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The Mod(mdg4) Component of the Su(Hw) Insulator Inserted in the *P* Transposon Can Repress Its Mobility in *Drosophila melanogaster*

Marina Karakozova, Ekaterina Savitskaya, Larisa Melnikova, Aleksandr Parshikov and Pavel Georgiev[1]

Department of the Control of Genetic Processes, Institute of Gene Biology, Russian Academy of Sciences, Moscow 119334, Russia

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ABSTRACT

Transposable element *P* of *Drosophila melanogaster* is one of the best-characterized eukaryotic transposons. Successful transposition requires the interaction between transposase complexes at both termini of the *P* element. Here we found that insertion of one or two copies of the Su(Hw) insulator in the *P* transposon reduces the frequency of its transposition. Inactivation of a Mod(mdg4) component of the Su(Hw) insulator suppresses the insulator effect. Thus, the Su(Hw) insulator can modulate interactions between transposase complexes bound to the ends of the *P* transposon in germ cells.

"INSULATORS" is the name given to a class of DNA sequence elements that have properties consistent with a role in limiting enhancer activity (Geyer and Clark 2002; West *et al.* 2002). In Drosophila, there is a well-characterized insulator within the 5′-untranslated region of the *gypsy* retrotransposon (Holdridge and Dorsett 1991; Geyer and Corces 1992; Roseman *et al.* 1993; Cai and Levine 1995, 1997). The *gypsy* insulator consists of reiterated binding sites for the Su(Hw) protein (Spana *et al.* 1988; Mazo *et al.* 1989). Genetic and molecular approaches have been used to identify and characterize two protein components of the Su(Hw) insulator. One of them, encoded by the *suppressor of Hairy wing* [*su(Hw)*] gene, is a zinc-finger protein that binds to insulator DNA (Dorsett 1990; Spana and Corces 1990). Modifier of mdg4 [Mod(mdg4)] is the second protein component of the *gypsy* insulator complex (Gerasimova *et al.* 1995; Georgiev and Kozycina 1996). The *mod(mdg4)* gene, also known as *E(var)3-93D*, encodes a large set of individual protein isoforms with specific functions in regulating the chromatin structure of different genes (Gerasimova *et al.* 1995; Buchner *et al.* 2000). Biochemical studies with purified Su(Hw) and Mod(mdg4) proteins indicate that one isoform, Mod(mdg4)-67.2, interacts with the enhancer-blocking domain of the Su(Hw) protein (Gause *et al.* 2001; Ghosh *et al.* 2001). All Mod(mdg4) isoforms share an amino-terminal 402-residue domain that includes a BTB/POZ motif (Gerasimova *et al.* 1995; Buchner *et al.* 2000). It was shown (Gause *et al.* 2001; Ghosh *et al.* 2001) that the BTB domain of Mod(mdg4)-67.2 is involved in self-interactions, whereas the C-terminal region of the protein is involved in interactions with the Su(Hw) protein. The interaction between BTB domains of Mod(mdg4) is postulated to be important for the insulator function (Gause *et al.* 2001; Ghosh *et al.* 2001). The homozygously viable *mod(mdg4)*^{u1} mutation is a *Stalker* transposon insertion into the "C-terminal" exon unique to the Mod(mdg4)-67.2 mRNA (Gerasimova *et al.* 1995; Buchner *et al.* 2000). Similarly to *mod(mdg4)*^{u1}, the *mod(mdg4)*^{T6} allele is homozygously viable and produces a Mod(mdg4)-67.2 protein truncated short of the C terminus (Mongelard *et al.* 2002). The truncated versions of the Mod(mdg4)-67.2 protein produced by either mutation do not interact with Su(Hw) (Gause *et al.* 2001; Ghosh *et al.* 2001).

