MOLECULAR AND CYTOGENETIC CHARACTERIZATION OF A METALLOTHIONEIN GENE OF DROSOPHILA

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ABSTRACT

A chromosomal DNA segment containing the metallothionein gene was isolated from a genomic library of *Drosophila melanogaster* using a previously characterized cDNA of this species as a probe. A segment of 1543 base pair (bp) was sequenced and found to include the cDNA sequence interrupted by one small intron. Several lines of evidence indicate that there is a single copy of the metallothionein gene (*Mtn*) in Drosophila; any other related genes, if they occur, must be sufficiently different that they are not detectable by our probe, even under hybridization conditions of reduced stringency. According to *in situ* hybridization and deletion mapping, *Mtn* is located in the right arm of the third chromosome in region 85E10-15. Within 300 bases upstream of the apparent site of transcription initiation, there are several short intervals very similar to the 12-bp segments considered to be responsible for metal regulation in mammalian systems.

METALLOTHIONEIN (MT), a low molecular weight, metal-binding protein is found in most major groups of organisms, often associated with cadmium, zinc or copper ions. A complete picture of its physiological role is not yet available; but it appears to protect against toxic metals, at least in some circumstances [for reviews see KÄGI and NORDBERG (1979) and KARIN (1985)]. In mammalian cells, the genes coding for MT are multiple, and they respond to metals by increased transcription (DURNAM and PALMITER 1981; HAMER and WALLING 1982; KARIN *et al.* 1984).

Thus, MT is a ubiquitous protein of potentially great biological importance, and genetic studies would contribute significantly to our understanding of its function and regulation. Drosophila affords an excellent opportunity for the type of molecular and functional genetic analysis necessary. It is particularly fortunate, in this respect, that in *Drosophila melanogaster* there appears to be, as we show here, a single copy of the MT gene.

We have demonstrated that Drosophila larvae respond to heavy metals by producing a 4000–5000 dalton protein similar to MT (MARONI and WATSON 1985). Similar observations were made by DEBEC, MOKDAD and WEGNEZ (1985) for Drosophila cells in culture. Further, we found that an RNA induced

by metal treatment has a sequence that could code for a 40-amino-acid protein strikingly similar to mammalian metallothionein (LASTOWSKI-PERRY, OTTO and MARONI 1985). In this report, we describe the molecular organization and cytogenetic localization of the gene that appears to code for MT. This gene will be designated *Mtn*.

MATERIALS AND METHODS

Molecular analyses: Unless otherwise specified, methods recommended by MANIATIS, FRITSCH and SAMBROOK (1982) or reported in LASTOWSKI-PERRY, OTTO and MARONI (1985) were used. A *D. melanogaster* genomic DNA library from a strain carrying the mutation cut^{Ns} was kindly provided by CHARLES H. LANGLEY and JOSEPH W. JACK. This library was prepared by partial digestion of imaginal DNA with *Mbo*I and cloning into the *Bam*HI site of the lambda derivative EMBL4. A probe for the MT structural sequence was obtained by nick-translation of the self-ligated insert of a cDNA clone, cDm51 (LASTOWSKI-PERRY, OTTO and MARONI 1985). *In situ* hybridizations were performed according to PARDUE and GALL (1975) with modifications described by HAYASHI *et al.* (1978); the probe was derived from a cloned genomic DNA fragment, λDm13, labeled with [^{3}H]dTTP by nick-translation. The sequence of a portion of λDm13 was determined using the strategy shown in Figure 2B; this involved subcloning into M13 vectors and sequence determination by the dideoxy chain termination method (SANGER, NICKLEN and COULSON 1977).

A 208-bp *Eco*RI-*Bam*HI fragment, the 5′ half of the cDNA clone cDm51 (LASTOWSKI-PERRY, OTTO and MARONI 1985), was used to establish which strand of genomic DNA is transcribed. Clones of this fragment—carried in M13mp8 and M13mp9, respectively—were isolated, and single-stranded, radioactive probes were prepared by primer extension.

To test whether a putative intron sequence was present in the cellular RNA at detectable levels, a *Bgl*II–*Aha*III fragment (208 bp), representing 78% of the proposed intron, was subcloned into pUC9 and was used as a probe on Northern blots.

