Insulation of Enhancer-Promoter Communication by a Gypsy Transposon Insert in the *Drosophila cut* Gene: Cooperation between Suppressor of Hairy-wing and Modifier of *mdg4* Proteins

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The *Drosophila mod(mdg4)* gene products counteract heterochromatin-mediated silencing of the *white* gene and help activate genes of the bithorax complex. They also regulate the insulator activity of the gypsy transposon when gypsy inserts between an enhancer and promoter. The Su(Hw) protein is required for gypsy-mediated insulation, and the Mod(mdg4)-67.2 protein binds to Su(Hw). The aim of this study was to determine whether Mod(mdg4)-67.2 is a coinsulator that helps Su(Hw) block enhancers or a facilitator of activation that is inhibited by Su(Hw). Here we provide evidence that Mod(mdg4)-67.2 acts as a coinsulator by showing that some loss-of-function *mod(mdg4)* mutations decrease enhancer blocking by a gypsy insert in the *cut* gene. We find that the C terminus of Mod(mdg4)-67.2 binds in vitro to a region of Su(Hw) that is required for insulation, while the N terminus mediates self-association. The N terminus of Mod(mdg4)-67.2 also interacts with the Chip protein, which facilitates activation of *cut*. Mod(mdg4)-67.2 truncated in the C terminus interferes in a dominant-negative fashion with insulation in *cut* but does not significantly affect heterochromatin-mediated silencing of *white*. We infer that multiple contacts between Su(Hw) and a Mod(mdg4)-67.2 multimer are required for insulation. We theorize that Mod(mdg4)-67.2 usually aids gene activation but can also act as a coinsulator by helping Su(Hw) trap facilitators of activation, such as the Chip protein.

The mechanisms by which enhancers transcriptionally activate promoters located several kilobases away are unknown. Some of these mechanisms are inhibited by insulator sequences. Insulators block activation of a promoter by an enhancer only when positioned between the enhancer and promoter (for reviews, see references 1, 12, 22, 43, and 56). Insulators block activation without inhibiting enhancers or repressing promoters. Thus, an enhancer prevented by an insulator from activating one promoter can still activate another promoter that is not separated by the insulator (4, 51). Similarly, a promoter insulated from some enhancers can still be activated by other enhancers that are not separated by the insulator. Insulators, therefore, interfere specifically with communication between enhancers and promoters.

Insulators help control gene expression. For example, multiple insulators organize enhancer-promoter interactions in the bithorax HOX gene complex of *Drosophila* (reviewed in references 39 and 56). Two insulators that bind BEAF-32 or the Zw5 protein flank a *Drosophila hsp70* heat shock gene cluster and may prevent activation of neighboring genes by heat shock factor (16, 33, 34, 58). Insulators flanking the chicken β -globin gene complex that bind the CTCF-binding factor (CTCF) protein are at the boundaries of the transcriptionally active domain (7, 50). An insulator that binds CTCF in a methylation-sensitive manner helps mediate imprinting of the mouse *Igf2* gene (2, 26, 53, 54).

Several models have been proposed to explain how insulators block activation. These include the suggestion that insulators regulate chromatin structure to form boundaries between domains of closed and open chromatin (7, 21, 33) and the idea that insulators are decoys that mimic a promoter and trap enhancers in futile interactions (22). An alternative idea is that insulators thwart proteins that act between enhancers and promoters to facilitate communication (11, 12, 41, 42). Such facilitator proteins could, for example, modify chromatin structure to bring an enhancer and promoter closer together or transmit an activation signal down the chromosome from an enhancer to a promoter.

The *Drosophila* proteins Chip and Nipped-B are putative facilitators of enhancer-promoter communication (41, 42, 48). Both proteins potentiate activation by diverse enhancers and have close mammalian homologues. They were originally identified by screening for mutations that increase insulation by gypsy transposon insertions in the *cut* and *Ultrabithorax* genes (41, 48).

The Su(Hw) protein is required for the gypsy transposon insulator to block enhancer-promoter interactions. Mutations in the *suppressor of Hairy-wing* [*su(Hw)*] gene decrease (suppress) the severity of the mutant phenotypes caused by gypsy insertions in many different genes (40, 49), and a sequence that binds Su(Hw) protein is the only part of gypsy required for insulation (23, 28). Su(Hw) interacts with the Chip protein in vitro, suggesting that Chip is a direct molecular target of the gypsy insulator (55).

The *modifier of mdg4* [*mod(mdg4)*] gene also determines how gypsy insertions alter gene expression. Unlike *su(Hw)*, however, the effects of *mod(mdg4)* differ from gene to gene. The

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mod(mdg4)^{u1} mutation was identified on the basis that it increases the severity of the mutant phenotype caused by a gypsy insertion at the *yellow* locus (18). The *y²* gypsy insertion is positioned such that it blocks only enhancers that activate *yellow* in wing and body cuticle (24). In *mod(mdg4)^{u1}* mutants, *yellow* expression is also reduced in bristles and larval mouthparts, and this repressive effect requires the Su(Hw) protein (18, 21). In this and other cases, the *mod(mdg4)^{u1}* mutation appears to convert Su(Hw) from an insulator to a repressor protein (5, 19, 21). In contrast to the effects on *yellow*, *mod(mdg4)^{u1}* has effects similar to those of *su(Hw)* mutations and suppresses the mutant phenotypes caused by gypsy insertions at other loci, such as *cut* (18). In these cases, the *mod(mdg4)^{u1}* mutation appears to reduce insulation by gypsy without causing repression.

The *mod(mdg4)* gene encodes several proteins (3, 9, 21, 27). Mod(mdg4)-67.2 is the major product and is present at the majority of the few hundred sites on polytene chromosomes that bind *mod(mdg4)* proteins (4) [Mod(mdg4)-67.2 is encoded by the mRNA designated *mod2.2* in reference 21]. Mod(mdg4)-67.2 interacts with Su(Hw) in vitro, and Su(Hw) is present at almost half the sites on polytene chromosomes that bind *mod(mdg4)* proteins (20, 21). The *mod(mdg4)^{u1}* mutation is a Stalker transposon insertion into the C-terminal exon unique to the Mod(mdg4)-67.2 mRNA (3, 21). It is likely, therefore, that a change in the activity of Mod(mdg4)-67.2 is responsible for the various effects that the *mod(mdg4)^{u1}* mutation has on the gypsy insulator.