The prevalent structural model suggests that boundary elements or insulators subdivide eukaryotic chromosomes into functionally and structurally autonomous domains (Gerasimova and Corces 2001; Geyer and Clark 2002; West *et al.* 2002; Kuhn and Geyer 2003). The insulators determine the limits of higher-order "looped" chromatin domains by interacting with each other or/and with some other nuclear structure. Consistent with the idea that pairing between Su(Hw) insulators is responsible for the boundary activity, duplication of the Su(Hw) insulator neutralized the enhancer-blocking activity and even strengthened activation by the enhancer (Cai and Shen 2001; Muravyova *et al.* 2001). At the same time, two Su(Hw) insulators flanking either the enhancer or the promoter are still capable of blocking enhancer-promoter communication. These results suggest that interaction between the Su(Hw) insulators may facilitate activation by bringing regulatory elements together or block the interaction between them. The main prediction of this model is that pairing between insulators can block interactions between all kinds of proteins.

[1]*Corresponding author:* Institute of Gene Biology, Russian Academy of Sciences, 34/5 Vavilov St., Moscow 119334, Russia.
E-mail: georgiev_p@mail.ru

Here we examined whether insertion of one or two copies of the Su(Hw) insulator in the *P* transposon would suppress the frequency of transposition, which depends on the efficiency of interaction between transposase complexes bound to *P* ends. Full-length *P* elements are 2.9 kb and encode an 87-kD transposase protein (O'Hare and Rubin 1983). *P*-element transposition requires ~150 bp of sequence at each end of the *P* element (Kaufman *et al.* 1989). These sequences include 31-bp terminal inverted repeats, internal transposase-binding sites, and internal 11-bp inverted repeats (Kaufman *et al.* 1989; Mullins *et al.* 1989). During *P*-element transposition, transposase catalyzes the cleavage and strand-transfer steps of the transposition reaction (Kaufman and Rio 1992; Beall and Rio 1997). The *P*-element transposase requires both 5′ and 3′ termini of the *P* element for efficient DNA cleavage, suggesting that a synaptic complex forms on the *P*-element termini prior to cleavage (Beall and Rio 1997, 1998). Insertion of one or two copies of the Su(Hw) insulator in the *P* transposon considerably diminishes the frequency of its transpositions. We suggest that the Su(Hw) insulator can interfere with proper interaction between the protein complexes involved in the *P* transposition.

MATERIALS AND METHODS

Drosophila strains, transformation, and genetic crosses: All flies were maintained at 25° on a standard yeast medium. The line bearing the *mod(mdg4)^{T6}* mutation in the *mod(mdg4)* gene was obtained from D. Dorsett. The structure and origin of the *mod(mdg4)^{u1}* and *mod(mdg4)^{T6}* mutations are described by Gerasimova *et al.* (1995) and Mongelard *et al.* (2002). All other mutant alleles and chromosomes used in this work and all balancer chromosomes are described in Lindsley and Zimm (1992).The transposon constructs, together with a transposase source, P25.7wc (Kares and Rubin 1984), were injected into *y^{-} ac^{-} w^{1118}* preblastoderm embryos. Transformed lines [*y^{-} ac^{-} w^{1118} P(y^{+}; w^{+})*] carrying a transposon insertion at the X chromosome were examined by Southern blot hybridization to check for transposon integrity and copy number. Lines with the homozygous *mod(mdg4)^{u1}* or *mod(mdg4)^{T6}* mutation were obtained as described in Georgiev and Kozycina (1996). The lines with Su(Hw) excisions were obtained by crossing the flies bearing the transposons with the line *yw^{i}; Cyo, P[w^{+},cre]/Sco* expressing Cre recombinase (Siegal and Hartl 2000). All excisions were confirmed by PCR analysis.

