Genetic and Molecular Complexity of the Position Effect Variegation Modifier *mod(mdg4)* in Drosophila

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ABSTRACT

mod(mdg4), also known as *E(var)3-93D,* is involved in a variety of processes, such as gene silencing in position effect variegation (PEV), the control of *gypsy* insulator sequences, regulation of homeotic gene expression, and programmed cell death. We have isolated a large number of *mod(mdg4)* cDNAs, representing 21 different isoforms generated by alternative splicing. The deduced proteins are characterized by a common N terminus of 402 amino acids, including the BTB/POZ-domain. Most of the variable C termini contain a new consensus sequence, including four positioned hydrophobic amino acids and a Cys_2His_2 motif. Using specific antibodies for two protein isoforms, we demonstrate different distributions of the corresponding proteins on polytene chromosomes. Mutations in the genomic region encoding exons 1–4 show enhancement of PEV and homeotic transformation and affect viability and fertility. Homeotic and PEV phenotypes are enhanced by mutations in other trx-group genes. A transgene containing the common 5′ region of *mod(mdg4)* that is present in all splice variants known so far partially rescues the recessive lethality of *mod(mdg4)* mutant alleles. Our data provide evidence that the molecular and genetic complexity of *mod(mdg4)* is caused by a large set of individual protein isoforms with specific functions in regulating the chromatin structure of different sets of genes throughout development.

DURING development of multicellular organisms, the spatial and temporal expression of many genes must be precisely regulated. Growing evidence implicates changes in chromatin structure as an important level in this control. Although the organization of the nucleosome is well understood (Luger *et al.* 1997), we lack substantial knowledge of higher-order chromatin structure. Chromatin proteins involved in structural changes during transcriptional regulation were identified by several approaches. In addition to biochemical analysis of chromatin remodeling complexes (Tsukiyama and Wu 1997; Kadonaga 1998), genetic dissection of chromatin structure has been performed successfully in Drosophila.

Several genes involved in the regulation of chromatin structure were identified as regulators of homeotic gene complexes. The Polycomb group of genes has been shown to be essential for silencing of homeotic genes outside their limits of expression. This function involves the formation of large multisubunit protein complexes, which limit the access of transcription factors to the genes to be silenced (Paro and Harte 1996; Pirrotta 1997). Trithorax (trx) group genes, on the other hand, encode positive regulators of homeotic gene expression that in some way counteract the effect of the Polycomb group silencers (Simon 1995).

Modification of position effect variegation (PEV) provides another powerful approach to identifying genes involved in the regulation of chromatin structure (for review see Reuter and Spierer 1992; Weiler and Wakimoto 1995; Wallrath 1998). More than 100 genes that, when mutated, either suppress or enhance PEV have been identified. The hypothesis that mutations in suppressors of PEV affect components of chromatin complexes involved in silencing is consistent with the molecular and cytological data available for this class of proteins (Wallrath 1998). The enhancers of PEV, which exert opposite effects, are less well studied. Insertional PEV enhancers have been isolated after remobilization of modified *P*-element transposons (Dorn *et al.* 1993a). The first enhancer gene of PEV was molecularly characterized using the *E(var)3-93D*^{neo129} mutation (Dorn *et al.* 1993b). In a study of modifiers of *gypsy*-induced mutations, it was shown that mutations in *mod(mdg4)* are also enhancers of PEV and are allelic to the *E(var)3-93D*^{neo129} mutation (Gerasimova *et al.* 1995; Gerasimova and Corces 1998). *Trithorax-like* (*Trl*), the second molecularly characterized PEV enhancer gene, encodes the GAGA factor (Farkas *et al.* 1994). Both genes encode transcriptional regulators and mutations display phenotypes characteristic of the trx group genes. Molecular

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analysis of both *mod(mdg4)* and *Trl* demonstrated the existence of several transcripts generated by alternative splicing (Dorn *et al.* 1993b; Gerasimova *et al.* 1995; Benyajati *et al.* 1997; Harvey *et al.* 1997). By immunocytology, proteins of both genes were shown to be associated with many sites along the polytene chromosomes. Analysis of two individual Trl isoforms revealed a complete colocalization and their physical interaction was demonstrated by coimmunoprecipitation (Benyajati *et al.* 1997).

Six different Mod(mdg4) isoforms have been identified so far (Dorn *et al.* 1993b; Gerasimova *et al.* 1995; Harvey *et al.* 1997). All of them contain a common N terminus, including the BTB/POZ domain, which is a conserved protein-protein interaction domain present in a number of genes involved in regulation of transcription (Bardwell and Treisman 1994; Ahmad *et al.* 1998). In most cases, the C terminus of such BTB/POZ-domain proteins contains one or more zinc finger motifs of the Cys_2His_2 type. Their involvement in DNA binding could be shown for two BTB zinc finger proteins, Trl and Tramtrack (Biggin and Tjan 1988; Harrison and Travers 1990).

Recently, we demonstrated genetic interactions between *mod(mdg4)* and several suppressors of PEV, suggesting that they were involved in a common process in the regulation of chromatin structure. Similarly, genetic interaction between *mod(mdg4)* mutations and mutations in trx group genes could be shown (Dorn *et al.* 1993b; Gerasimova and Corces 1998), indicating that the set of genes identified as homeotic or PEV regulators may functionally overlap. Because of the ability of *mod(mdg4)* alleles to modify the insulating effect of *gypsy* transposon insertions, Gerasimova *et al.* (1995) proposed a general role for *mod(mdg4)* in the control of chromatin insulators. *Doom*, another splice variant of *mod(mdg4)*, was identified as an interactor of the baculovirus inhibitor of apoptosis protein (IAP), whose involvement in apoptosis could be demonstrated directly (Harvey *et al.* 1997). Together, these data demonstrate a considerable molecular and functional complexity for the *mod(mdg4)* gene.

In this article, we describe a comprehensive molecular analysis of *mod(mdg4)*. We have identified 17 additional splice variants, that reflect the complexity of Mod(mdg4) protein expression seen on Western blots. The alignment of the deduced protein isoforms revealed the existence of a new conserved protein consensus motif in addition to the BTB/POZ domain in most of these isoforms. By the use of isoform-specific antibodies, we provide the first evidence for differential binding of Mod(mdg4) protein isoforms on polytene chromosomes. Genetic and molecular analyses of mutations demonstrate an essential maternal contribution of *mod(mdg4)* to early embryonic development. Significantly reduced amounts of Mod(mdg4) proteins in egg chambers of mutant females result in maternal effect lethality.

MATERIALS AND METHODS

Fly stocks and genetic analysis: Fly stocks were maintained under standard conditions. Chromosomes and mutations are described in Lindsley and Zimm (1992). All crosses, unless otherwise indicated, were carried out at standard temperature (25°). Isolation of *mod(mdg4)* mutant alleles is described in Dorn *et al.* (1993b).

The degree of homeotic transformation of abdominal segment A5 to A4 in males was quantified using arbitrary units. Flies were graded into seven classes on the basis of the size of the area without black pigmentation on A5. Unpigmented A5 equals grade 6; grade 5 corresponds to an unpigmented area of ~80%; grade 4 equals ~50% of unpigmented A5; ~30% unpigmented A5 was given grade 3; grade 2 represents A5 with isolated medium-sized unpigmented patches; A5 with small unpigmented spots corresponds to grade 1; and grade 0 represents completely pigmented A5. Quantification of PEV in w^{m4} was performed by pigment measurement as described in Ephrussi and Herold (1944). The mutant alleles *AI117* and *AI351* obtained from Dr. M. Frasch were renamed *mod(mdg4)*^{06} and *mod(mdg4)*^{07}, respectively.

Standard procedures: Restriction analysis, subcloning, and Southern blot analysis were performed according to Sambrook *et al.* (1989). Genomic DNA from adult flies was isolated as described in Jowett (1986). Hybridization probes were generated by random priming using the Multi Prime labeling kit (Amersham, Piscataway, NJ). For radioactive labeling, [α-^{32}P]dATP was used. Sequencing was performed using the T7 sequencing kit (Pharmacia, Piscataway, NJ) and [α-^{35}S]dATP for radioactive labeling or alternatively by cycle sequencing and analysis using the sequencer ABI 377 (Perkin Elmer, Norwalk, CT).

RNA isolation and analysis: RNA was isolated as described by Auffray and Rougeon (1980). The mRNA purification kit (Pharmacia) was used to purify poly(A)$^+$ RNA. RNA was hybridized in a solution containing 50% formamide at 42° after size fractionation on 1% agarose-formaldehyde gels and transfer to Hybond-N$^+$ (Amersham).

***P*-element-mediated germline transformation:** Germline transformation was performed as described in Rubin and Spradling (1982). The 7.5-kb *Bam*HI genomic fragment containing the 5′ region of *mod(mdg4)* was inserted in the transformation vector pW8 (Klemenz *et al.* 1986). One second chromosomal transgenic line was obtained. Mobilization of the insertion using *TM3, Sb Δ2-3* as a transposase source resulted in two additional lines with the w^+ insert on a second chromosome carrying the dominant marker *Sco*. All three transgenic lines were able to rescue the recessive lethality of *mod(mdg4)*^{02}. For complementation crosses in Table 3 and Figure 9, line *P(w*$^+$*7.5kb Bam*HI*)-2/1* was used.

Screening of embryonic cDNA libraries: cDNA clones have been isolated from two embryonic libraries: 12- to 24-hr embryonic cDNA library in λgt10 (Poole *et al.* 1985) and 2- to 12-hr embryonic cDNA library in λZAPII (Stratagene, La Jolla, CA). The 0.5-kb genomic *Sal*I fragment that is colinear to the earlier identified cDNA clones *mod(mdg4)-58.0* (*23gt*) and *mod(mdg4)-67.2* (*38gt*) (Dorn *et al.* 1993b) was used as a radioactive labeled probe. Isolated clones were analyzed by restriction analysis and partial sequencing. Some of the isolated clones contained sequences at the 5′ ends derived from other cloned genes, indicating that artificial clones resulted during construction of the libraries. The numbers of independent cDNA clones isolated were as follows:

one cDNA clone: mod(mdg4)46.3, 51.4, 52.0, 56.9, 57.4, 60.1, 65.0
two to four cDNA clones: mod(mdg4)53.1, 54.2, 54.6, 55.1, 55.3, 55.7, 56.3, 58.0, 58.6, 59.0, 62.3, 64.2
five to eight cDNA clones: mod(mdg4)55.6, 67.2.

The GenBank/EMBL numbers of the representative mod(mdg4) cDNA clones are AJ277174–AJ277194.

Library construction and screening: Genomic libraries were constructed from partially *Sau*3A-digested and size-fractionated genomic DNA prepared from heterozygous w^{m4}; $mod(mdg4)^{02}$/*TM3, Sb Ser* and w^{m4}; $mod(mdg4)^{03}$/*TM3, Sb Ser* mutant flies using the λZAPII cloning system (Stratagene). Screening and phage DNA purification was performed according to the manufacturer's protocol. The 7.2-kb genomic *Bam*HI fragment was used as a probe for screening.

Antibodies: The whole open reading frame (ORF) of the cDNA clone *mod(mdg4)-58.0* as well as the specific C-terminal parts of the clones *mod(mdg4)-58.0* [amino acids (aa) 403–534] and *mod(mdg4)-67.2* (aa 403–610) were introduced into the unique *Bam*HI site of the expression vector pGEX-2T (Pharmacia). The proteins were expressed as fusion protein with glutathione-*S*-transferase of *Schistosoma japonicum* (Smith and Johnson 1988) and purified from inclusion bodies via urea extraction (Küpper *et al.* 1982) or as soluble proteins via affinity chromatography using glutathione-Sepharose as matrix. Antibodies were generated in rabbits and mice (Eurogentec). The polyclonal rabbit antibody anti-Mod(mdg4)-58.0$^{403\text{-}534}$ was purified on an affinity column with the corresponding recombinant protein.