The position corresponding to the 5′ end of the mRNA was determined by the method of primer extension, using the procedures in BENYAJATI *et al.* (1983) with the following modifications: the primer hybridization reaction included 20 μg of total RNA isolated from cadmium-fed larvae and 0.1 μg of denatured restriction fragment (primer). The primer, derived from cDm51, was either a 106-base *Stu*I–*Bgl*I restriction fragment (5′ proximal primer) or a 106-base *Bgl*I-*Bgl*II fragment (distal primer). Reverse-transcription reactions contained 10 μCi [^{35}S] α-thio dATP. Extension products were analyzed on a 6% acrylamide-8 M urea gel. A sequencing ladder of the *Bgl*II–*Bam*HI fragment from pDm131, subcloned into the *Bam*HI site of M13mp19, was used for the size determination of extension products.

Drosophila strains: $Df(3R)by^{62}/In(3LR)TM1$ and $Df(3R)by^{10}/In(3LR)TM3$ were obtained from K. A. MATTHEWS and T. C. KAUFMAN (KEMPHUES, RAFF and KAUFMAN 1983). $Df(3R)\gamma B104/In(3LR)TM3$ was obtained from C. M. CHENEY (unpublished data). The exact positions of the distal breakpoints of the latter two deletions have not been established, but the best estimates (KEMPHUES, RAFF and KAUFMAN 1983; K. A. MATTHEWS and C. M. CHENEY, personal communications) place them between 85E10 and 85E15, immediately distal to one of the α-tubulin genes (at 85E6-10, KALFAYAN and WENSINK 1981; MISCHKE and PARDUE 1982). For these, and most other laboratory strains tested, the restriction pattern in the neighborhood of the *Mtn* gene is similar, with the *Mtn* gene included in a 3.5-kb *Eco*RI fragment; this is designated pattern A. For one of the laboratory strains used, however, the restriction pattern is different, and the *Mtn* gene is in a 5.8-kb *Eco*RI fragment; this is designated pattern B.

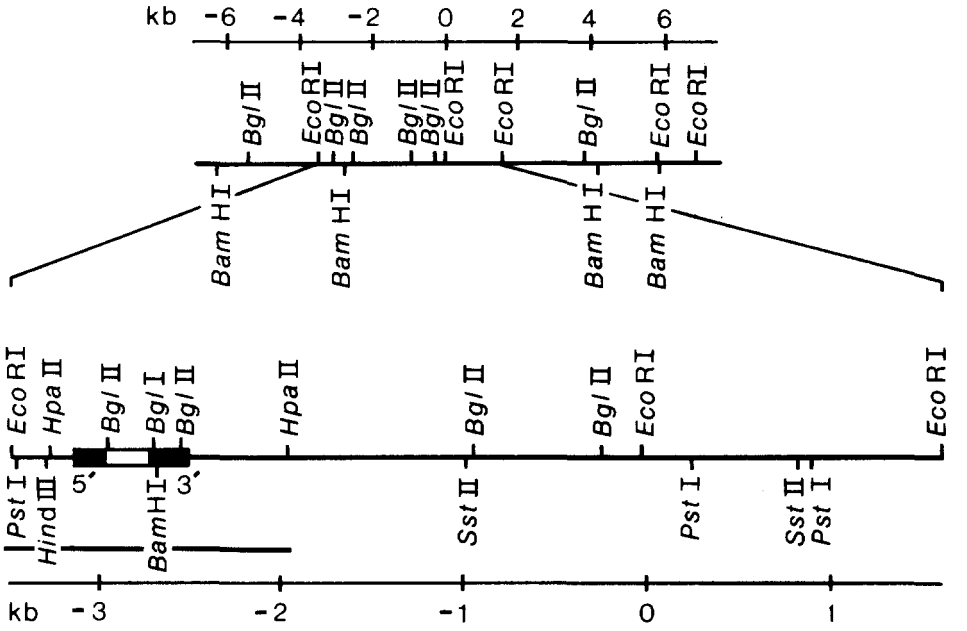

FIGURE 1.—Restriction enzyme map of the *Mtn* region. Top, genomic insert cloned in EMBL4 (λDm13). Bottom, fragment of λDm13 subcloned in pUC9 (pDm131). According to our interpretation of results presented in this paper, the box would represent the transcribed region, exons in black and intron in white. The cDNA clone (cDm51), which was used as a probe for many of these experiments, is coextensive with the black boxes except for the presence, in the former, of poly-A and poly-C tails. The bar indicates the segment sequenced. Only the most proximal *Hpa*II sites are included.

RESULTS

Organization and sequence of the metallothionein gene: Chromosomal DNA fragments containing *Mtn* were isolated using cDm51, a cDNA clone, to probe a genomic library of *D. melanogaster*. Between five and six genome equivalents of Drosophila DNA were screened, and six plaques gave a positive signal. From these, we obtained three phage strains that carried the MT structural gene plus different amounts of flanking DNA. In one of the three, λDm13, the flanking DNA extends 3.8 kb in the 5′ direction and 10 kb in the 3′ direction. Figure 1 shows a restriction map of the λDm13 insert. This map agrees with others obtained by digestion of total Drosophila DNA (data not shown).