As the source of *P* transposase (Robertson *et al.* 1988), we used the *w^{1}*, *P[ry^{+}Δ2-3]*(99B), *Sb e/TM1, e* strain. The transposase gene *P[ry^{+}Δ2-3]*(99B) was abbreviated as *Δ2-3*. The frequency of transposition from the X chromosome to autosomes was examined in the F_1 progeny of *y^{-} ac^{-} w^{1118} P(y^{+}; w^{+}); Δ2-3 Sb/+* males individually crossed to five to six *y w/y w* or *y^{1w1} sc^{D1} w/y^{1w1} sc^{D1} w* females. The frequencies of transposition were calculated as the number of *y w/Y; P(y^{+}; w^{+})* or *y^{1w1} sc^{D1} w/Y; P(y^{+}; w^{+})* males (with pigmented eyes and cuticle) divided by the total number of scored *y w/Y* or *y^{1w1} sc^{D1} w/Y* males (with yellow cuticle and white eyes). The frequencies of nondisjunction [appearance of *y^{-} ac^{-} w^{1118} P(y^{+}; w^{+})* males in the F_1 progeny] were in the range of 3–12%.The *y^{2} sc^{D1} w; P[ry^{+}Δ2-3]*(99B) *mod(mdg4)^{u1} Sb/TM6* line was constructed to examine the *P* transpositions on the *mod(mdg4)^{u1}* background. The frequency of transpositions on the *mod(mdg4)^{u1}* background was examined in the progeny of *y^{-} ac^{-} w^{1118} P(y^{+}; w^{+}); Δ2-3 Sb mod(mdg4)^{u1}/mod(mdg4)^{u1}* males individually crossed to five to six *y w/y w* or *y^{1w1} sc^{D1} w/y^{1w1} sc^{D1} w* females.

The dominant effect of the *mod(mdg4)^{u1}* mutation was examined in the progeny of the cross of *y^{-} ac^{-} w^{1118} P(y^{+}; w^{+}); Δ2-3 Sb mod(mdg4)^{u1}/mod(mdg4)$^{+}$* males with *y w* females. The frequency of transposition in the *mod(mdg4)^{u1}/mod(mdg4)^{T6} trans*-heterozygote was examined in the progeny of *y^{-} ac^{-} w^{1118} P(y^{+}; w^{+}); Δ2-3 Sb mod(mdg4)^{u1}/mod(mdg4)^{T6}* males crossed to *y w* females. Details of the crosses used for genetic analysis and for excision of the Su(Hw) insulator are available upon request.

Transgenic constructs: The ESY and ES(−893)YSW transgenic lines were obtained previously and described in Muravyova *et al.* (2001). The WS transgenic lines were obtained from A. Golovnin.

The 8-kb fragment containing the *yellow* gene was kindly provided by P. Geyer. The 3-kb *Sal*I-*Bam*HI fragment containing the *yellow* regulatory region (yr) was subcloned into *Bam*HI + *Xho*I-digested pGEM7 (yr plasmid). The 5-kb *Bam*HI-*Bgl*II fragment containing the coding region (yc) was subcloned into CaSpeR3 (C3-yc). The *yellow* regulatory region with the Su(Hw) inserted at −893 bp (yr-su) was described in Muravyova *et al.* (2001).

The 430-bp *gypsy* sequence containing the Su(Hw)-binding region was PCR amplified from the *gypsy* retrotransposon. After sequencing to confirm its identity, the product was inserted between two loxP sites [lox(su)]. The lox(su) fragment was blunt ligated into a CaSpeR2 vector treated with *Bgl*II [C2-lox(su)]. The 5-kb *Bam*HI-*Bgl*II fragment containing the coding region (yc) was subcloned into CaSpeR2-lox(su), (C2-lox(su)-yc, or CaSpeR3 (C3-yc). The modified Caspew15 vector (Caspew15-su) containing the Su(Hw) insertion from the 3′ side of the *mini-white* gene was obtained from A. Golovnin.