Most *mod(mdg4)* mutations are recessive lethal, but *mod(mdg4)^{u1}* is homozygous viable, indicating that it is not a null allele. It has not been determined whether *mod(mdg4)^{u1}* simply reduces the activity of Mod(mdg4)-67.2. This lack of knowledge about the effects of *mod(mdg4)^{u1}* on Mod(mdg4)-67.2 activity has made it difficult to understand the precise role of Mod(mdg4)-67.2 in Su(Hw) insulator activity. Until now, the effect of the *mod(mdg4)* gene on the gypsy insulator has been deduced from flies homozygous for *mod(mdg4)^{u1}* or heterozygous for *mod(mdg4)^{u1}* and another mutation in *mod(mdg4)*. Thus, Mod(mdg4)-67.2 may be a coinsulator that helps Su(Hw) block enhancers, or alternatively, Mod(mdg4)-67.2 may be a facilitator of enhancer-promoter communication that is targeted by Su(Hw). In the latter scenario, *mod(mdg4)^{u1}* would produce a mutant Mod(mdg4)-67.2 protein that is immune to Su(Hw).

Indeed, there is evidence that some *mod(mdg4)* products support gene activation. The first lethal *mod(mdg4)* alleles [originally named *E(var)3-93D* mutations] were isolated as dominant mutations that increase silencing of the *white* gene caused by heterochromatic position effect (10). This suggests that *mod(mdg4)* products promote *white* expression. *mod(mdg4)* mutations also increase the severity of homeotic transformations caused by mutations in trithorax group (*trxG*) genes, suggesting that *mod(mdg4)* gene products are themselves *trxG* proteins and promote expression of genes in the bithorax complex (9, 20). Consistent with this idea, expression of the Antennapedia complex is also reduced in *mod(mdg4)* mutants (20).

Here we report the results of experiments designed to clarify the role of Mod(mdg4)-67.2 in Su(Hw)-mediated insulation at the *cut* locus. Our results indicate that a multimer of wild-type

TABLE 1. Dominant effects of mutant *mod(mdg4)* alleles on the *cr^K* and *In(1)^{w^{m4}}* mutant phenotypes^a

Allele	Mutagen	Viability	Effect of allele on phenotype of ^b :		Source or reference
			<i>cr^K</i>	<i>In(1)^{w^{m4}}</i>	
<i>mod(mdg4)^{u1}</i>	Stalker	Viable	S	O	20
<i>mod(mdg4)^{T6}</i>	EMS	Viable	S	O	23
<i>mod(mdg4)^{neo129}</i>	P element	Semilethal	S ^c	E	12
<i>mod(mdg4)^{bpd1}</i>	P element	Viable	O	NT	27
<i>mod(mdg4)^{bpdEX57}</i>	P excision	Lethal	s	O	27
<i>mod(mdg4)²⁰</i>	EMS	Lethal	O	NT	M. Frasch
<i>mod(mdg4)¹¹⁷</i>	EMS	Lethal	O	NT	M. Frasch
<i>mod(mdg4)²⁶⁹</i>	EMS	Lethal	O	NT	M. Frasch
<i>mod(mdg4)³²⁴</i>	EMS	Lethal	O	NT	M. Frasch
<i>mod(mdg4)³³⁴</i>	EMS	Lethal	O	NT	M. Frasch
<i>mod(mdg4)³⁴⁰</i>	EMS	Lethal	s ^d	NT	M. Frasch
<i>Df(3R)e-BS2</i>	X ray	Lethal	s	E	3

^a EMS, ethyl methanesulfonate; S, strong suppression; s, weak suppression; O, no significant effect; E, strongly enhanced; NT, not tested.

^b Letters in the *cr^K* column indicate the dominant effect on the wing-nicking phenotype displayed by *cr^K* males. Selected wings representing the median effects of *mod(mdg4)^{u1}*, *mod(mdg4)^{T6}*, *mod(mdg4)^{bpdEX57}*, and *Df(3R)e-BS2* are shown in Fig. 1. Letters in the *In(1)^{w^{m4}}* column indicate the dominant effect on the variegated eye color phenotype caused by heterochromatin-mediated silencing of white displayed by *In(1)^{w^{m4}}* males. In most cases the effects were determined when the indicated allele was heterozygous with *mod(mdg4)^{u1}*, but the *mod(mdg4)^{neo129}* allele has previously been reported to enhance variegation when heterozygous with wild-type *mod(mdg4)* (12). *mod(mdg4)^{u1}* had no significant effect when homozygous or when heterozygous with the wild type. Selected wings representing the median phenotypes for the alleles tested are shown in Fig. 2.

^c At 29° some *mod(mdg4)^{neo129}* homozygotes were recovered, and these demonstrated strong but incomplete suppression of the *cr^K* mutant phenotype.

^d With *mod(mdg4)^{340/+}* only a small fraction of the wings showed weak suppression.

Mod(mdg4)-67.2 interacts with Su(Hw) and contributes to insulation. We find that *mod(mdg4)^{u1}* is antimorphic and interferes with wild-type *mod(mdg4)* activity in insulation but is virtually wild type with regard to counteracting heterochromatin-mediated variegation of *white* gene expression. We also present evidence that Chip may be a molecular target of the gypsy insulator.

MATERIALS AND METHODS

Genetic crosses. All crosses were conducted at 25°C unless indicated otherwise. Various *mod(mdg4)* mutant stocks and deficiencies were provided by Pavel Georgiev (Institute of Gene Biology, Moscow, Russia), Haini Cai (University of California, Berkeley), Manfred Frasch (Mt. Sinai School of Medicine, New York, N.Y.), Rainer Dorn (Martin Luther University, Halle, Germany), Mark Brennan (University of Louisville, Louisville, Ky.), Valerie Budnik (University of Massachusetts, Amherst), and the Indiana University (Bloomington) stock center (Table 1).