Subclones of λDm13 were used to obtain the primary structure of *Mtn*. The strategy used and the sequence of a 1543-bp segment are shown in Figure 2. The frequency of codon usage in the predicted amino acid sequence is compared in Table 1 to that of seven other Drosophila proteins compiled by O'CONNELL and ROSBASH (1984); the frequency with which G or C occupies the third codon position is 77% in the sequence under investigation and is 78% for the pool of Drosophila proteins. Statistical analysis carried out by K. BURTIS (Stanford University School of Medicine, personal communication) ac-

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                                                                -300.
GAATTCGTTGCAGGACAGGATGTGGTGCCCGATGTGACTAGCTCTTTGCTGCAGGCCGTCCTATCCTCTGGTTCCGATAA

GAGACCCAGAACTCCGGCCCCCCACCGCCCACCGCCACCCCCATACATATGTGGTACGCAAGTAAGAGTGCCTGCGCATG

          -200.
CCCCATGTGCCCCACCAAGAGTTTTGCATCCCATACAAGTCCCCAAAGTGGAGAACCGAACCAATTCTTCGCGGGCAGAA

                    -100.
CAAAAGCTTCTGCACACGTCTCCACTCGAATTTGGAGCCGGCCGGCGTGTGCAAAAGAGGTGAATCGAACGAAAGACCCG

                                        1.
TGTGTAAAGCCGCGTTTCCAAAATGTATAAAACCGAGAGCATCTGGCCAATGTGCATCAGTTGTGGTCAGCAGCAAAATC

                                                          100.
AAGTGAATCATCTCAGTGCAACTAAAGGCCTAAATAGCCCATACCTACCTTTTTTGTAAACAAGTGAACAAGTTCGAGGA

                    Met Pro Cys Pro Cys Gly Ser G
AATACAACTCAATCAAG ATG CCT TGC CCA TGC GGA AGC G GTAAGTTCGCAGTCTGGTGTGATCCTTTAGGA

          200.
TATCACAGATCTTTCAGAGAAATGGTATTATACTAGTATAAAAATTCAATGGTGATTCAATAGTATAAAAATTCAAGGCT

                              300.
GAAACTATCTGCAAAGTGAAATCTCTGAGTTCGTCTCTCTAAGAAAAGAAGTTCTTCAACTGCGTTTTATAAAATGGAAC

                                                    400.          Iy Cys
ACTAATGTTATATGGCTTATGGATTACAGGATGTACCAGCATGTACTAATTTTTAAATTCTACTTCTTTCCAG GA TGC

Lys Cys Ala Ser Gln Ala Thr Lys Gly Ser Cys Asn Cys Gly Ser Asp Cys Lys Cys Gly
AAA TGC GCC AGC CAG GCC ACC AAG GGA TCC TGC AAC TGC GGA TCT GAC TGC AAG TGC GGC

Gly Asp Lys Lys Ser Ala Cys Gly Cys Ser Glu Opa
GGC GAC AAG AAA TCC GCC TGC GGC TGC TCC GAG TGA GCTTTCCCCCAAAAAAGATCTGGAGTAGAGGC