Western blot analysis and immunoprecipitation: Nuclear extracts from 0- to 12-hr Drosophila embryos were prepared according to Elgin and Hood (1973). Nuclei were lysed in SDS protein gel loading buffer and incubated at 95° for 5 min.

For immunoprecipitation, 4 g of dechorionated embryos were homogenized in ice-cold 50 mm HEPES, pH 7.6; 385 mm NaCl; 0.1% Tween 20; 0.1% EGTA; 1 mm $MgCl_2$; 0.1 mg leupeptin, pepstatin, and aprotinin; 1 mm phenylmethylsulfonyl fluoride (PMSF) and incubated for 15 min at 4°. All subsequent steps were performed at 4°. After centrifugation for 1 hr at 15,000 × *g*, 10% glycerol was added to the supernatants, and the extracts were frozen in liquid nitrogen and stored at −70°. Embryonic extracts containing 15 mg protein were precleared with 25 μl 50% protein-A-Sepharose in binding buffer (50 mm HEPES, pH 7.6; 192.5 mm NaCl; 0.1% Tween 20; 7 mm DTT; 10% glycerol; 1 mm PMSF) for 1 hr. After centrifugation at 15,000 × *g* for 30 min, 1 μg affinity-purified anti-Mod(mdg4)-58.0$^{403\text{-}534}$ antibody was added to the supernatant, and the extracts were incubated for 2 hr. A total of 50 μl of 50% protein-A-Sepharose was added for binding of the complexes for 2 hr. The beads were washed four times for 10 min in 1 ml washing buffer (50 mm HEPES, pH 7.6; 150 mm NaCl; 0.1% Tween 20; 7 mm DTT; 10% glycerol; 1 mm PMSF) and resuspended in 30 μl SDS protein gel loading buffer. After incubation at 95° for 5 min, the eluted complexes were separated on a SDS-PAGE gel (Laemmli 1970).

After electrophoresis, the proteins were electroblotted to a polyvinylidene difluoride (PVDF) membrane. The membranes were blocked in 5% nonfat dry milk in PBS for at least 1 hr. The primary antibodies [anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ 1:4000; anti-Mod(mdg4)-67.2$^{403\text{-}610}$ 1:500; anti-Mod(mdg4)-58.0$^{403\text{-}534}$ 1:500] were diluted in blocking solution and incubated overnight at 4°. AP- or HRP-conjugated secondary antibodies (Dianova; 1:5000) were added and detection was performed using colorimetric reaction (AP) or ECL system (Amersham Pharmacia Biotech) for HRP-labeled antibodies.

Immunocytochemistry: Ovaries of adult flies were prepared in 3% paratormaldehyde, 0.1% Triton X-100 in PBS and incubated for 2 hr. The ovaries were washed in PBS and dehydrated in 10, 20, 20, 50, 70, 90, and 100% ethanol for 30 min each. The tissue was infiltrated with a mixture of polyethylene glycol (PEG) 1500 and PEG 4000 (2:1). The following steps were used: PEG/ethanol 1:3, 1 hr; PEG/ethanol 1:1, 1 hr; PEG/ethanol 3:1, 1.5 hr; and PEG, 2 hr. Ovaries were embedded and stored at 4°. Sections (2 μm) were performed using a microtome and mounted on a slide coated with 0.1% poly-l-lysine (Sigma, St. Louis). Slides were blocked in 1% BSA in PBS and the anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ antibody (1:1000 in blocking solution) was added overnight at 4°. The secondary antibody was anti-rabbit-FITC (1:100; Dianova).

Polytene chromosome squashes were prepared according to Silver and Elgin (1978). Glands were prepared in 0.7% NaCl, transferred in squashing solution (45% acetic acid; 1.85% formaldehyde) for 10 min, and squashed. Slides were incubated with primary antibodies [anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ 1:1000; anti-Mod(mdg4)-67.2$^{403\text{-}610}$ 1:500; anti-Mod(mdg4)-58.0$^{403\text{-}534}$ 1:250] overnight at 4°. The secondary antibodies were FITC or Texas red labeled and used at a dilution of 1:100. DNA staining for sections and squashes was performed with 4′, 6-diamidino-2-phenylindole (DAPI; 1 μg/ml) in PBS for 3–10 min.

RESULTS

***mod(mdg4)* codes for a large family of alternatively spliced transcripts:** The isolation of cDNA clones for *mod(mdg4)* sharing a common 5′ region but differing in 3′ sequences indicates alternative splicing (Dorn *et al.* 1993b; Gerasimova *et al.* 1995; Harvey *et al.* 1997). In Northern blot analysis, using a genomic 0.5-kb *Sal*I fragment as a probe (indicated in Figure 3), two abundant transcripts of ~2.0 and 2.3 kb have been detected (Figure 1). Sequences of the 0.5-kb *Sal*I fragment are present in all cDNA clones isolated. Both transcripts are found in all developmental stages of Drosophila analyzed, although their abundance is significantly decreased during early larval development (Figure 1). In Western blot analysis with a polyclonal antibody that should recognize all the protein isoforms, at least 12 polypeptides with apparent molecular sizes in the range 70–100 kD are detected in embryonic nuclear extracts

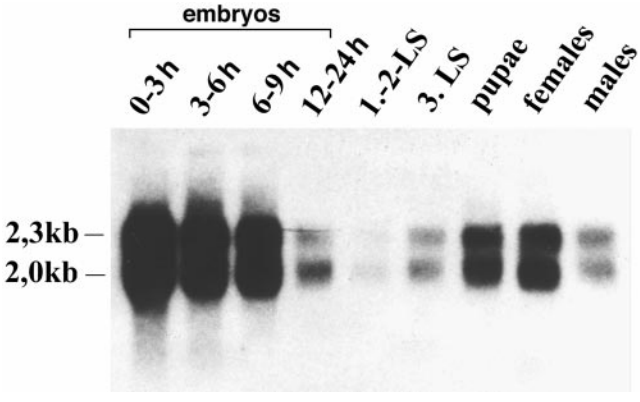


Figure 1.—Northern blot analysis of poly(A)$^+$ RNA isolated from indicated stages of Drosophila development. The genomic 0.5-kb *Sal*I fragment (*cf.* Figure 3) present in all isolated cDNA clones was used as a hybridization probe. In each lane, 5 μg RNA was loaded. Two abundant transcripts in the range 2.0–2.3 kb showing a similar developmental pattern are detected. Note that the transcripts detected represent overlapping signals from different splice variants.

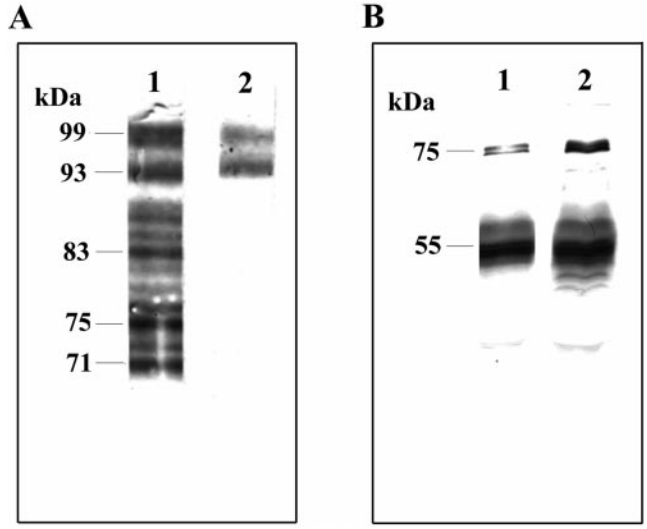


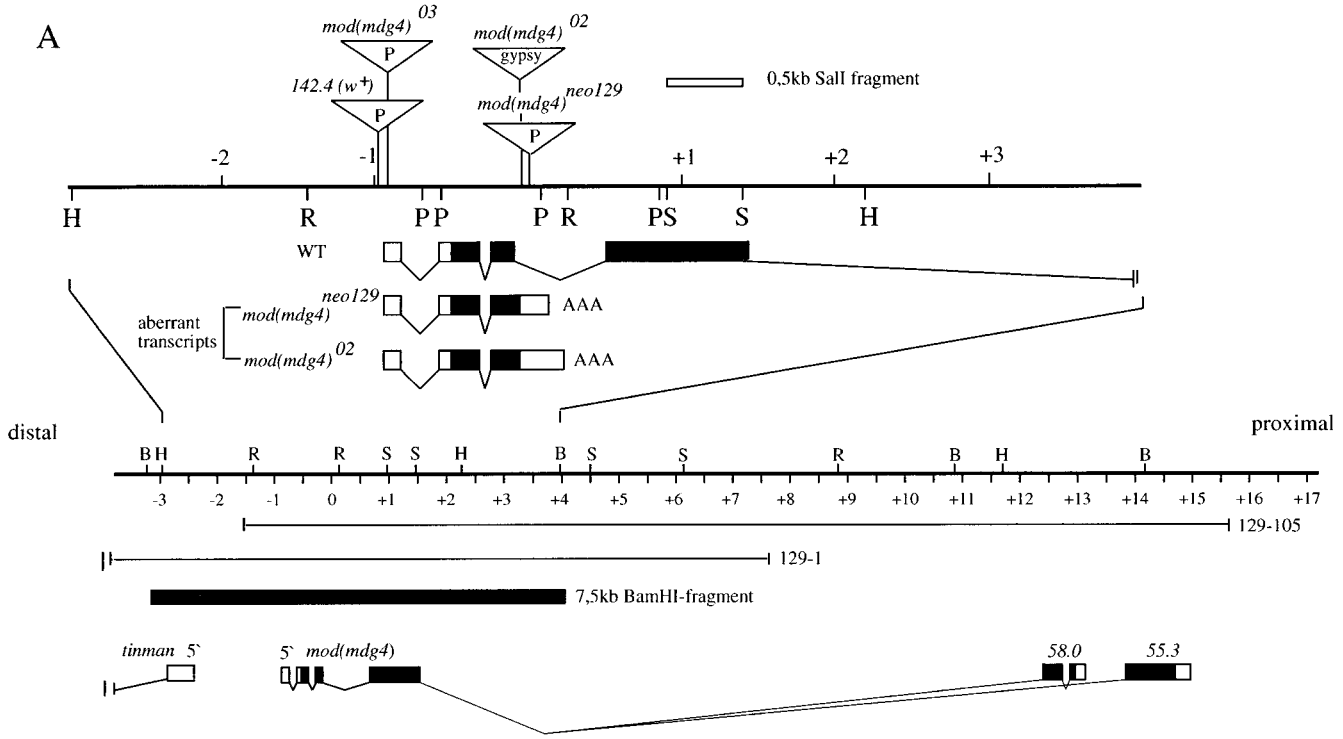
Figure 2.—Western blot analysis using antibodies detecting all Mod(mdg4) proteins and domain-specific antibodies. (A) Embryonic nuclear extract (0–12 hr) was fractionated on a 7.5% SDS-PAGE gel. Following transfer to PVDF membrane, two lanes were probed with anti-Mod(mdg4)-58.0$^{BTB-534}$ antibody detecting all Mod(mdg4) proteins (lane 1) and the domain-specific anti-Mod(mdg4)-67.2$^{403-610}$ antibody directed against the isoform Mod(mdg4)-67.2$^{403-610}$ (lane 2). (B) Immunoprecipitation of Mod(mdg4)-58.0 was performed with anti-Mod(mdg4)-58.0$^{403-534}$-specific antibody using embryonic extracts. The resulting immunoprecipitate was fractionated on a 7.5% SDS-PAGE gel, blotted onto PVDF membrane, and analyzed with anti-Mod(mdg4)-58.0$^{403-534}$-specific antibody (lane 1) and an affinity-purified anti-Mod(mdg4)-58.0^{BTB} antibody (lane 2) directed against a protein representing almost the BTB domain. The strong signal at 50 kD represents the antibody used for immunoprecipitation.