To observe the dominant effects of various *mod(mdg4)* mutations or deletions on the partial cut wing phenotype displayed by *cr^K*, virgin *cm cr^K* females were crossed to *mod(mdg4)* mutant males and progeny *cr^K* males with the *mod(mdg4)* mutation were scored for the cut wing phenotype. Similarly, to observe the dominant effects of *mod(mdg4)* mutations on the *cr^{53d}* cut wing phenotype, *y w^{67c23} cr^{53d}* females were crossed to *mod(mdg4)* mutant males. Wings were mounted and photographed as described previously (11). The representative wings displayed in Fig. 1 were selected by arranging several wings in order of the severity of their cut wing phenotype and choosing the median wings to photograph. To quantify the phenotypes displayed by some genotypes, 10 randomly selected wings for each genotype were mounted and photographed using a 4× objective and a digital camera (Qimaging Microimager II) mounted on a Nikon microscope. The percentage of margin lacking bristles was determined for each wing using the curve measuring tool in Northern Eclipse 6.0 software (Empix

FIG. 1. Dominant effects of *mod(mdg4)* mutations on the *ct^K* cut wing phenotype. (A) Representative wings from the indicated genotypes. The *mod(mdg4)^{u1}* and *mod(mdg4)^{T6}* mutations strongly suppress the cut wing phenotype, and the *mod(mdg4)^{bpdEX57}* mutation and the *Df(3R)e-BS2* deficiency that deletes the *mod(mdg4)* gene weakly suppress it. Rare *mod(mdg4)^{neo129}* homozygotes generated at 29°C show strong but incomplete suppression. The wings shown represent the median phenotypes for the indicated genotypes. (B) Box plot of the suppression of the *ct^K* mutant phenotype by selected *mod(mdg4)* alleles. The percentage of the wing margin lacking bristles was measured from 10 randomly chosen wings for each genotype, and a box plot was generated. The five horizontal lines from top to bottom for each genotype are the 90th, 75th, 50th, 25th, and 10th percentiles. The circles are the minimum and maximum values observed for each genotype.

Imaging). Statistical analysis was performed using Statview 5.0 software (SAS Institute).

To examine the effects of various *mod(mdg4)* mutations on the position-effect variegation of *white* expression displayed by *In(1)w^{m4}*, *In(1)w^{m4} ct^{L-32}* females were crossed to various *mod(mdg4)* mutant males and the male progeny were examined for the variegation of *white* expression in the adult eye. For each *mod(mdg4)* mutation, 20 or more flies were examined and compared side-by-side with controls. Initial comparisons were conducted by one person, and the flies were then coded and scored again in a blind fashion by a second person. In all cases the original scoring and rescoring were in agreement. The representative eyes shown in Fig. 2 were chosen by photographing several eyes for each genotype, arranging them in order of phenotypic severity, and selecting one with the median phenotype.

Structural analysis of *mod(mdg4)* mutations. The *mod(mdg4)^{bpd1}* and *mod(mdg4)^{bpdEX57}* mutations were balanced over *In(3LR)TM6,Tb ubi-GFP* (provided by Kathryn Anderson, Sloan-Kettering Institute, New York, N.Y.), and homozygous mutant embryos and larvae lacking green fluorescent protein were collected using a fluorescence dissection microscope. Genomic DNA was pre-

pared as described elsewhere (37). Genomic DNA samples were analyzed for the presence or absence of specific sequences by PCR using several different primers. These include a primer against the inverted repeat of the P element (5'-GAC GGG ACC ACC TTA TGT TA-3'), a primer against the first exon in the *mod(mdg4)*-67.2 mRNA (5'-GAC GCG TTC TGC GGG TCG-3'), and a primer against coding sequences present in the second exon (5'-CCA GCA CAA GCT GAA TTG CTC-3').

Northern blot hybridization. Total RNA from wild-type flies and *mod(mdg4)* mutants was isolated from different stages of development using the Trizma reagent (Sigma). The 2.3-kb *mod(mdg4)*-67.2 transcripts were detected by Northern blot hybridization performed as described elsewhere (13). Radioactively labeled single-stranded antisense RNA probes were prepared from a pGEM vector (Pharmacia) containing the *EcoRI*-*Bst*EII fragment from the *mod(mdg4)*-67.2 cDNA (provided by Elizabeth Blackwood, University of California, San Diego). This probe hybridizes only to sequences deleted from the terminal exon in the *mod(mdg4)^{u1}* mutant. As an internal standard, ethidium bromide-stained rRNA was measured by densitometric scanning of a photographic negative.

FIG. 2. Effects of *mod(mdg4)* mutations on the *In(1)w^{m4}* variation phenotype. The panels show representative eyes from *In(1)w^{m4}* males with the indicated combinations of *mod(mdg4)* alleles. *mod(mdg4)^{neo129}* and *Df(3R)e-BS2* strongly enhance the variation when heterozygous with *mod(mdg4)^{u1}*. *mod(mdg4)^{neo129}* and deficiencies also strongly enhance the variation when they are heterozygous with wild-type *mod(mdg4)* (10). The eyes shown represent the median phenotype displayed by the indicated genotype.

Affinity chromatography. Affinity chromatography experiments were conducted as described elsewhere (55) using various glutathione *S*-transferase (GST) fusion proteins (see below) expressed in *Escherichia coli* and bound to glutathione beads and various ³⁵S-labeled proteins prepared by translation in

vitro. Briefly, binding reactions were conducted at 4°C for 1 h in phosphate-buffered saline containing 1% Triton X-100, 2% nonfat milk, protease inhibitors, and 1 mM dithiothreitol. Each reaction mixture contained ~10⁻¹³ pmol of ³⁵S-labeled proteins and beads with ~10 to 20 μg of GST fusion protein in a total

volume of 200 μ l. After extensive washing, the beads were boiled in 40 μ l of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, and the amount of labeled protein that was retained was quantified with a phosphorimager after SDS-PAGE.

GST-fusion constructs. To make the GST-Mod(mdg4)-67.2 fusion, the open reading frame encoding amino acids 1 to 610 from the *Mod(mdg4)-67.2* cDNA clone was amplified by PCR (primers 5'-ATT GGA TCC AAG ATG GCG GAC GAC GAG CAA-3' and 5'-ATA TGA TAT CGG CAT ATT CCG TGT CAC TTC T-3') and cloned into the *Bam*HI and *Sma*I sites of pGEX-2T (Pharmacia).