For (S)WS, the lox(su) fragment was ligated into Caspew15-su treated with *Xba*I; for ESY(S)W, the yr-su fragment was ligated into C2-lox(su)-yc treated with *Xba*I and *Bam*HI; and for E(S)YW, the lox(su) fragment was ligated into yr treated with *Eco*47III at position −893 [yr-lox(su)]. The yr-lox(su) fragment was ligated into C3-yc treated with *Xba*I and *Bam*HI. For EY(S)W, the yr fragment was ligated into C2-lox(su)-yc treated with *Xba*I and *Bam*HI.

RESULTS

Insertion of one or two copies of the Su(Hw) insulator in the *P* transposon suppresses the frequency of transposition: In the first series of experiments, we used previously described transgenic lines carrying *P* transposons with the *yellow* and *white* marker genes or with only the *white* gene (Figure 1). The *yellow* gene is required for dark pigmentation of Drosophila larval and adult cuticle and its derivatives, while the *white* gene is required for eye pigmentation. In the ESY construct, the Su(Hw) insulator was inserted at −893 bp relative to the *yellow* transcription start site. In the ES(−893)YS construct, the *yellow* gene is flanked by the Su(Hw) insulators, one at position −893 and the other downstream of the *yellow* gene. The WS has an insertion of the Su(Hw) insulator from the 3′ end of the *white* gene. The *P* element was mobilized in three ESY, four ES(−893)YS, and two WS lines, which had the transposon insertion

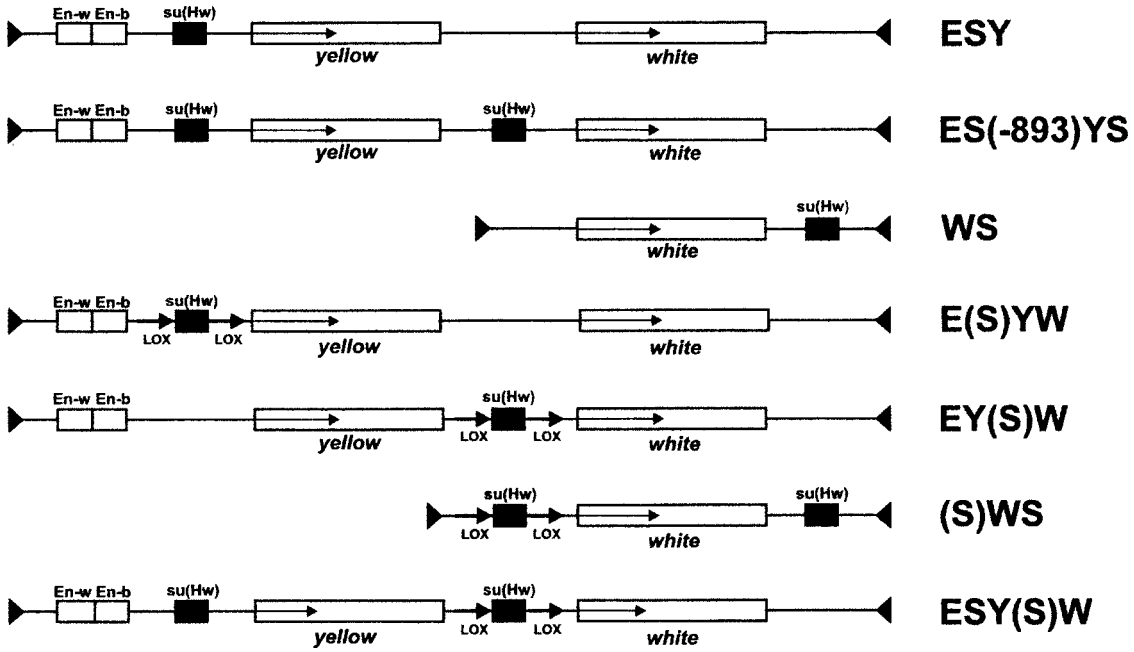

FIGURE 1.—Transposon constructs used to test the suppression of *P* transpositions. The maps of the constructs (not drawn to scale) show the *yellow* wing and body enhancers (En-w and En-b, respectively) as open boxes. The Su(Hw) insulator is shown as a solid box, and the *yellow* and *white* genes as open boxes with arrows indicating the direction of transcription. The thick arrows marked LOX represent the target sites of the Cre recombinase.