(Figure 2A, lane 1). The numerous polypeptides may be explained by complex post-translational protein modifications and/or by the existence of many alternatively spliced transcripts with similar sizes of 2.0 and 2.3 kb. To identify additional putative splice variants, two embryonic cDNA libraries were screened using the 0.5-kb *Sal*I fragment (Figure 1; materials and methods). Altogether, >75 individual cDNA clones have been isolated. These clones were grouped into 21 different cDNA families by restriction analysis and partial sequencing. Fourteen of the different cDNA families are represented by two or more independent clones, whereas 7 are represented by only a single clone, suggesting the existence of even more splice variants generated from the *mod(mdg4)* locus. With the exception of the cDNA clone *mod(mdg4)-58.6*, all of them possess poly(A) tails. All cDNAs are generated by alternative splicing using the same acceptor/donor site of exon 4. No alternative splicing was observed within exons 1–4 (*cf.* Figure 3). We did not identify any cDNA clone matching the sequence of *mod(mdg4)1.9* and *mod(mdg4)1.8* described by Gerasimova *et al.* (1995). For each of the 21 different cDNA families, the longest cDNA clone was sequenced on both strands and the putative proteins deduced from the ORFs were named according to the theoretical molecular weight. These sequences are deposited in the EMBL database (for accession numbers see material and methods).

To show that the two abundant signals obtained in Northern blot analysis are composed of several alternatively spliced transcripts, we used 3′-specific sequences of five different cDNAs as hybridization probes. Specific probes of cDNA clones *mod(mdg4)-55.6*, *mod(mdg4)-56.3*, and *mod(mdg4)-58.0* detected transcripts with low abundance with a similar size of 2.0 kb. A specific probe derived from cDNA clone *mod(mdg4)-67.2* identifies a significantly more abundant transcript with a size of 2.3 kb, whereas the specific cDNA probe from clone *mod(mdg4)-55.3* detects a transcript of very low abundance at 2.3 kb (data not shown). The existence of several alternatively spliced transcripts with similar sizes explains the detection of only two abundant transcripts in Northern analysis by probes that include sequences of the common 5′ region.

Genomic organization of the complex *mod(mdg4)* locus: The molecular structure of the common part of *mod(mdg4)*, the exon/intron structure of two *mod(mdg4)* cDNAs, and putative regulatory elements identified by sequencing of the corresponding genomic regions are shown in Figure 3. Conserved promoter elements, like two putative CAAT boxes, the TATAA box, a downstream promoter element (DPE), and the transcription start consensus sequence, could be identified at the appropriate distance 5′ from the longest cDNA clones isolated (Figure 3B). With both the TATAA box and the DPE element, *mod(mdg4)* closely resembles the Drosophila *hsp70* gene (Burke and Kadonaga 1997). The common part of all identified cDNA families is encoded by exons 1–4 separated by introns of 242, 77, and 713 bp, respectively. In contrast, larger introns of different sizes separate the common part from the alternatively spliced exons of the two cDNA clones *mod(mdg4)-58.0* and *mod(mdg4)-55.3*, which have been mapped by sequencing the corresponding genomic region shown in Figure 3A. Using the specific 3′ ends of all identified cDNA clones as probes in Southern blot hybridization experiments, five additional specific exons [*mod(mdg4)-53.2*, *mod(mdg4)-55.2*, *mod(mdg4)-55.6*, *mod(mdg4)-60.2*, and *mod(mdg4)-64.2*] could be mapped within intron 4 of *mod(mdg4)-58.0*. According to these results, the specific exons of the earlier described cDNA clones *mod-(mdg4)-67.2* [*mod(mdg4)2.2*] and *mod(mdg4)-56.3* (*Doom*) are localized downstream of exon 5 of cDNA clone *mod(mdg4)-55.3*. The putative colinear arrangement and the different abundance of alternatively spliced transcripts suggest a complex regulated splicing of a large primary transcript containing the *mod(mdg4)* exons.

***mod(mdg4)* encodes a family of related proteins containing a conserved protein consensus motive:** All identified cDNA families encode ORFs sharing the common



B

Promoter elements identified in the upstream region of *mod(mdg4)* at position -1

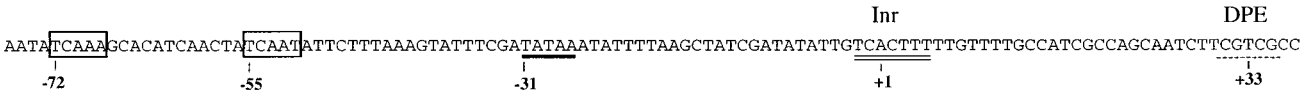


Figure 3.—Molecular map of the *mod(mdg4)* region and the putative promoter region of *mod(mdg4).* (A) Genomic region of *mod(mdg4)* encoding the two alternatively spliced transcripts *mod(mdg4)-58.0* and *mod(mdg4)-55.3* is shown; ORFs are indicated by solid bars. Below is the restriction map deduced from the two λ phages 129-1 and 129-105; the 7.5-kb *Bam*HI genomic fragment used for rescue experiments is indicated (solid bar). The top part represents a magnification of the region encoding exons 1–4 represented in all identified cDNA families. Insertional mutations identified in this region are indicated by open triangles. Truncated transcripts identified in mutations *mod(mdg4)*^{02} and *mod(mdg4)*^{neo129} by Northern blot analysis and RT-PCR are shown below the restriction map. Restriction sites indicated are: B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; R, *Eco*RI; and S, *Sal*I. (B) Genomic sequence located at position −1 in the restriction map shown above. Putative CAAT boxes, TATAA box, and DPE element are indicated. The consensus sequence for initiation of transcription (Inr) is underlined.

N-terminal part of 402 amino acids but differing in their C termini coding for an additional 28–208 amino acids. The molecular weight of the deduced proteins varies between 46 and 67 kD. The common N terminus contains the BTB/POZ domain, which has been found in >40 different proteins identified in Drosophila, humans, and viruses. Many of these proteins contain zinc finger motifs close to the C terminus (Bardwell and Treisman 1994; Zollmann *et al.* 1994). No canonical zinc finger motif could be identified in the known Mod(mdg4) proteins. Sequence comparison of the identified putative Mod(mdg4) proteins revealed a new consensus sequence, which is present in 15 out of 21 Mod(mdg4) proteins (Figure 4A). This consensus sequence consists of 4 hydrophobic amino acids in conserved positions and an additional Cys_2His_2 motif. The two His residues within the Cys_2His_2 motif are separated by 1 amino acid, mostly an asparagine. The Cys_2His_2 motif is represented by the consensus Cys-$X_{6\text{-}12}$-Cys-$X_{17\text{-}22}$-His-*X*-His (*X* is any amino acid) indicating variable spacing except the position of the two histidines separated by 1 amino acid, mostly an asparagine. Mod(mdg4) protein isoforms not containing the consensus sequence are shown in Figure 4B.

Harvey *et al.* (1997) demonstrated the interaction of the specific domain of DOOM, which represents the splice variant Mod(mdg4)-56.3, with the IAP of *Orgya pseudotsugata.* The presence of the identified consensus sequence in DOOM might indicate its putative function in protein-protein interaction. We could identify a new

A

Specific domains of Mod(mdg4) proteins

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56.3 DLGELNPSNLADFGNESFLPKTKGKRPQNVRCGLAPDQKCVRTLADWDRIR |YDRTRSGDV-LVYDGYRYDRRANYND-----IIYWGCA------KKRLSCNVYMITHK---NKPTYVAISG-VHNH| L.   514
54.6 -------------------VTFDVLTDPIVKPDQHQLMKRVRLSKSMEGVH |YVRTPAGNVVLHCGEHRYLRNAAYKD-----KVYWKCS------KWRKQCRSRVITHIL-PNGQSRYAVSG-VHNH| P.   496
51.4 --------------------------------------------GSVAYYS |YITGFRGSRKLKIGEFSFTRNKTSGL-----KTYWSCAR-----AGVHKCKARVVTAQ----DHDVTIKCG-QHNH| PPY.473
54.2 -----------------------------------------------ELAV |FGTGQRGRTVLLFQNEKFVKNRCSAS-----RTYWICSK-----KDVTVCRARVVTAVD-KNSQERIIKCTYEHDH| SRK
53.1 --------------------------------------------GDATQFF |FTKGQRESVKLNYCGHSYVKFMENGR-----GTKWICAT-----RSTTKCRARIRTTK---NNYLEVLYAS--HNH| GFP
52.0 --------------------------------------------GTSHLAT |FSCTRKKKRKLVIDRHEFVMDRKLKS-----SINWRCAR-----YRSSNCKVRATTHVQ-KNGLEVYRLKYAKHSH| L.   475
55.3 ----------------------------------DRKRYSKKFLNFDGPAE |FSLAAHRRPRLIIANKHFIVHRILGKDNL--IGSWRCM------YHHKGCKARATTFMV-DSEVKYRSTCS-SHNH| KNV
55.6 ----------------------GHLSTLRHLPVEAIFDADGKQMDFIPNIR |VIRSQRKTIKLMFKKYAYSKTNEHDT-----TTYWHCRSR---RNGRPACKARFSTKKLKNGSYKVYLTQP-EHNH| PPK
57.4 ----------------------------------EFDYGHGQYRGNNPQIQ |FSVSKRGGQLLWLDGMKFFRNNINRT-----NLYWRCHWY----YRHTKCPVLICMSKT--NSNDFRQIHD--HCH| IRP
58.6 ----------------------------------VVLANDEVPNPEDVLVF |FTQSLRGRPAIMANGIRFLIMSENKK-----KILWRCSS---MATKKLKCPARITMLK--ETPPKFIINKA-EHLH| AEL
55.7 --------------------------------GQFIGDIPRGQWIDKHEYF |FLKNQKQGFNLVFNGYMYKKEASFRA-----TVNWICSDGNGKRLNENKCSARAITKFD---GGIKLGKNP--HNH| PPR
64.2 ----------------------------------------------DTEIS |FIRSQKKNAQLVFRNYIYNKKLTQANG----QTTWRCAD-----VLKLRCKAVVITRD----GHFIDARR--QHNH| ESH
59.0 ---------------------------------------VTQHVRNCGPQM |FLISRKGGTLLTINNFVYRSNLKFFGKSNN-ILYWECVQ-----NRSVKCRSRLKTIG----DDLYVTNDV--HNH| MGD
60.1 ----------------------------------------------EDELV |FIESPWSTPCLVLNGYMYNCHSRKSN-----KQYWRCHNYS-KKAHEMRCRSRCVLEN----GRLKSVTGG-LHNH| QPH
62.3 ---------------------------------------------ESSVAI |YSATSRGRMQLIYGGQPFIFEKTLKLSSGEEKRFWRCNQ-----WWNQKCRSRVFTIN----GVVCPLNRF--HTH| EEI