The GST-Su(Hw)-CTD (C-terminal domain) fusion was made by cloning the *Eco*RI fragment from pGEM-3Z-SUHW* (35) into the *Eco*RI site of pGEX-1ZT (Patricia Cortes, Rockefeller University, personal communication), which was constructed by insertion of a polylinker into the *Eco*RI site of pGEX-1 λ 84 (Pharmacia). GST-Su(Hw)-CTD fusions with various deletions in the Su(Hw)-CTD were made by cloning *Eco*RI-*Sma*I fragments from the previously described deletion constructs in the pCasper vector (35) into the *Eco*RI-blunted *Hind*III sites of pGEX-1ZT.

In vitro translation constructs. The full-length Su(Hw) construct and the Su(Hw) construct lacking the CTD were described elsewhere (55). A full-length Mod(mdg4)-67.2 in vitro translation construct was made by cloning the *Bam*HI-*Eco*RV fragment of the PCR product described above into the *Bgl*II-*Eco*RV sites of the pING14.1 vector (S. Ingles and I. Brierly [55]). To make in vitro translation vectors for Mod(mdg4)-67.2^{u1} and the Mod(mdg4)-67.2-CTD (CTD residues 307 to 610), products generated by PCR [5'-ATT AGG ATC CAA GAT GGC GGA CGA C-3' and 5'-ATT AGG ATC CGC CAT GGA ATT GCC CAC GAA A-3' primers for Mod(mdg4)-67.2^{u1}; 5'-ATT AGA TAT CCT ACA CAT TGA AGA TTA GCT TGA-3' and 5'-ATA TGA TAT CGG CAT ATT CCG TGT CAC TTC T-3' primers for Mod(mdg4)-67.2-CTD] were cloned into the *Bgl*II-*Eco*RV sites of pING14.1. To generate Mod(mdg4)-67.2^{T6}, we used primers 5'-ATT AGG ATC CAA GAT GGC GGA CGA C-3' and 5'-TTC AAA TCT CAA ACT CCT CGA A-3', and the product was ligated to the pCRII-TOPO vector (Invitrogen). To produce the Mod(mdg4)-67.2 N-terminal domain (residues 1 to 308), the *Nco*I-*Eco*RI fragment was deleted from pGEX-2T-Mod(mdg4)-67.2 by digestion and blunt-end ligation, and the *Bam*HI-blunted *Eco*RI fragment of the resulting plasmid was cloned into the *Bgl*II-*Eco*RV sites of pING14.1. All clones produced by PCR were sequenced at the 5' end of the coding region, and the Mod(mdg4)-67.2^{u1} clone was sequenced at both the 5' and 3' ends.

RESULTS

Some *mod(mdg4)* mutations decrease insulation by the *ct^K* gypsy insertion. If Mod(mdg4)-67.2 is required for insulation by Su(Hw) and gypsy, loss-of-function mutations in *mod(mdg4)* should reduce insulation by a gypsy insertion. In contrast, if Mod(mdg4)-67.2 facilitates enhancer-promoter communication, then loss-of-function mutations in *mod(mdg4)* either would have no effect on insulation or might increase insulation. The results described below indicate that some loss-of-function alleles of *mod(mdg4)* can reduce insulation by gypsy at *cut*, but more weakly than does *mod(mdg4)*^{u1}.

We used the *ct^K* gypsy insertion in *cut* to compare the abilities of several mutant alleles of *mod(mdg4)* to alter insulation. Gypsy insertions in *cut* block activation of the *cut* promoter by a wing margin-specific enhancer located some 85 kb upstream of the promoter (11, 31). The *ct^K* gypsy only partially insulates the wing margin enhancer of *cut*, resulting in an intermediate cut wing phenotype, presumably because it contains fewer Su(Hw)-binding sites than most gypsy elements (Fig. 1A) (29). The *ct^K* mutant phenotype is more sensitive to the levels of Su(Hw) activity than most gypsy insertions and is almost completely suppressed by heterozygous *su(Hw)* mutations (Fig. 1A) (49). *ct^K* is also highly sensitive to the activities of the Chip and Nipped-B proteins, which facilitate activation by the wing margin enhancer, and is made more severe by heterozygous *Chip^{e5.5}* and *Nipped-B⁴⁰⁷* loss-of-function mutations (Fig. 1A). We reasoned therefore that *ct^K* might also be very sensitive to

Mod(mdg4)-67.2 activity and would allow us to test whether both viable and lethal alleles of *mod(mdg4)* have dominant effects on insulation.

As illustrated in Fig. 1 and summarized in Table 1, heterozygous *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6} mutations suppressed the cut wing phenotype of *ct^K*. Similar to *mod(mdg4)*^{u1}, the *mod(mdg4)*^{T6} allele is homozygous viable and produces a Mod(mdg4)-67.2 protein truncated near the C terminus (M. Brennan, personal communication). The suppression by heterozygous *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6} was not as strong as that observed with a heterozygous *su(Hw)* mutation (Fig. 1A). Homozygous *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6} mutations suppressed more strongly than did the heterozygous mutations, although the suppression was still incomplete (Fig. 1A).

In contrast to the suppression by *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6}, most lethal *mod(mdg4)* mutations tested had little or no dominant effect on the *ct^K* mutant phenotype (Table 1). However, the *mod(mdg4)*^{bpdEX57} allele and the *Df(3R)e-BS2* deficiency, which deletes *mod(mdg4)*, both suppressed *ct^K*, but more weakly than did *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6} (Table 1; Fig. 1). Although weak, the suppression was reproducible in repeated experiments.

Because most lethal *mod(mdg4)* alleles had no effect on the *ct^K* phenotype, we were concerned that the weaker suppression observed with *mod(mdg4)*^{bpdEX57} and *Df(3R)e-BS2* might be caused by mutations in other genes besides *mod(mdg4)*. The *mod(mdg4)*^{bpdEX57} mutation was generated by excision of a P element inserted 27 bp upstream of the putative transcription start site for the *mod(mdg4)-67.2* transcript in the *mod(mdg4)*^{bpd1} mutation (25). The *mod(mdg4)*^{bpdEX57} mutation also fails to complement mutations in *tinman*, the gene just upstream of *mod(mdg4)*. We performed PCR analysis on genomic DNA isolated from *mod(mdg4)*^{bpdEX57} and *mod(mdg4)*^{bpd1} homozygous mutant larvae and confirmed that the P element is absent in *mod(mdg4)*^{bpdEX57} (see Materials and Methods). Northern blot hybridization with a probe that hybridizes only to exon sequences downstream of the Stalker insertion in *mod(mdg4)*^{u1} revealed that *mod(mdg4)*^{bpdEX57}/*mod(mdg4)*^{u1} adults contain less than 5% wild-type levels of the 2.3-kb *mod(mdg4)-67.2* mRNA (not shown). These results confirm that *mod(mdg4)*^{bpdEX57} is a loss-of-function mutation. Although the *tinman* gene is mutant or absent in the *mod(mdg4)*^{bpdEX57} and *Df(3R)e-BS2* chromosomes, the *tin^{EC40}* null allele in the absence of *mod(mdg4)* mutations had no dominant effect on the *ct^K* mutant phenotype (not shown).