on the X chromosome. As a result (Table 1), we found that in seven tested transgenic lines carrying both *yellow* and *white* genes, the frequency of *P* transposition from the X chromosome to autosomes was in the range of 0.8–1.3%. Two transgenic lines with the WS transposon, containing only sequences of the *white* gene, had even lower transposition frequencies: 0.05 and 0.4%.

As Mod(mdg4)-67.2 is an important component of the Su(Hw) insulator, we examined the frequency of transposition on the $mod(mdg4)^{u1}$ mutant background that produces a nonfunctional version of the Mod(mdg4)-67.2 protein. On the $mod(mdg4)^{u1}$ background, the transposition frequency was elevated 3- to 7-fold in most of the transgenic lines (Table 1). The striking effect of the $mod(mdg4)^{u1}$ mutation was found in the case of the WS(1) transgenic line (Table 1), which showed a 100-fold increase in the *P* transpositions on the $mod(mdg4)^{u1}$ background. The results obtained suggest that the presence of one or two copies of Su(Hw) in transposons reduces the frequency of transposition, while inactivation of the Mod(mdg4) component of the Su(Hw) insulator reactivates transpositions.

Mod(mdg4)-67.2 is essential for suppression of *P* transpositions by the Su(Hw) insulator: To find out if other components of the Su(Hw) insulator in addition to Mod(mdg4) are required for blocking the transpositions, we compared the frequency of transposition of the *P* transposon inserted at the same site before and after deletion of the Su(Hw) insulator. The Su(Hw) insulator was flanked by Cre recognition target (LOX) sites to permit its excision from transgenic flies by crossing the latter with flies expressing Cre recombinase (SIEGAL and HARTL 2000). The Su(Hw) insulator flanked by LOX sites was inserted either at −893 bp, E(S)YW, or between the *yellow* and *white* genes, EY(S)W (Figure 1). Three EY(S)W and three E(S)YW transgenic lines that contain a single *P* transposon on the X chromosome were established. The frequency of transposition was examined in these transgenic lines and their derivatives were generated by deletion of Su(Hw) on the wild-type or the $mod(mdg4)^{u1}$ mutant background (Table 2). Deletion of the Su(Hw) insulator significantly increased the frequency of transposition only in the former case. The inability of the $mod(mdg4)^{u1}$ mutation to affect *P* transpositions when the Su(Hw) insulator has been deleted confirms that Mod(mdg4)-67.2 operates by interacting with the Su(Hw) insulator. The quite similar levels of *P* transposition on the $mod(mdg4)^{u1}$ background and after deletion of the Su(Hw) insulator suggest that mainly the Mod(mdg4)-67.2 component of the Su(Hw) insulator is required for suppression of *P* transposition.

As the $mod(mdg4)^{u1}$ mutation was obtained in a highly mutable line (GEORGIEV and GERASIMOVA 1989), it is possible that, in addition to $mod(mdg4)^{u1}$, another mutation that is responsible for the repression of the *P* transpositions has arisen. To eliminate the role of genetic background, we first examined the frequency of *P* transposition in three E(S)YW transgenic lines on the $mod(mdg4)^{u1}/mod(mdg4)^{+}$ background. The comparably low frequency of *P* transposition on the wild-type and the $mod(mdg4)^{u1}/mod(mdg4)^{+}$ backgrounds suggests that the effect of $mod(mdg4)^{u1}$ is recessive (Table 2). Next, we