                               Consensus     F/Y      L    F/Y          WXC  X6-12  C      X17-22        HXH

54.2 FPSNNVNLPVLIKREKALSLDAS.497
53.1 PQKKDRGRASQRM.486
55.3 RSQQQSLKMPWVFTD.503
55.6 KRRL.505
57.4 KRKENSGTGDGPKIRTPVVSNVRSLPQSMAHMFDM.520
58.6 KRNKYSSSKAQTLRDPHQMATKLDCEMEGAGGVTFDLHEEELNELTHDV.536
55.7 FLGGKVPAKLMPKDAFYPQY.510
64.2 ASRIGQRQLYKVEQELEEYIEICTSNPKISQYLGSSNIIVTAKDGKDCKLFLPAAEATEIEMQALVDAAEEELDEEERHAEERIRDRQRVGRWRTEEAKHRSLLKSEHP.580
59.0 NKRIEAAKAAGMLIHKKLSSLTAADKIQGSWKMDTEGNPGPSTQDVAAFYTFPTQTFVFC.541
60.1 TEKIDKIIQRNKMAAIGTGRKLSRTHSFTQLQLQEQKQEFIDEHQLTSDAATLQLTDQELMHASMMLIHE.544
62.3 VRRKKRVRRVPPVETIAKVVATTPRHPQHQQTTQQQQEIQLTSDAIAGAILDDESPATIDVSELGMHLKYEEIVADVTGIVGGTRVVSRRK.567
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B

Other Mod(mdg4) proteins

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67.2 AATSASATKIPPRKRGRPKTKVEDQTPKPKLLEKLQAATLNEEASEPAVYASTTKGGVKLIFNGHLFKFSFRKADYSVFQCCYREHGEECKVRVVCDQKRVFPYKGEHVHFMQASDK
     SCLPSQFMPGESGVISSLSPSKELLMKNTTKLEEADDKEDEDFEEFEIQEIDEIELDEPEKTPAKEEEVDPNDFREKIKRRLQKALQNKKK.610
65.0 VKIKMEPSPTPGHSSDAAVAALAVTYLSDEESFRKPFTLPKLLDGKFYKNIQPNQKTPGAIQATCTTCHGLISGTTKSTGNFLSHIKRRHKELLPLCQLYCQAKANGTVPAVKSSPP
     NPNHVLTSATPTPAMEMMTQVAQMPPPAAYATGPTHLGMPVTVPVPVSMSLAMPISLPHVQTPQMMALMQQHQAHGAVFISKDY.603
59.1 VCDDLDDMKGAIKHSLLTFIRGQRGCKLLAFNGHNYVRNRRSNLKTYWICSKKGSTKCNARVVTNVVEGVHKIVLESCHHTCLNTERKKRLSVTNVVGKARSKSEKSVSTGFIKEEG
     DEDLTLELRTLNLSIEDLNNLQ.541
58.0 DGPSKDTAIPKPAEHPRKPATDSVQKSPRDADAIPLFDGSRVFVSKVALAKAYIPMPMIYTCRVMDLVIGKDKLVRIAQHEETTDKDLIQDIITHVCKVFALRGNQLTPSAVQEFID
     HKLSTLKLMPIKEGK.534
55.1 GLIFKAARHIAPIQKVRQVRDDKFLATIIKLEPAGRLNLKNPDNIIRTSSNEHNFVYVGLPRMKGKCVNCLKKNRTGLRRINTLCNTCPGSNWMCEPCFEELHS.506
46.3 GCDGLQGSCRDRGGQKLTGANHQMHLRA.430
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C

Sequence comparison of Mod(mdg4)-56.3 (DOOM) and Mod(mdg4)-54.6

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56.3 DLGELNPSNLADFGNESFLPKTKGKRPQNVRCGLAPDQKCVRTLADWDRIRYDRTRSGDVLVYDG-YRYDRRANYNDIIYWGCAKKRLSCNVYMITHK--NKPTYVAISGVHNHL.515
                 : :       : .: .. :         . ::  . . ..: :: .::... : .:: :.: :.: .:: :.: : .:.  .:::   :  .  :.:::::
54.6 ----VTFDVLTD-------PIVKPDQHQ--------LMKRVRLSKSMEGVHYVRTPAGNVVLHCGEHRYLRNAAYKDKVYWKCSKWRKQCRSRVITHILPNGQSRYAVSGVHNHP.499
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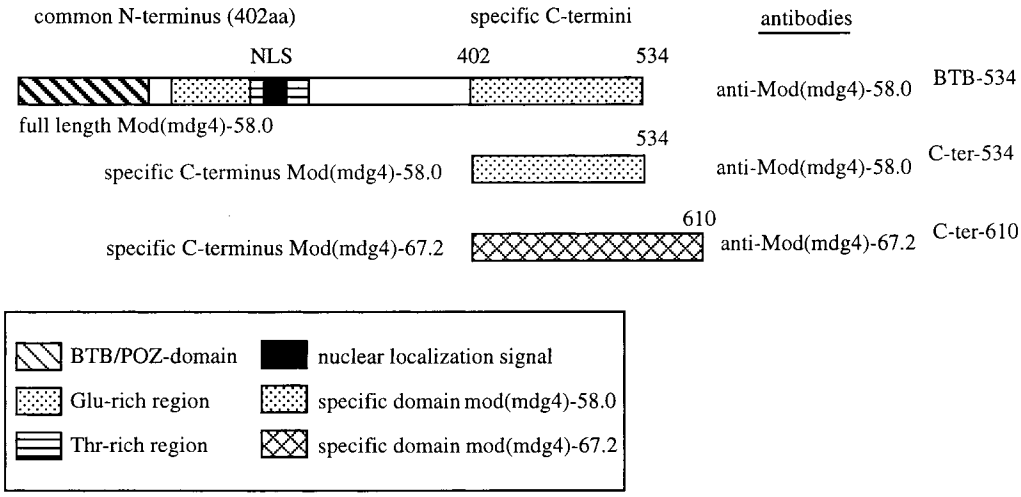

Figure 5.—Schematic representation of Mod(mdg4) proteins used for generating antibodies. The common antibody anti-Mod(mdg4)-58.0$^{BTB-534}$ directed against the full-length protein Mod (mdg4) 58.0 should detect all isoforms, whereas the antibodies Mod(mdg4)-58.0$^{403-534}$ and Mod(mdg4)-67.2$^{403-610}$ directed against the specific domains of the corresponding protein detect single isoforms.

protein isoform, Mod(mdg4)-54.6, with significant homology to DOOM/Mod(mdg4)-56.3. A sequence comparison of the specific domains of both proteins is shown in Figure 4C. The degree of overall identity is 37% (63% similarity). A functional role of Mod(mdg4)-54.6 in apoptosis has not been shown. Although putative AT-hook motifs in Mod(mdg4)-67.2 and Mod(mdg4)-58.6 and a cluster of acidic amino acids in several proteins have been detected, no other significant homology to known proteins represented in the databases has been identified.

Mod(mdg4) proteins do not colocalize on polytene chromosomes: Using the antibody anti-Mod(mdg4)-58.0$^{BTB-534}$ [directed against the full-length Mod(mdg4)-58.0 isoform; *cf.* Figure 5], a large number of sites on polytene chromosomes were detected (Dorn *et al.* 1993b). Since the common domain was included in the antigen, this antibody should recognize all Mod(mdg4) protein isoforms identified so far. We studied the chromosomal distribution of individual protein isoforms using newly produced specific antibodies generated against fusion proteins containing specific epitopes of two different isoforms, Mod(mdg4)-67.2 (aa 403–610) and Mod(mdg4)-58.0 (aa 403–534; Figure 5). Mod(mdg4)-67.2 is encoded by an abundant 2.3-kb transcript, whereas cDNA clone *mod(mdg4)-58.0* represents a significantly less abundant 2.0-kb transcript.

The anti-Mod(mdg4)-58.0$^{BTB-534}$ antibody detects at least 12 polypeptides in embryonic nuclear extracts (Figure 2A, lane 1), whereas the specific antibody anti-Mod(mdg4)-67.2$^{403-610}$ detects 2 polypeptides at molecular weights of 93 and 99 kD (Figure 2A, lane 2), although a single polypeptide of 67 kD was expected. Both the existence of 2 cross-reacting polypeptides and their increased apparent molecular size in SDS-PAGE gels suggest post-translational modifications. This is supported by the presence of several putative phosphorylation sites within the common part of the N-terminal 402 amino acids. Comparison of the theoretical molecular weight of the identified Mod(mdg4) proteins in the range of 46 to 67 kD and the apparent molecular weight of polypeptides detected in Western blot analysis (70–100 kD) suggests a general shift in electrophoretic mobility of ~25 kD. The second specific antibody, anti-Mod(mdg4)-58.0$^{403-534}$, did not detect a polypeptide in Western blot of embryo nuclear extracts. However, after immunoprecipitation by the anti-Mod(mdg4)-58.0$^{403-534}$ antiserum using embryonic extracts, 2 polypeptides at 75 kD are clearly detected by this antibody (Figure 2B, lane 1). The 2 polypeptides also react with the affinity-purified anti-BTB antiserum (Figure 2B, lane 2). No cross-reaction of the immunoprecipitate with the specific anti-Mod(mdg4)-67.2$^{403-610}$ antibody was found, indicating the selectivity of both specific antisera.

Figure 4.—Sequence comparison of the specific C-terminal protein domains of Mod(mdg4) proteins. (A) Alignment of 15 out of 21 Mod(mdg4) proteins containing the consensus sequence. The proteins are named according to their theoretical molecular weight to the left. Note that the common N-terminal part of 402 amino acids containing the BTB domain (*cf.* Dorn *et al.* 1993a) is not shown. Therefore, all specific protein domains start with amino acid position 403. The alignment (inside the indicated box) was performed by the CLUSTAL X program (Thompson *et al.* 1994) with some minor corrections. The consensus sequence (below the indicated box) represents positions where >90% of the aligned proteins have identical amino acids. Because of the varying location of the consensus relative to the C terminus of the different isoforms, the extending C-terminal protein parts are shown below the consensus sequence. (B) Sequence of Mod(mdg4) protein isoforms that do not contain the consensus sequence. Cys and His residues are indicated by boldface letters. Note the presence of at least four Cys or His in all proteins. (C) Comparison of the two protein isoforms Mod(mdg4)-56.3/DOOM and Mod(mdg4)-54.6. Identical amino acid positions are indicated by a colon; chemically similar amino acids are indicated by a point.