                                        600.
GCTGCATCTTGTCTCTCTACACACCCTGCAATAAATGTCCAATTAAAGTAATTGATGCCTAACTGCGTCTTTTCGGGTTG

                                                          700.
CATAATCAATTGGTCTGCGGCATTCTAGGTTAGATTCGCTTTTATTGGAGGTAGCTTCTAGCTACGTGGTCGGCAATATG

CGTCGTGGAAATGGGATGGTCAAGTGTTTTCCACAATGTGCATATACATATGTACATAACACTAAAGTCAGTTGAGCAAT

          800.
ATGGTAATCTGAGATGACTACTTCTGAAGCGACTGAGGGATGAGTTCAAACACACGGCTGACCATGACTGTAGATAAAAA

                              900.
TACAGTTCGGCGTTAGAATATAGCCGCTATCGAATGGATAATATTAAAGAATACTAGCTTTAGAAATAATAAAAATATAT

                                                    1000.
TACCCTATCAAATTTAAAACGATTTTAGGCATAACAACGAAATGGGTAATGAAAGTTCATATTTAAATCGGCTTCCATTA

                                                          1100.
TTTTATAGGTGATTCATAGAAATATATGATTGTAGACTTATTATTGCTCAGTCTGTTTTGTGAAATGCCTCGTTTATAGC

GCAAAAGTGCCATATAGTTTTAGATGTAATATGATCGCGCAATTAACATGAAAGTGTAAGAACCCG                A
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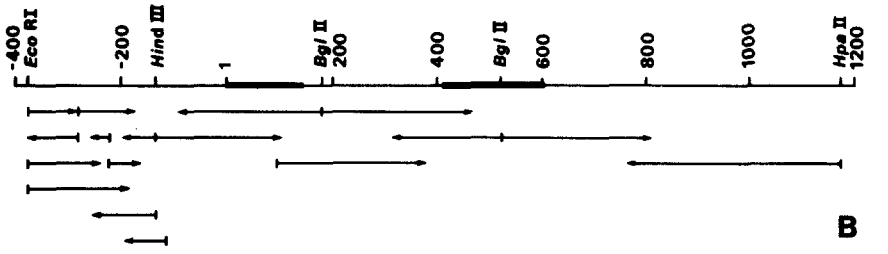

TABLE 1

Codon usage in Drosophila metallothionein

Amino acid	Codon	*Dm*	MT	Amino acid	Codon	*Dm*	MT
Ser	TCT	10	1	Gln	CAA	9	0
	TCC	64	3		CAG	100	1
	TCA	5	0				
	TCG	25	0				
	AGT	2	0	Lys	AAA	3	2
	AGC	37	2		AAG	98	3
Pro	CCT	15	1	Asp	GAT	50	0
	CCC	82	0		GAC	68	2
	CCA	20	1				
	CCG	9	0	Glu	GAA	14	0
					GAG	128	1
Thr	ACT	10	0				
	ACC	94	1	Cys	TGT	3	0
	ACA	0	0		TGC	17	10
	ACG	6	0				
Ala	GCT	34	0	Gly	GGT	48	0
	GCC	126	3		GGC	74	3
	GCA	8	0		GGA	37	4
	GCG	12	0		GGG	1	0

The column headed *Dm* includes the number of times that different codons are present in a pool of genes for seven different Drosophila proteins (O'CONNELL and ROSBASH 1984). The data for the MT column are from Figure 2.

cording to the method of STADEN and MCLACHLAN (1982) showed no significant difference in the bias of codon usage between the MT and other Drosophila genes. The GC content of the exons is very high, 64%; GC content is much lower for the untranslated regions of the transcript—40% for the 3′ and 5′ ends and 32% for the intron.

Representation of *Mtn* sequences in metal-induced, abundant RNA: When total nucleic acids were hybridized to single-stranded probes derived from the *Eco*RI—*Bam*HI 5′ fragment of the cDNA, cloned in opposite orientations in M13 vectors, the fragment carried by M13mp9, but not by M13mp8, showed hybridization to RNA in a Northern blot (data not shown). This demonstrates that the strand shown in Figure 2 is the antisense strand.

To determine whether the presumptive intron is present in larval RNA, an intron-specific probe was used for hybridization to a Northern blot. Under

FIGURE 2.—A, Sequence of a segment of λDm13 which includes the *Mtn* gene. The underlined portions are present in a previously sequenced cDNA clone; the only mismatch between the two sequences is that the C in position 2 went undetected in the cDNA sequence (LASTOWSKI-PERRY, OTTO and MARONI 1985). The boxes identify the Hogness-Goldberg and polyadenylation sequences. The arrows mark 12-mers in which at least nine bases correspond to the consensus sequence identified as the metal regulatory regions in mammals (see also Figure 8). B, Sequencing strategy. The region about −200 was sequenced several times in different subclones because band compression (due probably to secondary structures) made sequence determination difficult.