If the effects of the *mod(mdg4)*^{bpdEX57} and *Df(3R)e-BS2* chromosomes on the *ct^K* phenotype are the result of defects in other genes that regulate *cut*, it is likely that they would not be specific for gypsy insertions but would also modify the phenotypes of other sensitive mutations in *cut*. The *ct^{53d}* lesion is a small deletion in the wing margin enhancer that partially reduces enhancer strength, and the *ct^{53d}* phenotype is dominantly enhanced by mutations in several genes that regulate *cut*, including *Chip*, *scalloped*, *vestigial*, *mastermind*, *Nipped-A*, and *l(2)41Af*, but is not affected by *su(Hw)* mutations (30, 41, 48). None of the several heterozygous *mod(mdg4)* mutations tested, including *mod(mdg4)*^{u1} and *mod(mdg4)*^{bpdEX57}, suppressed or enhanced the partial cut wing phenotype displayed by *ct^{53d}* males (not shown).

The inability of most lethal *mod(mdg4)* mutations to sup-

FIG. 3. The Mod(mdg4)-67.2 protein interacts with a region of Su(Hw) that is required for insulation. The Su(Hw) protein contains 12 zinc fingers (gray boxes) and an enhancer-blocking region within residues 737 to 880 (black box). The Su(Hw)-CTD and the indicated mutant forms fused to GST were expressed in *E. coli* and bound to glutathione beads. The autoradiograms of SDS-PAGE gels show the amount of ^{35}S -Mod(mdg4)-67.2 and ^{35}S -luciferase control protein (luc) prepared by translation in vitro that bound to the various GST-Su(Hw)-CTD beads and to GST-only control beads. The left-hand lane (load, lane 1) shows the amount of input labeled proteins. Mod(mdg4)-67.2 binds to the GST-Su(Hw)-CTD beads (lane 3), but deletions that affect the enhancer-blocking region of Su(Hw) prevent binding of Mod(mdg4)-67.2 (lanes 4, 5 and 6). All the results were reproduced in multiple independent experiments.

press the ct^K mutant phenotype suggests that they may affect essential Mod(mdg4) proteins that are not involved in insulation or that they may not reduce Mod(mdg4) protein activity enough to suppress the mutant phenotype. Experiments with the $mod(mdg4)^{neo129}$ cold-sensitive allele are consistent with the notion that the level of $mod(mdg4)$ activity can be critical. At 25°C, $mod(mdg4)^{neo129}$ is homozygous lethal, but at 29°C, it is semilethal and a few $mod(mdg4)^{neo129}$ homozygotes survive to adulthood (9). At 25 and 29°C, we found that $mod(mdg4)^{neo129}$ had only a very slight dominant effect on the ct^K mutant phenotype, but the few $mod(mdg4)^{neo129}$ homozygotes produced at 29°C displayed strong but incomplete suppression (Fig. 1A). Based on this and the weak dominant suppression of the ct^K mutant phenotype by $mod(mdg4)^{bpdEX57}$ and $Df(3R)e\text{-BS2}$ loss-of-function alleles, we conclude that at least some Mod(mdg4) proteins contribute to insulation by the ct^K gypsy insertion.

The results portrayed in Fig. 1 also indicate that the $mod(mdg4)^{u1}$ and $mod(mdg4)^{T6}$ mutations are not simple loss-of-function (hypomorphic) mutations. They suppressed ct^K

more strongly than did the loss-of-function alleles, suggesting that their products actively interfere with the activities of the remaining dose of wild-type Mod(mdg4) proteins. We considered the possibility that the $mod(mdg4)^{u1}$ and $mod(mdg4)^{T6}$ chromosomes might contain other mutations that increase their effects on ct^K . It is unlikely, however, that both chromosomes, which were isolated independently and are homozygous viable, would contain similar additional mutations. It is much more likely that they have similar effects because they both produce similarly truncated Mod(mdg4)-67.2 proteins. We conclude, therefore, that the $mod(mdg4)^{u1}$ and $mod(mdg4)^{T6}$ mutations are antimorphic and inhibit the function of wild-type $mod(mdg4)$ in insulation by gypsy in a dominant-negative manner.

The $mod(mdg4)^{u1}$ and $mod(mdg4)^{T6}$ mutations do not substantially alter position-effect variegation of $In(1)w^{m4}$. Some alleles of $mod(mdg4)$ were isolated as dominant mutations that increase heterochromatin-mediated silencing [position effect variegation (PEV)] of the *white* gene in the $In(1)w^{m4}$ chromosomal rearrangement (10). The dominant-negative effects of

FIG. 4. The truncated Mod(mdg4)-67.2^{u1} and Mod(mdg4)-67.2^{T6} proteins do not interact with Su(Hw). The Mod(mdg4)-67.2 protein contains a BTB/POZ motif (gray box) at the N terminus common to most if not all Mod(mdg4) proteins, a glutamine-rich (Q-rich) region, and a unique C terminus (cross-hatched box) (3). ³⁵S-labeled fragments of Mod(mdg4)-67.2, including fragments that mimic the predicted mutant proteins produced by the *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6} alleles, were prepared by translation in vitro and tested for their ability to bind GST-Su(Hw)-CTD beads and GST-only control beads. The autoradiograms of SDS-PAGE gels show the amount of input labeled protein (load, lane 1) and the amount that bound to the beads indicated at the tops of lanes 2 and 3. The full-length Mod(mdg4)-67.2 and the C-terminal fragment of Mod(mdg4)-67.2 both bound to the GST-Su(Hw)-CTD beads (lane 3), while the truncated Mod(mdg4)-67.2^{u1}, Mod(mdg4)-67.2^{T6}, and N-terminal fragment of Mod(mdg4)-67.2 did not (lane 3). As expected, the luciferase (luc) control protein also did not bind to any of the beads. In the lowest panel, nearly equal amounts of labeled full-length Mod(mdg4)-67.2 and Mod(mdg4)-67.2^{u1} were prepared by cotranslation in vitro. In this case, a small but significant amount of Mod(mdg4)-67.2^{u1} was retained (lane 6, arrow). All the results shown were reproducible in independent experiments.