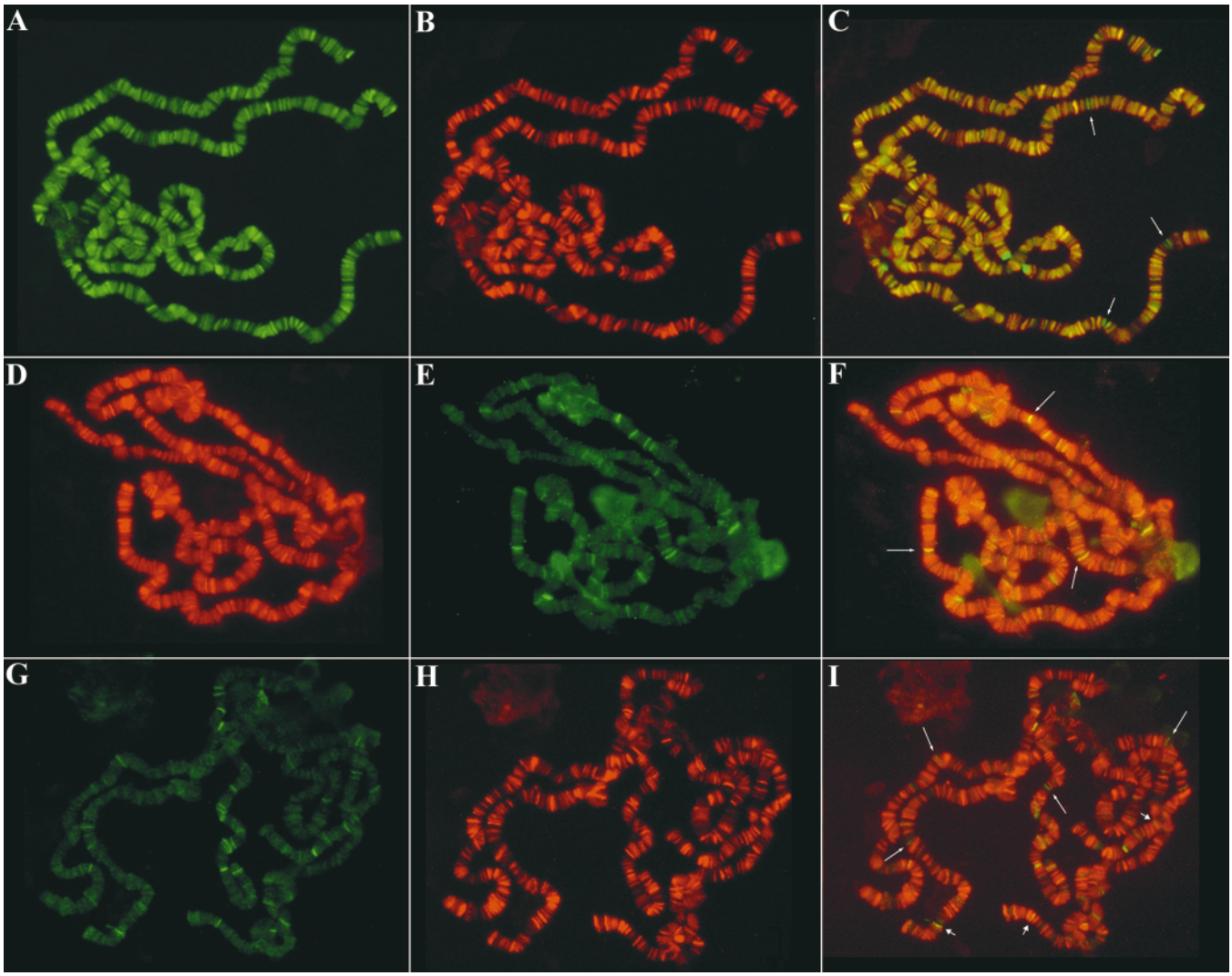

Figure 6.—Localization of Mod(mdg4) proteins and the specific distribution of the two protein isoforms Mod(mdg4)-58.0 and Mod(mdg4)-67.2 along polytene chromosomes. Double staining of wild-type chromosomes using affinity-purified anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ antibody (A) and anti-Mod(mdg4)-67.2$^{403\text{-}610}$ (B). Merged images (C) indicate the overlapping sites (yellow). Staining of polytene chromosomes using antibodies anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ (D) and anti-Mod(mdg4)-58.0$^{403\text{-}534}$ detecting the isoform Mod(mdg4)-58.0 (E). Overlapping signals (yellow) are shown in the merged image (F). Costaining of polytene chromosomes using the two domain-specific antibodies anti-Mod(mdg4)-58.0$^{403\text{-}534}$ (G) and anti-Mod(mdg4)-67.2$^{403\text{-}610}$ (H). Double exposure (I) demonstrates the partial (yellow) but not complete overlap of both Mod(mdg4) protein isoforms. Mod(mdg4)-58.0-specific signals are indicated by long arrows, and putative overlapping signals of Mod(mdg4)-58.0 and Mod(mdg4)-67.2 are indicated by short arrows.

The chromosomal distribution of the Mod(mdg4)-67.2 protein isoform was studied by double staining of polytene chromosomes with the common antibody anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ and the specific antibody anti-Mod(mdg4)-67.2$^{403\text{-}610}$. Both antibodies detect a large number of sites (Figure 6, A and B). The merged images (Figure 6C) show an overlap at most (yellow signals) but not all sites. A small number of sites, which are exclusively detected by the anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ antibody (green signals), correspond to binding sites of Mod(mdg4) proteins other than Mod(mdg4)-67.2. These are located at the telomeres of the autosomes and the X chromosome and a number of loci like 2C, 7D, and 95D (labeled by arrows in Figure 6C). In contrast, the other specific antibody anti-Mod(mdg4)-58.0$^{403\text{-}534}$ detects only ~50 sites on polytene chromosomes (Figure 6, E and G Table 1). A total of 24 sites have been found in all preparations analyzed so far, with 3 sites standing out by a very prominent staining. Of the sites listed in Table 1, 23 stain more weakly and/or were not detected in all nuclei analyzed.

Costaining of polytene chromosomes, using anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ and the specific anti-Mod(mdg4)-58.0$^{403\text{-}534}$ antibody, results in a complete overlap of sites detected by the specific antibody (yellow signals, Figure 6F). However, the majority of sites are recognized exclu-

TABLE 1

Binding sites of Mod(mdg4)-58.0 on polytene chromosomes

Chromosome	Cytology[a]
X	2B, 4C, 5A, 15C, 20
2L	22C, 28C, 30A
2R	42A, 42E, 47F, 48AB, 48F, *50A*, 50D, 51C, 53F, 54A, 54C, 55F, *56E*, 57D, 59A, 60A, 60E, 60F
3L	63B, 63EF, 64D, 64F, 66A7, 68C, 70F, 72D, 74EF, 75B
3R	*84A*, 84B, 84E, 84F, 89B, 90A, 93A, 94A, 97D, 99F

[a] Three types of signals are differentiated. Italic, prominent signals; underlined, regular, less strong signals; all other signals, weak and not found in all nuclei.

sively by the antibody detecting all Mod(mdg4) proteins (red signals). This result indicates that Mod(mdg4)-58.0 is present in a small subset of Mod(mdg4) binding sites. Next, we compared the distribution of the two protein isoforms Mod(mdg4)-58.0 and Mod(mdg4)-67.2 by costaining with the two specific antibodies (Figure 6, G and H, respectively). In merged images only, a subset of sites is recognized by both antibodies (Figure 6I, yellow signals). However, at many sites both proteins exclude each other, clearly indicating a differential distribution of the two protein isoforms Mod(mdg4)-58.0 and Mod(mdg4)-67.2.

Isolation and genetic analysis of *mod(mdg4)* mutations: First mutations of *mod(mdg4)* have been identified by their modifying effect on several *gypsy*-induced mutations (Georgiev and Gerasimova 1989). The independently isolated *P*-transposon-induced insertional enhancer of position effect variegation $E(var)3\text{-}93D^{neo129}$ was used for molecularly defining the locus (Dorn *et al.* 1993b). Molecular cloning of the *mod(mdg4)* locus revealed that both *E(var)3-93D* and *mod(mdg4)* are identical genes (Gerasimova *et al.* 1995; Gerasimova and Corces 1998). These studies also proved that the previously isolated *mod(mdg4)* mutations show a dominant enhancer effect on PEV. It was concluded that *mod(mdg4)* behaves as a typical enhancer of position effect variegation, which connects insulator function with changes in chromatin structure.

Mutations of *mod(mdg4)* are characterized not only by their dominant enhancer effect on PEV, but also by their result in lethality or semilethality and female sterility (Dorn *et al.* 1993b; Gerasimova *et al.* 1995). To identify new alleles of *mod(mdg4)*, we tested >100 independently isolated dominant enhancers of PEV mutations (Dorn *et al.* 1993a) for viability and female fertility in *trans*-heterozygotes with $mod(mdg4)^{neo129}$ and *Df(3R)GC14*. In these studies, four new *mod(mdg4)* alleles were identified. Two mutations, $mod(mdg4)^{03}$ and $mod(mdg4)^{05}$, were induced by *P*-element mutagenesis and two, $mod(mdg4)^{02}$ and $mod(mdg4)^{04}$, were spontaneous in origin (Table 2).

Deficiency *Df(3R)GC14* uncovers *mod(mdg4)* and several lethal complementation groups (Azpiazu and Frasch 1993). Two of the mutations isolated in a screen for EMS-induced recessive lethals uncovered by *Df(3R)GC14* (Azpiazu and Frasch 1993) proved to be allelic to mutations $mod(mdg4)^{06}$ and $mod(mdg4)^{07}$ (Table 2). The $P(w^+)142$ transposon-induced mutation affects the same complementation group, and several deficiencies have been generated by imprecise excision of the transposon (Azpiazu and Frasch 1993). All these deficiencies, including *P142Δ32*, delete sequences in the common part of the *mod(mdg4)* locus and display a strong dominant enhancer effect on PEV (Table 2; data not shown).

The mutations differ in the strength of their dominant enhancer effect on white variegation in w^{m4} and can be arranged in an allelic series. The strongest enhancer effect is shown by mutations $mod(mdg4)^{R32}$, $mod(mdg4)^{04}$, $mod(mdg4)^{06}$, $mod(mdg4)^{07}$, and deletion *P142Δ32*. Weaker enhancer effects are found in genotypes carrying $mod(mdg4)^{neo129}$, $mod(mdg4)^{02}$, and $mod(mdg4)^{03}$, whereas mutation $mod(mdg4)^{05}$ shows only a weak enhancer effect (Table 2). Enhancement of position effect variegation has been quantified by red eye pigment measurements in w^{m4} flies also carrying the strongly dominant suppressor of position effect variegation $Su(var)2\text{-}1^{01}$.

Complementation analysis between the newly identified *mod(mdg4)* mutations and $mod(mdg4)^{neo129}$ revealed a rather complex complementation pattern (Table 3). The complementation analysis showed that two mutations, $mod(mdg4)^{05}$ and $mod(mdg4)^{03}$, complement or partially complement most of the other alleles (Table 3). Allele $mod(mdg4)^{05}$ is viable with all mutations, including *Df(3R)GC14*. *Trans*-heterozygous females are fertile, with the exception of $mod(mdg4)^{05}/mod(mdg4)^{03}$ females (Table 3). $mod(mdg4)^{03}$ partially complements most alleles, and all *trans*-heterozygotes tested are female sterile. These results suggest a weak hypomorphic nature of mutations $mod(mdg4)^{05}$ and $mod(mdg4)^{03}$. Both mutations belong to the group of alleles that display an intermediate or weak enhancer effect.

In an excision analysis of the insertional mutation $mod(mdg4)^{neo129}$, the recessive lethal allele $mod(mdg4)^{R32}$ was isolated by screening for ry^--associated phenotype and is lethal with all *mod(mdg4)* alleles except $mod(mdg4)^{05}$ and $mod(mdg4)^{03}$. An almost identical com-

TABLE 2

PEV enhancer effect and homeotic effects of *mod(mdg4)* mutations

Allele	Enhancer effect[a] (R1)	Paternal effect[b] (R2)	Viability	Homeotic transformation of A5 to A4[c]	Origin
mod(mdg4)^{neo129}	++(0.17)	+(0.47)	−	94.2(73)	*P*-induced
mod(mdg4)^{R32}	+++(0.05)	++(0.26)	−	96.4(86)	Revertant of *neo129*
mod(mdg4)^{02}	++(0.16)	+(0.65)	−	28.8(52)	Spontaneous
mod(mdg4)^{03}	++(0.16)	++(0.15)	−	100(43)	*P*-induced
mod(mdg4)^{04}	+++(0.07)	+(0.52)	−	50.1(96)	Spontaneous
mod(mdg4)^{05}	+(0.32)	+(0.69)	−	55.7(61)	*P*-induced
mod(mdg4)^{06}	+++(0.05)	++(0.33)	−	17.7(113)	EMS
mod(mdg4)^{07}	+++(0.04)	++(0.24)	−	13.4(127)	EMS
142.4 Δ32	+++(0.03)	+++(0.03)	ND[d]	ND	Rev. of *P(w^{+})142*[d]
DfGC14	+(0.30)	−(0.79)	−	12.7(94)	Deficiency[d]

The strength of the PEV enhancement and the paternal effect have been classified as strong (+++; $R <$ 0.10), intermediate (++; R between 0.10 and 0.30), and weak (+; R between 0.30 and 0.75) alleles; $R > 0.75$ represents no significant effect.