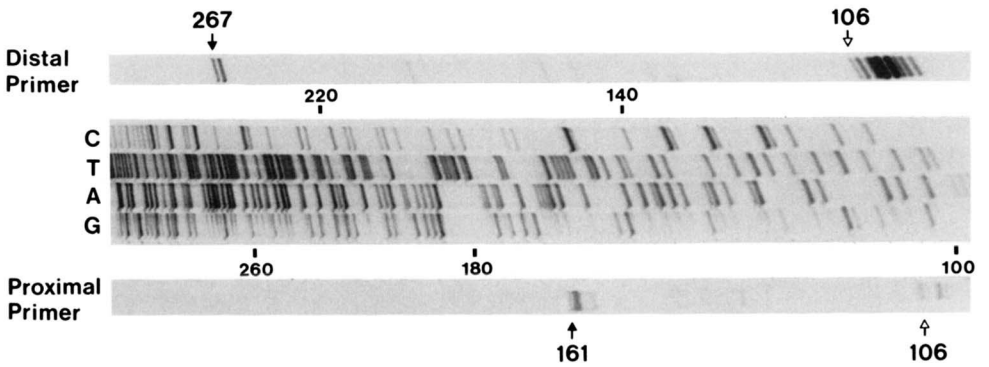

FIGURE 3.—Identification of the putative cap site. The designation of proximal and distal (primers) is relative to the 5′ end of the mRNA. Open arrows indicate the position of migration of the primers; filled arrows indicate that of the longest extension products. The sequencing ladder was used as a size marker. All numbers indicate fragment length in bases. Given the length of the primers (106 bases), one can estimate that the primer with 5′ end at 56 was extended by 55 (+/−1) bases and the one with 5′ end at 427 by 161 (+/−1), from which we deduce the same initiation site if we exclude the intron (265 bases).

conditions that show strong hybridization of the cDNA probe to RNA of metal-treated larvae (LASTOWSKI-PERRY, OTTO and MARONI 1985), hybridization by the intron probe to the putative MT mRNA was not detectable (data not shown).

To define the 5′ end of the transcript, two different primers derived from the cDNA clone were used in separate primer extension reactions. The proximal primer extended from position 56 to position 426 to include portions of both exons, but not the intron, and the 5′ distal primer extended from position 427 to position 532 to include only a portion of the second exon (Figure 2). After hybridization to total RNA from induced larvae and reverse-transcription, the majority of the extended fragments, from both primers, reached lengths that brought their ends to the neighborhood of position 1, when allowance is made for excision of the proposed intron in the case of the more distal primer (Figure 3). In both cases, the longest of the fragments reached position 1 (+/−1 base).

Absence of sequences cross-hybridizing to metallothionein cDNA: To discover whether other sequences, related to the cloned *Mtn*, occur in *D. melanogaster*, total genomic DNA was subjected to Southern analysis. The DNA was digested with *Eco*RI or *Hpa*II, fractionated by agarose gel electrophoresis and transferred onto nitrocellulose membranes. Hybridization was performed using a radioactive cDNA probe and three different stringency conditions. A single fragment was labeled at the two highest temperatures; at the lowest temperature, cross-hybridization occurred in a broad smear reflecting the distribution of the bulk of DNA (Figure 4).

Determination of the number of copies of the metallothionein gene: Genomic reconstruction experiments were performed by mixing λDm13 (Figure 1) with *E. coli* DNA in proportions simulating Drosophila genomes having one, two or four copies of the MT gene. The DNA mixtures were digested with