mod(mdg4)^{u1} and *mod(mdg4)*^{T6} on insulation by gypsy raised the question of whether these mutations are also antimorphic with regard to enhancement of PEV. Unexpectedly, *mod(mdg4)*^{u1}+, *mod(mdg4)*^{T6}/*mod(mdg4)*^{u1}, and *mod(mdg4)*^{bpdEX7}/*mod(mdg4)*^{u1} did not detectably enhance the PEV displayed by *In(1)*w^{m4} (Fig. 2). In contrast, *mod(mdg4)*^{neo129}/*mod(mdg4)*^{u1} and *Df(3R)e-BS2/mod(mdg4)*^{u1} both strongly enhanced variegation (Fig. 2). *mod(mdg4)*^{u1/mod(mdg4)^{u1} flies displayed a weak enhancement of the PEV, but this is likely to be a background effect, because *mod(mdg4)*^{T6/mod(mdg4)^{u1} had the opposite effect and mildly suppressed the PEV. Previously it had been observed that *mod(mdg4)*^{neo129} and deficiencies that remove *mod(mdg4)* also dominantly enhanced the PEV displayed by *In(1)*w^{m4} when heterozygous with wild-type *mod(mdg4)* (10). The enhancement of PEV that was observed when *mod(mdg4)*^{neo129} and *Df(3R)e-BS2* were heterozygous with *mod(mdg4)*^{u1} indicates that the *mod(mdg4)*^{u1} chromosome does not contain a counteracting dominant suppressor-of-variegation mutation that would mask any effects of *mod(mdg4)*^{u1} on PEV. We conclude, therefore, that *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6} are essentially wild type with regard to their effects on variegation of *In(1)*w^{m4}.}}

The Mod(mdg4)-67.2 protein interacts with the insulation domain of Su(Hw). It has previously been reported that Mod(mdg4) proteins interact with full-length Su(Hw) in vitro (21). Our laboratory has previously shown that a region of some 140 amino acids (amino acids 737 to 880) in Su(Hw)

contains the residues required for strong enhancer-blocking activity in vivo (35). To test whether this insulation region interacts with the Mod(mdg4)-67.2 protein, we expressed a fusion protein in which GST was fused to the CTD of Su(Hw) [Su(Hw)-CTD, residues 673 to 943] in *E. coli* and bound the fusion protein to glutathione beads to make an affinity matrix. We also constructed a series of similar affinity matrices with various deletion mutants of Su(Hw)-CTD (Fig. 3). Full-length ³⁵S-Mod(mdg4)-67.2 protein was prepared by translation in vitro and incubated with the wild-type and mutant GST-Su(Hw)-CTD beads. Consistent with previous reports that Mod(mdg4) proteins interact with full-length Su(Hw) (23), Mod(mdg4)-67.2 bound to the wild-type GST-Su(Hw)-CTD beads (Fig. 3, lane 3) but not to GST control beads (Fig. 3, lane 2). In contrast, Mod(mdg4)-67.2 did not bind to any of the mutant forms of GST-Su(Hw)-CTD in which portions of the insulation domain (residues 737 to 880) were deleted (Fig. 3, lanes 4, 5, and 6).

To test for other Su(Hw) domains that may interact with Mod(mdg4)-67.2, GST was fused to full-length Mod(mdg4)-67.2 and the fusion protein bound to beads. Full-length Su(Hw) prepared by translation in vitro bound to the GST-Mod(mdg4)-67.2 beads, but Su(Hw) with the C-terminal residues (673 to 943) deleted did not (data not shown). These results indicate that the CTD is the only portion of Su(Hw) that interacts with Mod(mdg4)-67.2.

Mutant Mod(mdg4)-67.2 proteins produced by *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6} do not interact with Su(Hw). As shown above,

FIG. 5. Mod(mdg4)-67.2 interacts with the Chip facilitator protein. The Chip protein contains multiple protein interaction regions (gray boxes) including the SID, the LID, and the OID that interacts with several homeodomain proteins and the Su(Hw) zinc finger region (55). ^{35}S -labeled N-terminal and C-terminal fragments of Mod(mdg4)-67.2 (see Fig. 4) and a luciferase control protein (luc) were prepared by translation in vitro and tested for their ability to bind to GST control beads and GST-Chip fusion protein beads (55). The autoradiograms of SDS-PAGE gels on the left (lanes 1, 2, and 3) show the amount of input protein (load, lane 1) and the amount that was retained by the indicated beads. The N-terminal fragment bound to the GST-Chip beads, but the C-terminal fragment and the luciferase control did not (lane 3). The autoradiogram of an SDS-PAGE gel on the right (lanes 4 through 10) shows the binding of full-length ^{35}S -labeled Mod(mdg4)-67.2 prepared by in vitro translation to beads containing fusions of GST to wild-type Chip and various deletion mutants of Chip (55). The load lane (lane 4) contains a tenth of the input protein. Deletions affecting only the SID (lane 10) or only the LID (lane 7) did not affect binding of Mod(mdg4)-67.2, while deletions affecting the OID (lanes 8 and 9) displayed substantially reduced binding. All the results shown were reproduced in independent experiments.

the viable *mod(mdg4)^{u1}* and *mod(mdg4)^{T6}* mutations more strongly reduce insulation by *ct^K* gypsy than do lethal and null alleles of *mod(mdg4)*. The experiments of Fig. 4 show that the residues of Mod(mdg4)-67.2 truncated by the *mod(mdg4)^{u1}* and *mod(mdg4)^{T6}* mutations are required to interact with Su(Hw).