TABLE 1

Role of Mod(mdg4) in repression of transposition of the *P* constructs containing one or two copies of the Su(Hw) insulator

	mod(mdg4)$^{+}$/*mod(mdg4)*$^{+}$		*mod(mdg4)*^{u1}/*mod(mdg4)*^{u1}		
Transposon	Total (Nu)	*Q* (%)	Total (Nu)	*Q* (%)	*P*
ESY					
(1)	1240 (16)	1.3	3360 (127)	3.8	<0.0001
(2)	3620 (47)	1.3	2310 (126)	5.5	<0.0001
(3)	4940 (41)	0.8	4240 (245)	5.8	<0.0001
ES(−893)YS					
(1)	2120 (21)	1.0	970 (68)	7.0	<0.0001
(2)	2000 (14)	0.7	2750 (136)	4.9	<0.0001
(3)	1930 (21)	1.1	730 (47)	6.4	<0.0001
(4)	4190 (39)	0.9	890 (38)	4.3	<0.0001
WS					
(1)	1960 (1)	0.05	1120 (65)	5.8	<0.0001
(2)	1090 (8)	0.4	1320 (54)	4.1	<0.0001

Total, the total number of scored males; Nu, the total number of males with a transposon insertion at an autosome; *Q*, the average frequency of transposition. The probability value (*P*) was determined with a 2 × 2 contingency test (the Statistica 6.0 program, StatSoft, 1984–2001) comparing frequencies of *P* transposition on the *mod(mdg4)*$^{+}$ and *mod(mdg4)*^{u1} backgrounds.

examined *P* transpositions in the same transgenic lines on the *mod(mdg4)*^{T6}/*mod(mdg4)*^{u1} background, where *mod(mdg4)*^{T6} produced a truncated version of the Mod(mdg4)-67.2 protein similar to that produced by the *mod(mdg4)*^{u1} mutation (Mongelard *et al.* 2002). The *mod(mdg4)*^{T6}/*mod(mdg4)*^{u1} *trans*-heterozygote restored transpositions in E(S)YW transgenic lines similar to those restored by the *mod(mdg4)*^{u1} homozygotes (Table 2). Because *mod(mdg4)*^{u1} and *mod(mdg4)*^{T6} have different origins (Gerasimova *et al.* 1995), we conclude that inactivation of Mod(mdg4) rather than another unidentified mutation in the *mod(mdg4)*^{u1} line is responsible for the effect of the *mod(mdg4)*^{u1} mutation.

The pairing between two Su(Hw) insulators does not neutralize the repression of transpositions: We initially observed (Table 1) that insertion of one or two copies of the Su(Hw) insulator into the *P* transposon had a similar effect on its transpositions. As pairing between two Su(Hw) insulators neutralizes each other's enhancer-blocking activity (Gause *et al.* 1998; Cai and Shen 2001; Muravyova *et al.* 2001; Melnikova *et al.* 2002; Kuhn *et al.* 2003), it is possible to explain the lack of significant difference in the transposition frequency between transposons with either one or two Su(Hw) insulators by location of the ESY and ES(−893)YS insertions in different regions of the X chromosome.

To further examine whether the pairing between the Su(Hw) insulators can inhibit the repression of *P* transpositions, one of the Su(Hw) insulators in the (S)WS and ESY(S)W constructs was flanked by LOX sites (Figure 1). Two (S)WS and three ESY(S)W transgenic lines bearing a single transposon insertion at the X chromosome were examined before and after excision of the Su(Hw) insulator (Table 2). In only one [(S)WS (2)] line, the frequency of transposition was significantly reduced after deletion of the Su(Hw) insulator. In four other transgenic lines, the frequency of transposition was approximately the same for transposons with one and two copies of the Su(Hw) insulator. As in the previous experiments, the frequency of *P* transposition was markedly elevated on the *mod(mdg4)*^{u1} mutant background, confirming that the Su(Hw) insulator is responsible for the repression of transpositions in all transgenic lines tested. Thus, the pairing between two Su(Hw) insulators inserted between the ends of the *P* transposon does not appreciably neutralize the blocking of its transposition.