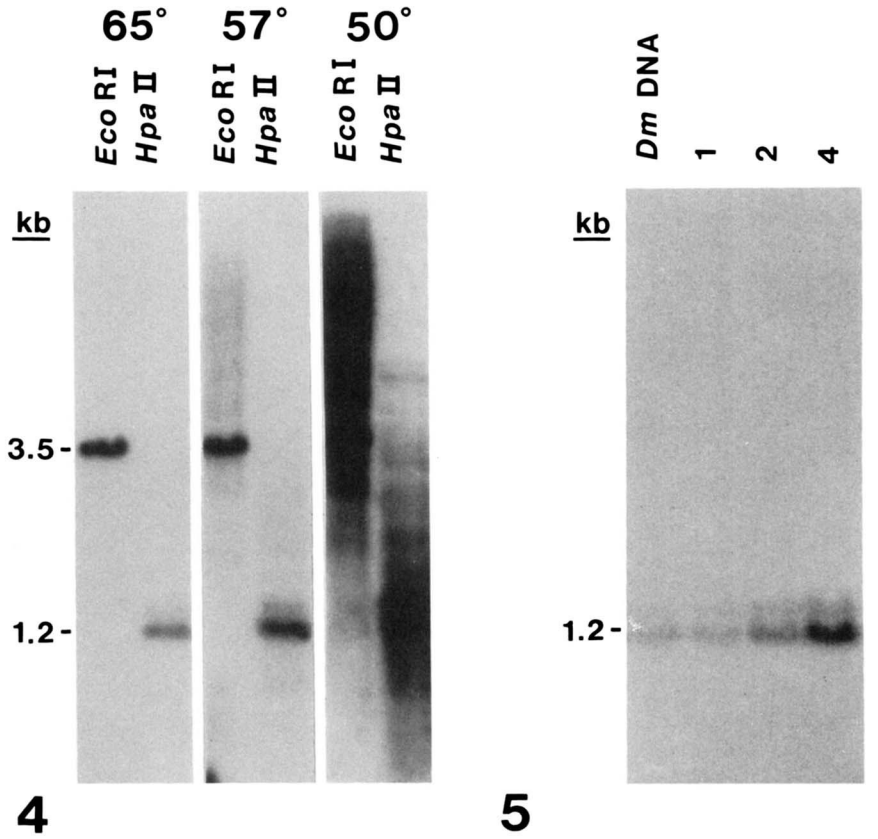

FIGURE 4.—Test for genomic fragments capable of cross-hybridizing with the *Mtn* structural sequence. Triplicate filters were hybridized under the least stringent conditions (3× SSC, 50°) and were then washed separately at three different temperatures and at the same ionic strength. Labeling in the 50° lanes reflects the distribution of the bulk of DNA (except for the 3.5- and 1.2-kb bands). The faint band in the *Hpa*II lanes is due to incomplete digestion (there is a third *Hpa*II site 180 bp upstream of this fragment).

FIGURE 5.—Genomic reconstruction experiment. Each lane contains either 3 μg Drosophila DNA (Dm) or 3 μg of a mixture of *E. coli* and λDm13 DNA (in the proportion of 1, 2 or 4 copies per haploid genome equivalent). The cDNA probe was used. The value used for the Drosophila haploid genome was 1.6×10^8 bp.

*Hpa*II, fractionated on agarose gels and transferred to nitrocellulose membranes for hybridization with a cDNA probe. Drosophila DNA was treated in parallel with the simulation mixtures. The intensity of hybridization of the Drosophila sample was comparable to that of the reconstructed genome with one copy (Figure 5).

Cytogenetic and genetic localization of the metallothionein gene: *In situ* hybridization to polytene chromosomes was carried out using λDm13 as a probe. Only one site, between 85E10 and 85E15 in chromosomal arm *3R*, displayed hybridization (Figure 6).

This localization was confirmed genetically by comparing the restriction profiles for different deletion heterozygotes; this approach takes advantage of the

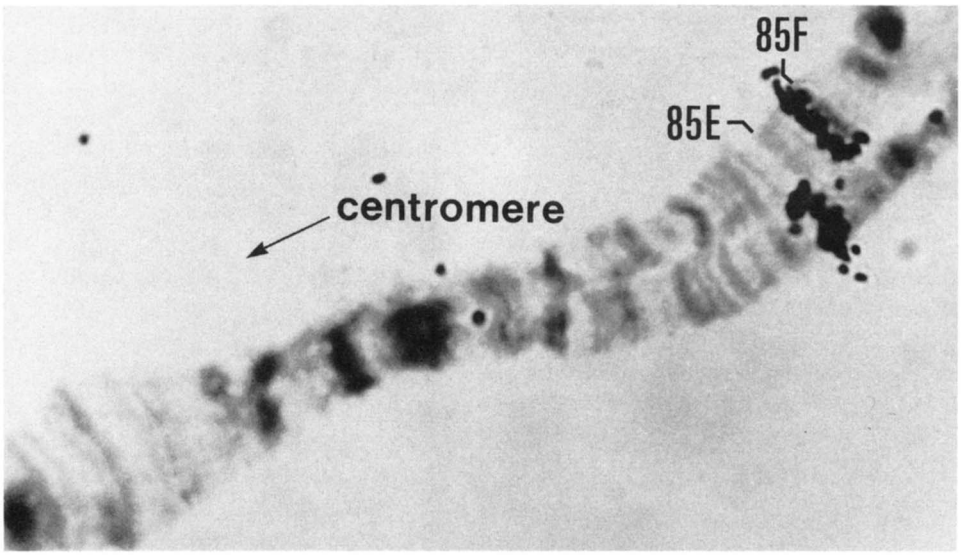

FIGURE 6.—*In situ* hybridization of λDm13 to polytene chromosomes. Proximal segment of chromosomal arm *3R*. In this nucleus, the two homologues are not paired in the region of hybridization.

existence of a laboratory strain having an unusual pattern of restriction sites in the region of *Mtn* (pattern B). Individuals from the pattern B strain were crossed with individuals from three different, balanced third-chromosome deletion stocks, each of which shows the restriction pattern A. The restriction patterns of the resultant heterozygotes were as follows: *Df*(3*R*)γ*B*104/*pattern B* had a mixed pattern AB, which indicates that this deficiency chromosome carries a *Mtn* gene (with pattern A). Heterozygotes *Df*(3*R*)*by*^{10}/*pattern B* and *Df*(3*R*)*by*^{62}/*pattern B* both gave only pattern B, indicating that neither of the deficiency chromosomes in question is contributing an *Mtn* gene (Figure 7A). These results localize *Mtn* to a region of the right arm of the third chromosome between the distal breakpoints of γ*B104* and *by*^{10} (85E10 and 85E15, Figure 7B).