The *mod(mdg4)^{u1}* mutation is a Stalker transposon insertion (18, 21). The residues affected are unique to the Mod(mdg4)-67.2 protein and are not present in other *mod(mdg4)* products. The Stalker insertion results in replacement of several C-terminal residues of Mod(mdg4)-67.2 by a single residue encoded by Stalker sequences (M. Brennan, personal communication). Thus, the Mod(mdg4)-67.2^{u1} protein contains wild-type residues 1 to 465 out of 610 (Fig. 4). The *mod(mdg4)^{T6}* mutation was induced by chemical mutagenesis and, similar to *mod(mdg4)^{u1}*, is also homozygous viable (21; Mark Brennan, personal communication). The *mod(mdg4)^{T6}* mutation also results in truncation of Mod(mdg4)-67.2, resulting in a protein that contains wild-type residues 1 to 567 (Fig. 4; M. Brennan, personal communication). In contrast to the full-length Mod(mdg4)-67.2 protein, the Mod(mdg4)-67.2^{u1} and Mod(mdg4)-67.2^{T6} mutant proteins prepared by in vitro translation did not bind to GST-Su(Hw)-CTD beads (Fig. 4, lane 3).

The Mod(mdg4)-67.2^{u1} mutant protein interacts with wild-type Mod(mdg4)-67.2. The genetic data described above suggest that the *mod(mdg4)^{u1}* mutant products interfere in a dominant-negative (antimorphic) fashion with the remaining dose of wild-type *mod(mdg4)* products. The predicted *mod(mdg4)* proteins contain a BTB/POZ domain at the N terminus (3). BTB/POZ domains of other proteins have been shown to self-interact (8, 32, 38), leading us to postulate that the Mod(mdg4)-

67.2^{u1} protein might form inactive multimers with wild-type Mod(mdg4)-67.2.

An experiment depicted in Fig. 4 provides evidence that the Mod(mdg4)-67.2^{u1} protein interacts with wild-type Mod(mdg4)-67.2. When wild-type Mod(mdg4)-67.2 and Mod(mdg4)-67.2^{u1} were translated together and incubated with GST-Su(Hw)-CTD beads, a small but significant amount of Mod(mdg4)-67.2^{u1} protein was retained (Fig. 4, lane 6). Because the truncated Mod(mdg4)-67.2 protein bound to the Su(Hw) C-terminal region only in the presence of full-length Mod(mdg4)-67.2 protein, we infer that the two Mod(mdg4)-67.2 proteins interact with each other, presumably through the BTB/POZ domain or other N-terminal residues.

Mod(mdg4)-67.2 interacts with the Chip facilitator protein. The putative facilitator protein Chip interacts with the zinc finger region of Su(Hw) in vitro, supporting the notion that the in vivo antagonism between Chip and Su(Hw) insulator activity is direct (41, 55). The experiments portrayed in Fig. 5 further support this idea by indicating that Chip also interacts with Mod(mdg4)-67.2. An N-terminal fragment of Mod(mdg4)-67.2 (residues 1 to 308) containing the BTB/POZ domain bound to GST-Chip beads, but a C-terminal fragment (residues 307 to 610) did not (Fig. 5, lane 3).

Experiments depicted in Fig. 5 also indicate that Mod(mdg4)-67.2 interacts with the same region of Chip that interacts with Su(Hw). The Chip self-interaction domain (SID) mediates Chip-Chip interactions, the LIM interaction domain (LID) interacts with LIM domains, and the other interaction domain (OID) supports self-interactions, interactions with several homeodomain proteins, and interactions with the zinc finger region of Su(Hw) (55). Full-length Mod(mdg4)-

FIG. 6. Proposed model for the role of Mod(mdg4)-67.2 protein in insulation. (A) To explain why truncated Mod(mdg4)-67.2 proteins have dominant-negative (antimorphic) effects on insulator activity, we propose, as shown on the left, that a multimer of Mod(mdg4)-67.2 interacts with more than one DNA-bound molecule of Su(Hw) to form a stable insulator complex. As shown on the right, truncated Mod(mdg4)-67.2^{u1} or Mod(mdg4)-67.2^{T6} protein would destabilize the complex, leading to reduced interaction between Mod(mdg4)-67.2 and Su(Hw) and/or reduced binding of Su(Hw) to DNA. (B) To explain how the Mod(mdg4)-67.2 protein contributes to the insulator activity of Su(Hw), we propose, as depicted on the left, that it traps facilitators such as Chip and blocks the previously postulated Chip-assisted spread of homeodomain protein (HD) binding between the enhancer and promoter (12, 55). As depicted on the right, this spread could create a series of loops that brings the enhancer and promoter closer together or could aid the binding of a surrogate activator near the promoter. Like the Mod(mdg4) proteins, GAGA factor is a group of BTB/POZ-containing trxB proteins that can also both aid activation and help insulate (44, 45). GAGA factor binds proximal to several promoters and potentiates activation of the *engrailed* promoter by a distal enhancer (46). We speculate, as shown with the promoter on the right, that when BTB/POZ proteins such as GAGA bind just upstream of a promoter, they anchor activators close to the promoter and thereby aid activation.

67.2 prepared by translation in vitro bound to beads containing mutant GST-Chip fusion proteins that lack the LID or SID (Fig. 5, lanes 7 and 10) but substantially less to beads containing GST-Chip mutants that lack the OID (Fig. 5, lanes 8 and 9). As shown previously, luciferase prepared by translation in vitro did not bind to any of the GST-Chip beads (55) (Fig. 5).

DISCUSSION

Mod(mdg4) proteins contribute to the insulator activity of Su(Hw). We found that certain loss-of-function alleles of *mod(mdg4)* reduce insulation by the Su(Hw) protein in the *cut* gene. This is evidence that *mod(mdg4)* products are not simply targets of Su(Hw) insulator activity but contribute to the insulator activity of Su(Hw). We also found that wild-type Mod(mdg4)-67.2, the major protein product of *mod(mdg4)*, interacted with a region of Su(Hw) that our laboratory has previously shown is required for insulation in vivo, but the truncated versions of the Mod(mdg4)-67.2 proteins produced by the viable *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6} alleles did not. This is consistent with the previous report that binding of Mod(mdg4) proteins to Su(Hw) binding sites on salivary gland polytene chromosomes is greatly reduced in *mod(mdg4)*^{u1} mutants (20). We also found that *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6} more strongly reduce insulator activity than do null alleles of *mod(mdg4)* and that this antimorphic nature of *mod(mdg4)*^{u1} may stem from the ability of the mutant protein to interact with wild-type Mod(mdg4)-67.2 protein. To explain these observations, we propose a model in which a multimer of Mod(mdg4)-

67.2 interacts with more than one Su(Hw) molecule to form the active insulator complex, and the truncated Mod(mdg4)-67.2 proteins produced by *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6} destabilize this complex (Fig. 6A).

