fig. 3) have been located in the exon regions in the genomic DNA; no additional restriction sites have been found. The mapping data allow us to approximate the location of the exon boundaries within the cDNA sequence. As indicated in Fig. 3, the junction of exons 2 and 3 maps very close to the *Alu* I site which is present in the cDNA but absent in the genomic DNA, suggesting that this *Alu* I site may be the result of RNA splicing. Determination of the sequence of this region of the genomic DNA has verified this hypothesis (N. Glanville, personal communication). The position of exon 1 has been confirmed by determining the sequence of the region between *Sst* I and *Xba* I of the genomic DNA. A T-A-T-A-A-A box homologous (10 out of 11 of nucleotides are identical) to that of the conalbumin gene (28) is located ≈100 nucleotides from the *Ava* II site of exon 1 (Fig. 5).

Our analyses indicate that we have isolated a cDNA clone and 15.5 kb of genomic DNA containing the MT-I gene. Availability of these DNA sequences will allow: (*i*) an approach to the isolation of the MT-II gene; (*ii*) an analysis of the molecular mechanisms involved in the regulation of this "housekeeping" gene by heavy metals and steroids; and (*iii*) exploration of the mechanism(s) responsible for Cd resistance.

We thank M. Davis for the mouse myeloma λ library, R. Maki for the mouse embryo λ library, L. Loeb for providing DNA polymerase I, and A. Sarthy for helping with the mapping of genomic DNA. The technical assistance of L. Hager, C. Wasylyk, B. Boulay, and E. Sittler is appreciated. R.D.P. is especially grateful to Dr. Pierre Chambon for providing a stimulating laboratory environment during the fall of 1979. This work was supported by a grant from the National Institutes of Health (HD-09172) and a travel grant from the International Cancer Research Data Bank Programme (ICRETT) of the National Cancer Institute (N01-CO-65341).

1. Margoshes, M. & Vallee, B. L. (1957) *J. Am. Chem. Soc.* **79,** 4813–4814.
2. Kägi, J. H. R. & Nordberg, M. (1979) *Metallothionein* (Birkhaser, Basel).
3. Anderson, R. D. & Weser, U. (1978) *Biochem. J.* **175,** 841–852.
4. Etzel, K. R., Shapiro, S. G. & Cousins, R. J. (1979) *Biochem. Biophys. Res. Commun.* **89,** 1120–1126.
5. Rudd, C. J. & Herschman, H. R. (1978) *Toxicol. Appl. Pharmacol.* **44,** 511–521.
6. Karin, M. & Herschman, H. R. (1979) *Science* **204,** 176–177.
7. Rugstad, H. E. & Norseth, T. (1978) *Biochem. Pharmacol.* **27,** 647–650.
8. Hildebrand, C. E., Tobey, R. A., Campbell, E. Q. & Enger, M. D. (1979) *Exp. Cell Res.* **124,** 237–246.
9. Rowe, D. W., Moen, R. C., Davidson, J. M., Byers, P. H., Bornstein, P. & Palmiter, R. D. (1978) *Biochemistry* **17,** 1581–1590.
10. Palmiter, R. D., Gagnon, J., Ericsson, L. H. & Walsh, K. A. (1977) *J. Biol. Chem.* **252,** 6386–6393.
11. Cherian, M. G. (1974) *Biochem. Biophys. Res. Commun.* **61,** 920–926.
12. Wickens, M. P., Buell, G. N. & Schimke, R. T. (1978) *J. Biol. Chem.* **253,** 2483–2495.
13. Goodman, H. M. & MacDonald R. J. (1979) *Methods Enzymol.* **68,** 75–90.
14. Norgard, M. V., Keem, K. & Monahan, J. J. (1978) *Gene* **3,** 279–292.
15. Elwell, L. P., de Graaf, J., Seibert, D. & Falkow, S. (1975) *Infect. Immun.* **12,** 404–410.
16. Cochet, M., Perrin, F., Gannon, F., Krust, A., Chambon, P., McKnight, G. S., Lee, D. C., Mayo, K. E. & Palmiter, R. D. (1979) *Nucleic Acids Res.* **6,** 2435–2452.
17. Early, P. W., Davis, M. M., Kaback, D. B., Davidson, N. & Hood, L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 857–861.
18. Maki, R., Traunecker, A., Sakano, H., Roeder, W. & Tonegawa, S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2138–2142.
19. Garapin, A. C., Lepennec, J. P., Roskam, W., Perrin, F., Cami, B., Krust, A., Breathnach, R., Chambon, P. & Kourilsky, P. (1978) *Nature (London)* **273,** 349–354.
20. Thomashow, M. F., Nutter, R., Montoya, A. L., Gordon, M. P. & Nester, E. W. (1980) *Cell* **19,** 729–739.
21. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
22. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3683–3687.
23. Wellauer, P. K., David, I. B., Brown, D. D. & Reeder, R. H. (1976) *J. Mol. Biol.* **105,** 461–486.
24. Probst, G. S., Bousquet, W. F. & Miya, T. S. (1977) *Toxicol. Appl. Pharmacol.* **39,** 51–60.
25. Huang, I., Yoshida, A., Tsunoo, H. & Nakajima, H. (1977) *J. Biol. Chem.* **252,** 8217–8221.
26. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
27. Benoist, C., O'Hare, K. & Chambon, P. (1980) *Nucleic Acids Res.* **8,** 127–142.
28. Cochet, M., Hen, R., Maroteaux, L., Perrin, F. & Chambon, P. (1979) *Nature (London)* **282,** 567–574.