[a] The enhancement of PEV was determined by measuring the absorbance of extracted red eye pigment of w^{m4}/Y males containing the *mod(mdg4)* allele and the suppressor mutation *Su(var)2-1^{01}* at 480 nm. These offspring males were obtained in a cross of w^{m4}; *Cy/ap^{Xa}*; *Su(var)2-1^{01}/Sb* females to w^{m4}/Y; *mod(mdg4)$^{-}$/TM3* males. (R1) represents the ratio of eye pigment content of w^{m4}; *+/ap^{Xa} Su(var)2-1^{01}/mod(mdg4)$^{-}$* males and w^{m4}; *+/ap^{Xa} Su(var)2-1^{01}/+* males obtained in an independent cross of w^{m4}; *Cy/ap^{Xa} Su(var)2-1^{01}/Sb* females to w^{m4}/Y males.

[b] Paternal enhancer effects were studied in w^{m4}; *+/ap^{Xa} Su(var)2-1^{01}/TM3* offpring males produced by a cross of w^{m4}; *Cy/ap^{Xa} Su(var)2-1^{01}/Sb* females to w^{m4}/Y; *mod(mdg4)$^{-}$/TM3* males. (R2) represents the ratio of eye pigment content of w^{m4}; *+/ap^{Xa} Su(var)2-1^{01}/TM3* males obtained in a cross of w^{m4}; *Cy/ap^{Xa} Su(var)2-1^{01}/Sb* females to w^{m4}/Y; *mod(mdg4)$^{-}$/TM3* males and w^{m4}; *+/ap^{Xa} Su(var)2-1^{01}/+* males obtained in an independent cross of w^{m4}; *Cy/ap^{Xa} Su(var)2-1^{01}/Sb* females to w^{m4}/Y males.

[c] Homeotic transformation of A5 to A4 was determined by examination of *trans*-heterozygous *Df(3R)red^{trx}/mod(mdg4)$^{-}$* males obtained in a cross of *Df(3R)red^{trx}/TM3, Ser* females to *mod(mdg4)$^{-}$/TM3, Sb Ser* males. Percentage of transformed flies is given; number of males examined is shown in parentheses.

[d] Mutations *mod(mdg4)^{06}* and *mod(mdg4)^{07}* were obtained from M. Frasch, and *P142Δ32* and *Df(3R)GC14* are described in Azpiazu and Frasch (1993) and Mohler and Pardue (1984), respectively. The *P142Δ32* mutation was induced on a *TM3, Sb Δ2–3* chromosome.

plementation pattern is found for alleles *mod(mdg4)^{04}*, *mod(mdg4)^{06}*, and *mod(mdg4)^{07}*. All of these alleles show the strongest enhancer effect and are suggested to represent amorphic or strongly hypomorphic mutations in respect to dominant enhancement of PEV and recessive lethality.

Mutations of *mod(mdg4)* were furthermore shown to display dominant homeotic effects characteristic for trx group genes in adult flies (Dorn *et al.* 1993b; Gerasimova and Corces 1998). All of the newly identified alleles show significant homeotic effects (Table 2). In males *trans*-heterozygous for *Df(3R)red^{trx}* and the mutations *mod(mdg4)^{neo129}*, *mod(mdg4)^{R32}*, *mod(mdg4)^{03}*, *mod(mdg4)^{04}*, or *mod(mdg4)^{05}*, homeotic transformation

TABLE 3

Complementation analysis of *mod(mdg4)* alleles

	Males							
Females	*02*	*R32*	*04*	*03*	*05*	*06*	*07*	*GC14*
mod(mdg4)^{neo129}	−	−	−	sl[a]	+	−	−	−
mod(mdg4)^{02}	−	−	−	sl[a]	+	−	−	−
mod(mdg4)^{R32}		−	−	sl[a]	+	−	−	−
mod(mdg4)^{04}			−	−	+	sl	−	−
mod(mdg4)^{03}				−	+[a]	sl[a]	sl[a]	sl[a]

A minimum of 250 offspring flies were scored in each cross. For viability: +, viable, on average >85% of the expected progeny survive to eclose as adults; sl, semilethal, on average 5–50% of the expected progeny survive to eclose as adults; −, lethal, on average <5% of the expected progeny survive to eclose as adults.

[a] Female sterile.

TABLE 4

Interaction of *mod(mdg4)^{neo129}* and mutations in other trx group genes in homeotic transformation

Genotype	Homeotic transformation of A5 to A4[a]
modmdg4)^{neo129} / +	0.2
Df(3R)red^{trx} / +	0.4
Df(3R)red^{trx} mod(mdg4)^{neo129} / +	3.1
brm^{2} / +	0.1
Df(3R)red^{trx} mod(mdg4)^{neo129}/brm^{2}	3.7
E(var)3–4^{01} / +	0.1
E(var)3–4^{01} mod(mdg4)^{neo129} / +	2.1

[a] Homeotic transformation of A5 to A4 in males was determined by artibrary units (materials and methods); 0, no transformation; 6, complete transformation.

TABLE 5

Interaction of *mod(mdg4)^{neo129}* and mutations in other trx group genes in enhancement of PEV

Genotype	Enhancer effect[a] (R1)	Paternal effect[b] (R2)
mod(mdg4)^{neo129} / +	++(0.18)	+(0.40)
Df(3R)red^{trx} mod(mdg4)^{neo129} / +	+++(0.05)	++(0.11)
Df(3R)red^{trx} / +	−(1.00)	−(1.00)
E(var)3–4^{01} / +	+++(0.02)	++(0.18)
E(var)3–4^{01} mod(mdg4)^{neo129} / +	+++(0.03)	+(0.45)
brm^{2} / +	+(0.40)	ND
brm^{2} mod(mdg4)^{neo129} / +	++(0.17)	++(0.28)
brm^{2} Df(3R)red^{trx} / +	++(0.15)	++(0.30)
vtd^{2} / +	+(0.62)	ND
vtd^{2} mod(mdg4)^{neo129} / +	+++(0.06)	++(0.12)

The strength of the PEV enhancement and the paternal effect have been classified as strong (+++; $R < 0.10$), intermediate (++; R between 0.10 and 0.40), and weak (+; R between 0.40 and 0.75) alleles; $R > 0.75$ represents no significant effect.

[a] The enhancer effect is shown as the ratio (R1) of eye pigmentation level of offspring males containing the indicated combination of trx group alleles and the suppressor mutation *Su(var)2–1^{01}* after crossing balanced trx group males to *w^{m4}; Cy/T(2;3)ap^{Xa} Su(var)2–1^{01}/Sb* females.

[b] The paternal enhancer effect represents the ratio (R2) of the E^{+} offspring males [*w^{m4}; Cy/ap^{Xa} Su(var)2–1^{01}/TM3*] resulting from crosses of *w^{m4}; Cy/T(2;3)ap^{Xa} Su(var)2–1^{01}/Sb* females to *w^{m4}/Y; trx group/TM3* and *w^{m4}/Y; Pr Dr/TM3* males (control), respectively.

of abdominal segment A5 to A4 is strongly increased compared to *trans*-heterozygotes with *mod(mdg4)^{02}*, *mod(mdg4)^{06}*, or *mod(mdg4)^{07}*. However, no direct correlation between the strength of the PEV enhancer effect and the frequency of homeotic transformation is found. Considering the molecular complexity of the locus, the mutations might differentially affect various isoforms of the *mod(mdg4)* locus.

Mutations in *mod(mdg4)* and other trx group genes additively interact in homeotic transformation as well as enhancement of PEV: To test for interaction between *mod(mdg4)^{neo129}* and mutations in trx group genes in their effect on homeotic transformation and dominant enhancement of PEV, we constructed several *cis* combinations between *mod(mdg4)^{neo129}* and mutations of other trx group genes, including *brahma*, *vertandi*, *trithorax*, and *E(var)3-4*. Phenotypic effects on PEV were quantified in *w^{m4}* flies also carrying the strong dominant PEV suppressor mutation *Su(var)2-1^{01}* by red eye pigment measurements. Homeotic transformation of abdominal segments A5 to A4 was quantified by arbitrary units (materials and methods).

Strong additive effects on homeotic transformation of A5 to A4 were found in *Df(3R)red^{trx} mod(mdg4)^{neo129} / +* males (Table 4). In these males, about one-third of the abdominal segment A5 was transformed to A4. *Df(3R)red^{trx} mod(mdg4)^{neo129} / bx^{34e}* flies showed strong transformation of T2 to T3 in both females and males (data not shown).

No further enhancement of homeotic transformation was found in *Df(3R)red^{trx} mod(mdg4)^{neo129} / brm^{2}* males (Table 4). The two dominant enhancer mutations, *E(var)3-4^{01}* and *mod(mdg4)^{neo129}*, alone cause only weak homeotic transformation. A significant increase of A5 to A4 transformation can be observed in the double heterozygous *E(var)3-4^{01} mod(mdg4)^{neo129} / +* males (Table 4).

Although none of the *trx* alleles nor *Df(3R)red^{trx}* affects PEV, strong enhancement of *white* variegation was found in *w^{m4}; Df(3R)red^{trx} mod(mdg4)^{neo129} / +* flies. Elevated enhancer effects on PEV were also detected in *vtd^{2} mod(mdg4)^{neo129} / +* males, whereas *brm^{2} mod(mdg4)^{neo129} / +* males were not significantly different compared to *mod(mdg4)^{neo129} / +* controls (Table 5). Our results show that a deletion of *trx* as well as a mutation in *vtd* strongly elevate the enhancer effect of *mod(mdg4)^{neo129}* on position effect variegation in *w^{m4}*. Therefore, these mutations not only show additive effects on homeotic transformation but also in their effect on position effect variegation. Different alleles of *brahma* and *vertandi* including *brm^{2}* and *vtd^{2}* show only weak enhancer effects on PEV (Table 5). Additive effects on enhancement of PEV were found between *brm* and *trx* in *w^{m4} / Y; brm^{2} Df(3R)red^{trx} / +* males and enhancement of PEV is about three times stronger compared to *w^{m4} / Y; brm^{2} / +* males. No significant difference in enhancement of PEV was observed in combinations between *E(var)3-4^{01}* and *mod(mdg4)^{neo129}*, which may be attributed to the strong enhancer effect of the *E(var)3-4^{01}* mutation.