DISCUSSION

Throughout this paper we have referred to the segment of pDm131 that hybridizes to cDm51, a cDNA clone, as the metallothionein gene or *Mtn*. This was done for the sake of convenience; we will now attempt to justify the designation. We previously showed (LASTOWSKI-PERRY, OTTO and MARONI 1985) that cDm51 has all of the sequence elements expected for an mRNA and that it could code for a small protein with remarkable sequence similarity to mammalian MT. We also showed that cDm51 cross-hybridizes to a cloned cDNA of the mouse metallothionein I. Furthermore, we demonstrated that cDm51 is complementary to a polyadenylated RNA (localized in the larval midgut), the concentration of which increases more than tenfold by the ingestion of cadmium or copper ions. Based on this structural and functional evi-

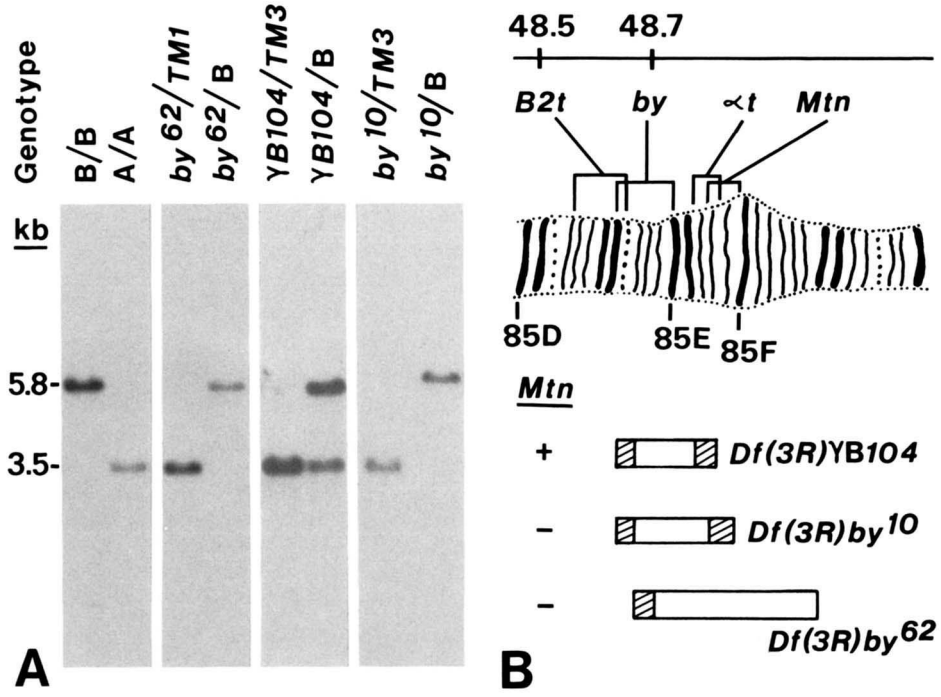

FIGURE 7.—Cytogenetic localization of *Mtn*. A, Southern blot analysis of different deletion heterozygotes. DNA was digested with *Eco*RI, the probe used was cDm51. B, Schematic representation of a segment of the salivary gland polytene chromosomes [according to LEFEVRE (1976)] and the three deficiencies used (hatched areas represent uncertainties in the break-points). The numbering of bands in this paper follows the map of Bridges, although in this figure not all the minor bands are represented. The numbers on the upper line correspond to genetic map positions. Parts of this figure are after KEMPHUES, RAFF and KAUFMAN (1983).

dence, we proposed that cDm51 was derived from the mRNA of a Drosophila MT. Evidence for the production of a Drosophila protein similar to MT was also obtained (MARONI and WATSON 1985): a 4000–5000 dalton cadmium-binding protein fraction, detected in the larval midgut, is inducible by cadmium and copper and has spectroscopic similarities to mammalian MT (see also DEBEC, MOKDAD and WEGNEZ 1985).

As is shown in this paper, cDm51 hybridizes to a Drosophila genomic DNA of identical sequence (disregarding a putative intron). Given that cDm51 is, thus, complementary to a genomic fragment apparently present as a single copy (see below), as well as to a polyadenylated RNA, it is reasonable to conclude that this genomic fragment codes for the RNA in question and constitutes a functional metallothionein gene. This conclusion is corroborated by the primer extension experiments, which indicate that the most probable site of initiation of transcription is 28 bp downstream of a TATA box and 50–200 bp downstream of a series of segments very similar to sequences associated with metal regulation in mammals (see below). The genomic clone hybridizes to a single site on polytene chromosomes, in section 85E10-15; therefore, we take this to be the site of the metallothionein gene.