An alternative explanation for why *mod(mdg4)*^{u1} has a stronger effect on insulation than does deletion of *mod(mdg4)* is that Mod(mdg4)-67.2 may support both activation of *cut* and insulation by Su(Hw). In this case, *mod(mdg4)*^{u1} would suppress the *cut*^K mutant phenotype more strongly than a null allele because the truncated Mod(mdg4)-67.2^{u1} protein can still activate *cut* but does not contribute to insulator activity, while a null allele would simultaneously reduce both insulation and activation. Our data suggest, however, that *mod(mdg4)* does not normally regulate *cut* in the absence of a gypsy insertion. Mutations in most genes known to regulate *cut* dominantly enhance the weak *cut* wing phenotype of *cut*^{53d} mutants (41, 48), but none of the several mutant *mod(mdg4)* alleles tested had an effect on *cut*^{53d}.

Interactions between Mod(mdg4)-67.2 and Su(Hw) are not required for the critical functions of Mod(mdg4)-67.2 and Su(Hw). The available evidence supports the notion that the insulation region of Su(Hw) interacts with other proteins besides Mod(mdg4)-67.2. Although viable, females homozygous for most *su(Hw)* mutations are sterile because of a block in oogenesis (36). Our laboratory has previously reported that the insulation region of Su(Hw) shown here to interact with Mod(mdg4)-67.2 is required for female fertility (35). Females homozygous for *mod(mdg4)*^{u1} are fertile, indicating that interactions between the insulation region of Su(Hw) and

Mod(mdg4)-67.2 are not necessary to support oogenesis. This raises the possibility that the insulation region of Su(Hw) interacts with other proteins besides Mod(mdg4)-67.2. Indeed, the viability of both *su(Hw)* and *mod(mdg4)^{u1}* mutants indicates that the interactions between Su(Hw) and Mod(mdg4)-67.2 are also not required for any of the essential functions of *mod(mdg4)*.

Is Chip a target of the Su(Hw)-Mod(mdg4)-67.2 insulator?

In this study we observed a direct *in vitro* interaction between Mod(mdg4)-67.2 and the Chip protein. Our laboratory has previously shown by genetic experiments that Chip and the Su(Hw) insulator protein are specifically antagonistic to each other *in vivo* (41). It was suggested, therefore, that Chip may act between enhancers and promoters to aid communication. Consistent with this idea, it was found that Chip is widely expressed and potentiates activation by diverse enhancers in several genes (41, 42).

Our laboratory has proposed a model in which Chip aids enhancer-promoter communication by assisting the binding of homeodomain proteins to several sites between enhancers and promoters (Fig. 6B) (12, 55). This model was based in part on *in vitro* interactions between Chip and diverse homeodomain proteins and the positive effects that Chip has on Bicoid homeodomain protein activity *in vivo* (55). It was also motivated by the *in vivo* binding of several homeodomain proteins to many sites between enhancers and promoters (6). The domain of Chip that interacts with homeodomain proteins also interacts with the zinc finger region of Su(Hw), consistent with the notion that the Chip-homeodomain interaction is a direct target of Su(Hw) (55). The *in vitro* interactions between Chip and the Mod(mdg4)-67.2 protein reported here suggest that Chip is a direct target of both proteins in the Su(Hw)-Mod(mdg4)-67.2 insulator complex (Fig. 6B).

In our experiments the N-terminal region of Mod(mdg4)-67.2 interacted with Chip, while the C-terminal portion interacted with Su(Hw). Both Chip and Mod(mdg4)-67.2 appear to form multimers *in vitro*. These observations suggested that it might be possible to form higher-order *in vitro* complexes containing Chip, Mod(mdg4)-57.2, and Su(Hw). Our preliminary attempts, however, to form such complexes by simultaneously binding Mod(mdg4)-67.2 and Su(Hw) to GST-Chip beads were unsuccessful. It appeared that in the presence of both Su(Hw) and Mod(mdg4)-67.2, Chip preferred to interact with Su(Hw). Because Su(Hw) and Mod(mdg4)-67.2 both interact with the same region of Chip, it is possible that they compete with each other. It is also feasible that interactions between Chip and Mod(mdg4)-67.2 are transitory in the context of the complete insulator protein complex.

The Mod(mdg4)-67.2 and GAGA BTB/POZ proteins have both insulator and activator activities. The evidence that Mod(mdg4)-67.2 is an active component of the gypsy insulator that blocks gene activation appears at first glance to be contradictory to the evidence indicating that the *mod(mdg4)* gene is a member of the *trxG* of genes that activate genes in the bithorax complex (9, 20). Another *trxG* protein, however, also appears to have insulator activity. The GAGA factor encoded by the *Trithorax-like* (*Trl*) gene is similar to Mod(mdg4)-67.2 in that it contains a BTB/POZ motif at the N terminus, self-interacts, and supports activation of the bithorax complex (14, 15, 32, 47, 52). GAGA factor is also required for enhancer

blocking by the insulator associated with the *even-skipped* (*eve*) promoter (45). This insulator activity requires GAGA binding sites just proximal to the transcription start site and is diminished by *Trl* mutations. Potential GAGA binding sites are found just proximal to many promoters in *Drosophila*, including sequences associated with insulator activity in the $\alpha 1$ *tubulin* gene promoter (44).

The GAGA-dependent insulator just proximal to the *eve* promoter does not prevent activation of the *eve* promoter by upstream enhancers even though it is positioned between them. Indeed, GAGA binding sites just proximal to the *engrailed* gene promoter potentiate activation by an upstream enhancer (46). To resolve the paradoxical insulator and activator activities of the GAGA and Mod(mdg4)-67.2 BTB/POZ proteins, therefore, we theorize that the function of promoter-proximal insulators is to aid activation of the promoters that contain them by helping to capture and anchor distal activator or facilitator proteins near the promoter (Fig. 6B). If so, it is feasible that the Mod(mdg4)-67.2 protein has a promoter-anchoring function in the bithorax complex, but when bound to Su(Hw), it anchors activator or facilitator proteins far from the promoter, thereby preventing activation.

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