Our data indicate that *mod(mdg4)* not only interacts with trx group genes in transcriptional regulation of homeotic genes but also in gene silencing caused by PEV. In earlier studies of *mod(mdg4)^{neo129}*, we reported paternal imprinting-like enhancer effects (Dorn *et al.* 1993b). Male offspring produced by *mod(mdg4)^{neo129} / +*

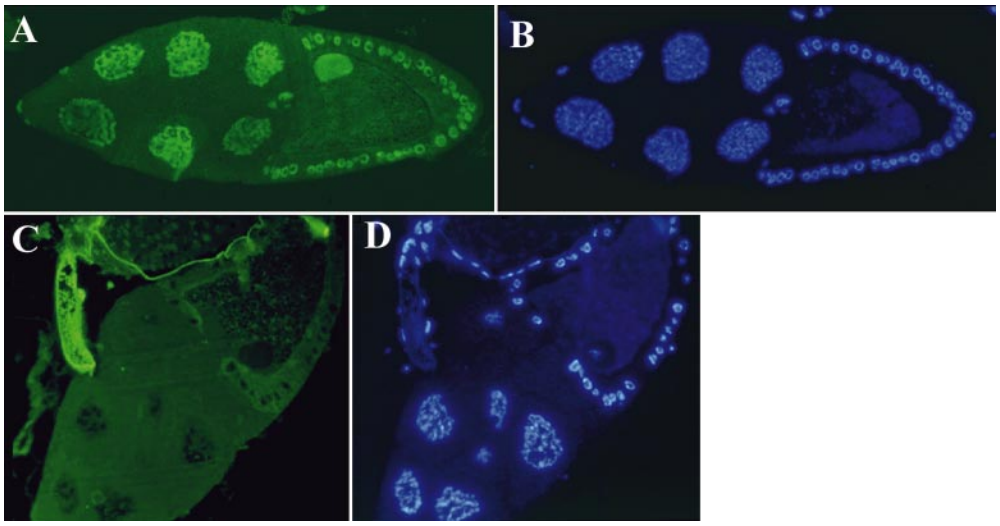

Figure 7.—Antibody staining of thin-sectioned stage 10 egg chambers from Drosophila wild-type ovaries (A) and ovaries from homozygous *mod(mdg4)*^{neo129} females (C) using the anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ antibody detecting all Mod(mdg4) proteins. Note the significantly reduced staining in all nuclei of mutant egg chambers. Images shown in B and D represent DNA staining using DAPI of the same egg chambers shown in A and B, respectively.

heterozygous fathers show PEV enhancement independent of whether they are *mod(mdg4)*^{neo129}/+ or +/+ in genotype. These effects can be observed for all other *mod(mdg4)* mutant alleles (Table 2). Strongly elevated paternal enhancer effects are found in offspring males produced by crosses with fathers of *cis*-heterozygotes of *mod(mdg4)*^{neo129} and the tested mutations of trx group genes (Table 5). The two *cis* combinations, *Df(3R)red*^{trx} *mod(mdg4)*^{neo129} and *vtd*^{2} *mod(mdg4)*^{neo129}, show the strongest paternal imprinting-like enhancer effect. Interestingly, a significant paternal enhancer effect is also detected in crosses with *brm*^{2} *Df(3R)red*^{trx}/+ fathers. These results suggest a functional interaction of several trx group genes not only in the control of homeotic gene expression but also in gene silencing caused by a change in higher order chromatin organization in PEV.

Molecular analysis of *mod(mdg4)* mutations: Because of the molecular complexity of *mod(mdg4)*, it is important to know the molecular nature of *mod(mdg4)* mutant alleles. Mutations involved in sequences encoding the common exons 1–4 should affect all identified protein isoforms. Five of the nine *mod(mdg4)* mutant alleles are transposon induced. The *mod(mdg4)*^{neo129} mutation was induced by insertion of the pUChsneory^{+} transposon 121 bp downstream from the 3′ junction of the third exon. Northern blot analysis using poly(A)$^{+}$ RNA isolated from heterozygous flies revealed a truncated transcript of ~1.0 kb slightly less abundant compared to the wild-type transcripts (data not shown). For a second *P*-induced allele, *mod(mdg4)*^{03}, restriction fragment length polymorphism within the genomic region encoding the common part of all transcripts was identified by Southern blot analysis. Isolation and sequencing of appropriate recombinant phages from a genomic library constructed from this mutant revealed the insertion of a truncated *P*-element 120 bp downstream from the putative TATAA box within the 5′ untranslated region (UTR). However, in Northern blot analysis of poly(A)$^{+}$ RNA isolated from heterozygous *mod(mdg4)*^{03} adults, no aberrant transcript could be detected. The insertion of the w^{+} transposon in mutation *P(w*$^{+}$*)142* could be located 40 bp downstream from the TATAA box in the 5′ UTR (M. Frasch, personal communication). Both mutations might result in reduced amounts of *mod(mdg4)* transcripts. In flies carrying the allele *mod(mdg4)*^{02}, which was obtained as a spontaneous mutation in a hybrid dysgenic cross, an abundant truncated transcript of ~1.2 kb was detected in Northern blot analysis (data not shown). Cloning and sequencing of the mutant allele revealed the insertion of a *gypsy*-like retrotransposon within the third intron, 84 bp downstream from the exon/intron junction of exon 3. All mutations represent independent insertions of transposable elements within a region of ~1 kb of the *mod(mdg4)* locus, indicating a hot spot for insertional mutations.

The truncated transcripts detected in mutations *mod(mdg4)*^{neo129} and *mod(mdg4)*^{02} are expected to result from aberrant splicing. Sequencing of DNA fragments obtained by RT-PCR revealed that the aberrant transcripts contain transposon sequences. Putative polyadenylation signals within the transposons are used to produce the detected truncated transcripts (Figure 3A). However, the coding capacity of both truncated transcripts is too small to encode functional Mod(mdg4) proteins.

Mod(mdg4) proteins are essential in oogenesis and early embryogenesis: The mutant allele *mod(mdg4)*^{neo129} shows temperature-sensitive pupal lethality. Homozygous females, obtained at 29°, produce a small number of eggs that do not show signs of further development. Ovaries of these homozygous females are of variable size, and ovarioles are frequently reduced in number and contain fewer egg chambers. In whole-mount *in situ* hybridization, large amounts of *mod(mdg4)* transcripts could be detected in nurse cells of wild-type egg chambers (data not shown). To determine if the female sterility of homozygous *mod(mdg4)*^{neo129} females is due to the reduced amount of Mod(mdg4) proteins during oogen-

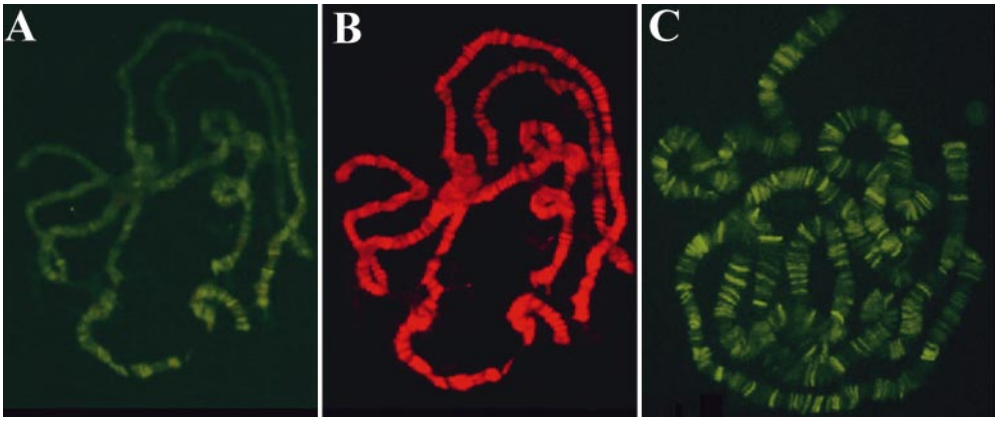

Figure 8.—Reduced levels of Mod(mdg4) proteins in larval stages of homozygous *mod(mdg4)* mutants. (A) Staining of polytene chromosomes of homozygous *mod(mdg4)*^{02} larvae using the anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ antibody, which detects all Mod(mdg4) protein isoforms. (B) Staining of the same set of chromosomes with anti-histone H1 antibody. (C) Fluorescent micrograph of polytene chromosomes from homozygous *mod(mdg4)*^{neo129} larvae stained with anti-Mod(mdg4) 58.0$^{BTB\text{-}534}$ antibody. Note the significantly reduced intensity of signals.

esis, we compared the distribution of Mod(mdg4) proteins in thin sections of wild-type and mutant vitellogenic egg chambers. In immunocytological analysis, using the anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ antibody, we found Mod(mdg4) proteins in follicle and nurse cell nuclei of wild-type stage 10 egg chamber (Figure 7A). In contrast, we found only very weak staining in egg chambers of homozygous *mod(mdg4)*^{neo129} females (Figure 7C). In nurse cell nuclei, only very little staining can be observed, whereas the nuclei of follicle cells, which surround the oocyte at this stage, do not stain at all. Additionally, in wild-type oocytes, we identified Mod(mdg4) proteins in the germinal vesicle, whereas no protein can be detected in the germinal vesicle of mutant homozygotes (Figure 7, A and C). In whole-mount *in situ* hybridization analysis using the full-length cDNA clone *mod(mdg4)-58.0* as a hybridization probe, we also found strongly reduced levels of *mod(mdg4)* transcripts in homozygous mutant egg chambers (data not shown). The reduced amount of Mod(mdg4) proteins in mutant egg chambers correlates with the observed maternal effect lethality, indicating an essential function of Mod(mdg4) proteins in oogenesis and early embryonic development.

Late larval/early pupal lethality of *mod(mdg4)* mutants is due to reduced levels of Mod(mdg4) proteins: The mutant alleles *mod(mdg4)*^{02} and *mod(mdg4)*^{neo129} show late larval/early pupal lethality. Immunocytological analysis of polytene chromosomes from homozygous third instar larvae should prove the correlation between lethality and reduced levels of Mod(mdg4) proteins at this developmental stage. Using the antibody anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$, little or no protein can be detected by immunostaining of polytene chromosomes from homozygous *mod(mdg4)*^{02} larvae (Figure 8A). The same result was obtained with a specific antibody directed against the Mod(mdg4)-67.2 protein (data not shown), whereas the costaining with an anti-histone H1 antibody as a control does not differ significantly from staining of wild-type chromosomes (Figure 8B). Salivary gland polytene chromosomes from homozygous *mod(mdg4)*^{neo129} third instar larvae showed significantly reduced staining with the anti-Mod(mdg4)-58.0$^{BTB\text{-}534}$ antibody (Figure 8C). A plausible hypothesis to explain the differences in immunostaining of polytene chromosomes from the two insertional mutants could be the strength of transcription termination within the different transposons, allowing the production of functional transcripts at low frequency in the case of the pUChsneory^{+} transposon in *mod(mdg4)*^{neo129} mutation but not (or only very rarely) in *mod(mdg4)*^{02} homozygotes containing the *gypsy*-like insertion. This hypothesis could also explain the observed semilethality of *mod(mdg4)*^{neo129} at 29°, assuming an increased accumulation of correctly spliced *mod(mdg4)* transcripts during development at elevated temperature. We suppose that insufficient amounts of functional Mod(mdg4) proteins result in developmental arrest.

***mod(mdg4)* mutant phenotypes can be partially rescued by a genomic fragment encoding the common part of *mod(mdg4)* transcripts:** The common N terminus of 402 amino acids, shared by all identified Mod(mdg4) proteins, contains a Glu/Thr-rich domain in addition to the BTB/POZ domain (Dorn *et al.* 1993b; *cf.* Figure 5). To study whether this common N-terminal region contains intrinsic functions, the 7.5-kb genomic *Bam*HI fragment (indicated in Figure 3A) containing the coding region for the common part of 402 amino acids and the identified conserved promoter elements was used for *P*-element-mediated transformation. Several second chromosomal transgenic lines containing this genomic fragment could be established (materials and methods). Surprisingly, the lethality of *mod(mdg4)*^{neo129} and *mod(mdg4)*^{02} mutant homozygotes and several *trans*-heterozygotes containing other *mod(mdg4)* alleles could be partially rescued in the presence of the genomic 7.5-kb

TABLE 6

Partial rescue of recessive lethality of *mod(mdg4)* alleles in the presence of the transgene *P* (w^+*7.5kb Bam*HI)

	Males					
Females[a]	neo129	02	rev32	04	06	07
P (w^+*7.5kb Bam*HI); *mod(mdg4)*^{neo129}	sl	sl	sl	sl	−[b]	−[b]
P (w^+*7.5kb Bam*HI); *mod(mdg4)*^{02}		sl	sl	−[b]	−[b]	−[b]

[a] w^{m4} females carrying the *P* (w^+*7.5kb Bam*HI) transgene and the indicated *mod(mdg4)* allele balanced over *TM3, Sb Ser* were crossed to heterozygous w^{m4}/*Y*; *mod(mdg4)/TM3* males. For viability: −, lethal, on average <5% of the expected progeny survive to eclose as adults; sl, semilethal, on average 5–50% of the expected progeny survive to eclose as adults; all *trans*-heterozygous flies are w^+ in phenotype, indicating the presence of the transgene.