Several of the experiments reported here could have indicated the existence of multiple copies had we been dealing with a gene family. This, however, was not the case; and based on the following lines of evidence, we conclude that there is a single sequence in the *D. melanogaster* genome capable of cross-hybridizing to cDm51 and probably a single metallothionein gene:

1. Only one sequence related to the cloned *Mtn* was revealed by Southern analysis of genomic DNA, even under conditions of reduced stringency. KARIN and RICHARDS (1982) were able to detect 11 or 12 different fragments in a similar experiment done with human metallothionein.
2. The 17 cDNA clones that were isolated independently from a cDNA library on the basis of copper inducibility were, very likely, derived from transcription products of the same gene as indicated by restriction enzyme analyses [LASTOWSKI-PERRY, OTTO and MARONI (1985) and data not shown]. This suggests that, if there are related sequences in the genome, they are poorly inducible by metals.
3. There is a single copy of the gene within the segment cloned in λDm13, according to the restriction map. This, of course, does not rule out the possibility that there are multiple copies of the entire 14-kb insert in the Drosophila genome.
4. Although different restriction-enzyme patterns in the neighborhood of *Mtn* (patterns A and B, for instance) occur, only one of the patterns is found in each strain. This implies that if there were multiple copies of the 14-kb segment, these would have to be corrected to maintain sequence identity within a strain.
5. *In situ* hybridization experiments show labeling at only one site.
6. Only six *Mtn*-carrying clones were detected by hybridization to filter-lifts of lambda plaques carrying the equivalent of between five and six Drosophila genomes.
7. Results of genomic reconstruction experiments indicate the occurrence of a single copy of this segment per haploid genome.

The existence of a single *Mtn* gene in Drosophila, and its localization within a narrow chromosomal segment defined by partially overlapping deficiencies, would simplify the search for *Mtn*-null mutations.

That the sequence presented in Figure 2 is, in fact, the complement of the transcribed strand is demonstrated by tests with single-stranded probes and by the primer extension experiments. (The primer located further downstream from the proposed 5′ end produced the longer extended fragment.)

The absence of putative intron sequences in mature MT-RNA was demonstrated with an intron-specific probe. This was corroborated by the results of the primer extension experiments: primers before and after the intron agreed on the 5′ end, if we assume that the intervening sequence is not present in the RNA. The site corresponding to the 5′ end of the mature mRNA, which is at, or in the neighborhood of, the base-pair numbered one (Figure 2) was thus identified. The 3′ end of the gene was deduced from the position of the poly-A tail in the cDNA.

The 265-bp intron found in this structural gene includes the splicing signals

Mammalian	C Y T T T G C R Y Y C G
Drosophila	(GC)T T T T G C A Y N C G
-55	G c T T T a C A C A C G
-88	C T T T T G C A C A C G
-127	C T T c T G C A C A C G
-133	G T T c T G C c C G C G
-193	G T T T T G C A T C C G

FIGURE 8.—Sequences suspected of being responsible for metallothionein metal regulation. The top line is the consensus sequence responsible for metal regulation of mammalian metallothionein (STUART *et al.* 1984). The second line is a consensus of related sequences found in Drosophila. The rest are five examples (different from the Drosophila consensus in not more than two positions; some of them occur as an inverse repeat of the sequence given). The numbers on the left column correspond to the position of the base, in each fragment, which is most distant from the TATA box.

GT-AG and interrupts the coding region at the Gly-8 codon. In the mouse and human MT genes there are two introns, one in the codon corresponding to Gly-10 and the other in Ser-32 (GLANVILLE, DURNAM and PALMITER 1981; KARIN and RICHARDS 1982). According to our proposed alignment, the mammalian Ser-32 corresponds to Ser-34, in Drosophila, the seventh residue from the C-terminus.

In the mouse MT-I gene there is a 12-bp consensus sequence that is repeated several times within the promoter region; these segments have been shown to be directly responsible for metal regulation of that gene. This sequence is also present in the several human MT genes studied so far (STUART *et al.* 1984; CARTER *et al.* 1984). We found seven occurrences of a similar sequence, or its inverse complement, within the 300 bp preceding the TATA box; these are identical to at least 9 of 12 bp of the mammalian segment. A consensus sequence of the repeats present in the Drosophila gene is shown in Figure 8. There are nine 12-mers within 200 bp upstream of the TATA box with 75% identity to the Drosophila consensus; five of these, with identity of at least 83%, are shown. Experiments to test whether this DNA segment is capable of supporting metal-regulated transcription in Drosophila are currently under way.

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