[b] *Trans*-heterozygotes with a frequency of 1–3% adults eclosed; without the transgene, no *trans*-heterozygous escapers were found.

*Bam*HI fragment (Table 6). However, no rescue was found for EMS-induced mutations *mod(mdg4)*^{06} and *mod(mdg4)*^{07}.

To determine whether homozygous *mod(mdg4)*^{neo129} mutants containing the transgene were sterile, we examined eggs deposited from these females after mating with wild-type males. Development of eggs is arrested at different stages of embryogenesis, although occasional first larval instar escapers were found. This result indicates a partial rescue of the maternal effect lethal phenotype.

Significant homeotic transformation of abdominal segment A5 to A4 was found in *P*(w^+*7.5kb Bam*HI)*; mod(mdg4)*^{02}/*mod(mdg4)*^{02} males and *P*(w^+*7.5kb Bam*HI)*; mod(mdg4)*^{neo129}/*mod(mdg4)*^{neo129} males (Figure 9, A and B), indicating that the transgene does not rescue this mutant phenotype. Elevated additive effects of homeotic transformation in *Df(3R)red*^{trx} *mod(mdg4)*^{neo129}/*mod(mdg4)*^{neo129} males are also not affected by the *P*(w^+*7.5kb Bam*HI*)* transgene (Figure 9C). Two conclusions can be drawn from these results. First, the presence of a transgene containing sequences encoding the common part of *mod(mdg4)* but lacking the alternatively spliced exons is able to partially rescue recessive lethality. Second, the partial rescue of recessive lethality results in females with maternal effect lethality and males with significant homeotic transformation, indicating that sequences encoding the specific domains and/or their expression at sufficient levels are important for wild-type function of Mod(mdg4) proteins.

DISCUSSION

Mod(mdg4) protein isoforms are differentially distributed on polytene chromosomes: Our molecular analysis revealed that *mod(mdg4)* produces a large number of transcripts by alternative splicing. We have identified 17 new splice variants. All deduced proteins contain a common N terminus of 402 amino acids encompassing the most N-terminal BTB/POZ domain and different C termini. Our results clearly indicate a differential distribution of at least two Mod(mdg4) proteins, Mod(mdg4)-58.0 and Mod(mdg4)-67.2, along polytene chromosomes. Whereas Mod(mdg4)-67.2 is found at the majority of sites, labeled by the antibody anti-

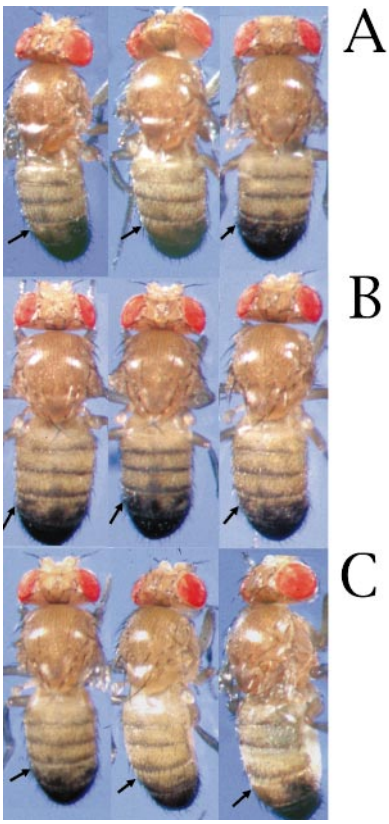

Figure 9.—Homeotic transformation of abdominal segment A5 to A4 in homozygous *mod(mdg4)* mutant males containing the 7.2-kb *Bam*HI transgene. (A) *P*(w^+*7.5kbBam*HI)/+; *mod(mdg4)*^{02}/*mod(mdg4)*^{02} males, (B) *P*(w^+*7.5kbBam*HI)/+; *mod(mdg4)*^{neo129}/*mod(mdg4)*^{neo129} males, and (C) *P*(w^+*7.5kb Bam*HI)/+; *Df(3R)red*^{trx} *mod(mdg4)*^{neo129}/*mod(mdg4)*^{neo129} males.

Mod(mdg4)-58.0$^{BTB\text{-}534}$ detecting all protein isoforms, the other isoform is restricted to a small subset of sites. The binding of Mod(mdg4)-58.0 and Mod(mdg4)-67.2 at different sites suggests that at least these two Mod(mdg4) isoforms participate in transcriptional regulation of different sets of genes. We suppose that the specific C-terminal domains play a critical role in directing the isoforms to different binding sites, possibly through specific interactions with other proteins. Two other observations are consistent with this hypothesis. Gerasimova *et al.* (1995) demonstrated by genetic and *in vitro* binding assays an interaction of Mod(mdg4)-67.2 [Mod(mdg4)2.2] with Su(Hw), a zinc finger protein that binds to *gypsy* sequences. Both proteins are implicated in the function of chromatin insulator sequences present in the *gypsy* transposon. Using the yeast two-hybrid system, Harvey *et al.* (1997) identified one of the Mod(mdg4) isoforms, DOOM [Mod(mdg4)-56.3], by its ability to interact with the baculovirus inhibitor of apoptosis protein. In coimmunoprecipitation experiments, this interaction could be clearly localized to the specific C-terminal domain of DOOM. Together these results suggest that the large number of protein isoforms generated from *mod(mdg4)* reflects the functional diversity of individual Mod(mdg4) proteins. The GAGA factor, encoded by the *Trl* gene, was shown to be involved in nucleosome remodeling in regulatory regions of many genes (reviewed in Wilkins and Lis 1997). Mutations in *Trl* and *mod(mdg4)* display very similar genetic properties, *e.g.*, enhancement of PEV, paternal effects, and homeotic transformation. The generation of different GAGA isoforms containing a common N terminus of 377 amino acids with an N-terminal BTB/POZ domain has been demonstrated. However, in contrast to *mod(mdg4)*, a colocalization of two different GAGA isoforms on polytene chromosomes and their ability to form heterodimers could be demonstrated by coimmunoprecipitation (Benyajati *et al.* 1997).

That the protein consensus sequence contains a Cys_2His_2 motif within the specific protein domains of most Mod(mdg4) isoforms may be of functional importance. In contrast to canonical zinc-finger motifs of the Cys_2His_2 type, the one found here has distinct features. The two histidine residues are separated by only one amino acid residue, and the consensus sequence extends N-terminal with additional conserved aromatic amino acid positions. The presence of the conserved sequence in the specific protein domain of DOOM implicates its putative involvement in protein-protein interaction with IAP. Disruption of this interaction by mutagenesis of the highly conserved amino acid positions could test this hypothesis. However, five of the different isoforms do not contain the identified consensus sequence, including Mod(mdg4)-58.0 and Mod(mdg4)-67.2. The functional significance of the presence of several Cys and His residues in these isoforms remains unknown.

Pleiotropic effects of *mod(mdg4)* mutations: Genetic analysis of several *mod(mdg4)* mutant alleles revealed pleiotropic effects. All mutations are dominant enhancers of PEV and display paternal enhancer effects. Additionally, *mod(mdg4)* mutations have been demonstrated to display properties typical for trx group genes (Dorn *et al.* 1993b; Gerasimova and Corces 1998). Enhanced homeotic transformation has been observed in *trans* and *cis* combinations with several mutations in other trx group genes, suggesting a possible interaction of the corresponding proteins (Gerasimova and Corces 1998; this work). This is supported by the observed interactions in PEV enhancement. Double heterozygous *Df(3R)red*^{trx} *mod(mdg4)*^{neo129}/++ males strongly enhance PEV in *w*^{m4} flies, whereas the *trx* mutation itself has no effect. In our earlier study, we described paternal enhancer effects (Dorn *et al.* 1993b). Furthermore, the finding of strong paternal enhancer effects in +/+ male offspring derived from *Df(3R)red*^{trx} *mod(mdg4)*^{neo129}/++ fathers also implies an interaction of the corresponding proteins in gene silencing in PEV. Based on the finding of different distribution of Trx and Mod(mdg4) proteins in diploid interphase nuclei and the altered distribution of Mod(mdg4) proteins in the background of *trx* mutations, Gerasimova and Corces (1998) proposed a two-tier model for chromatin assembly. According to this model, the formation of complexes containing Trx precedes the assembly of Mod(mdg4) proteins.

Most of the molecularly characterized *mod(mdg4)* mutations involve sequences within the common 5′ region. These mutations would be expected to affect all Mod(mdg4) protein isoforms, explaining the observed pleiotropic mutant effects. Although we have demonstrated the differential distribution of two protein isoforms, we do not know if the loss of single isoforms causes distinct mutant phenotypes. This would be expected if Mod(mdg4) proteins have specific functions in chromatin. Mutations within the specific protein domains of different isoforms should allow a further functional dissection of *mod(mdg4)*.

***mod(mdg4)* is expressed at high levels during oogenesis:** The presence of large amounts of Mod(mdg4) proteins in all stages of oogenesis and early embryogenesis indicates a strong maternal component (this work; K. Büchner and R. Dorn, unpublished results). The significantly reduced amounts of Mod(mdg4) proteins detected in egg chambers of homozygous *mod(mdg4)*^{neo129} females and the failure of eggs to foster further development indicate important functions of *mod(mdg4)* during oogenesis and early embryonic development. The presence of Mod(mdg4) in both nurse cell and follicle cell nuclei and the supposed role as a general transcriptional regulator suggest that *mod(mdg4)* is required for control of maternal genes during oogenesis. This is in agreement with the supposed role of Mod(mdg4) protein(s) in mediating the function of chromatin insulator sequences as a prerequisite for correct promoter-

enhancer interactions (Gerasimova and Corces 1998). In the embryo, Mod(mdg4) proteins do not become localized to the nuclei until cleavage cycle 9 (K. Büchner and R. Dorn, unpublished results), further arguing against a function in chromatin organization during early cleavage cycles.

We have shown that a transgene containing the common part of Mod(mdg4) can partially rescue the recessive lethality of *mod(mdg4)* mutant alleles. This result can be explained by the expression of a truncated protein containing the 402-amino-acid common N-terminal region and the ability to partially replace the function of full-length Mod(mdg4) proteins. However, we were not able to detect a protein of the expected molecular size in the transgenic animals, which may be due to the limited sensitivity of Western blot analysis. Expression of a tagged protein under control of the *hsp70* promoter from a transgene will be required to prove the proposed function of the common N-terminal peptide.

We are grateful to Dr. M. Frasch for providing mutations *AI117* and *AI351* [corresponding to $mod(mdg4)^{06}$ and $mod(mdg4)^{07}$, respectively] and $P(w^{+})142$ and revertants of this insertional mutation, including *P142Δ32*. The anti-histone H1 antibody was kindly provided by Dr. U. Grossbach. We would like to thank M. Reich for isolation of revertants from $mod(mdg4)^{neo}$, G. Hause for advice in immunocytological analysis of ovaries, and M. Kube and C. Frost for technical assistance. We thank V. Pirrotta for critical reading of the manuscript. This work was supported by grants from the Deutsche Forschungsgemeinschaft to R.D. (Do407/1-2 and 1-3).

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