DISCUSSION

The results obtained show that the Su(Hw) insulator affects the *P* transpositions in germ cells. It is most likely that the Su(Hw) insulator interferes with the interaction between protein complexes bound to the ends of the *P* transposon. Previous studies have shown that the loss of Mod(mdg4)-67.2 attenuates enhancer blocking by the Su(Hw) insulator at some genes but not at others (Geyer and Clark 2002; Kuhn and Geyer 2003). In several cases, the absence of Mod(mdg4)-67.2 converts the Su(Hw) insulator into a repressor (Georgiev and Kozycina 1996; Cai and Levine 1997; Wei and Brennan 2001). These data suggest that the Mod(mdg4)-67.2 isoform is involved in only some Su(Hw) insulator functions. In contrast, Mod(mdg4)-67.2 fulfills the main

TABLE 2

Correlation between the frequency of transposition and the number of copies of the Su(Hw) insulator in the *P* constructs

	ΔSu(Hw)				
Transposon	Total (Nu)	*Q* (%)	Total (Nu)	*Q* (%)	*P*
E(S)YW (1)	1820 (8)	0.4	4046 (71)	1.8	<0.0001
mod(mdg4)^{u1}/mod(mdg4)^{u1}	2040 (47)	2.3	3940 (87)	2.2	0.81
mod(mdg4)^{u1}/mod(mdg4)$^{+}$	2900 (17)	0.6	1230 (38)	3.1	<0.0001
mod(mdg4)^{u1}/mod(mdg4)^{T6}	3850 (123)	3.2	1910 (55)	2.9	0.52
E(S)YW (2)	3130 (14)	0.4	2760 (151)	5.5	<0.0001
mod(mdg4)^{u1}/mod(mdg4)^{u1}	2390 (117)	4.9	1890 (70)	3.7	0.06
mod(mdg4)^{u1}/mod(mdg4)$^{+}$	2440 (19)	0.8	940 (44)	4.7	<0.0001
mod(mdg4)^{u1}/mod(mdg4)^{T6}	1230 (51)	4.1	1220 (46)	3.8	0.63
E(S)YW (3)	2200 (2)	0.1	1990 (123)	6.2	<0.0001
mod(mdg4)^{u1}/mod(mdg4)^{u1}	1540 (75)	4.9	1400 (78)	5.6	0.42
mod(mdg4)^{u1}/mod(mdg4)$^{+}$	4300 (13)	0.3	3100 (152)	4.9	<0.0001
mod(mdg4)^{u1}/mod(mdg4)^{T6}	3820 (225)	5.9	1700 (85)	5.0	0.21
EY(S)W (1)	2560 (9)	0.4	1876 (19)	1.0	<0.01
mod(mdg4)^{u1}/mod(mdg4)^{u1}	ND	ND	2055 (60)	2.9	ND
EY(S)W (2)	4200 (21)	0.5	3090 (59)	1.9	<0.0001
mod(mdg4)^{u1}/mod(mdg4)^{u1}	1900 (31)	1.6	2860 (51)	1.8	0.69
EY(S)W (3)	2050 (8)	0.4	2410 (66)	2.7	<0.0001
mod(mdg4)^{u1}/mod(mdg4)^{u1}	2010 (54)	2.7	2790 (86)	3.1	0.42
(S)WS (1)	3730 (89)	2.4	4210 (103)	2.4	0.86
mod(mdg4)^{u1}/mod(mdg4)^{u1}	2770 (199)	7.2	4210 (616)	14.6	<0.0001
(S)WS (2)	2980 (74)	2.5	3050 (19)	0.6	<0.0001
mod(mdg4)^{u1}/mod(mdg4)^{u1}	1870 (97)	5.2	4460 (346)	7.8	<0.001
ESY(S)W (1)	4720 (33)	0.7	2940 (26)	0.9	0.61
mod(mdg4)^{u1}/mod(mdg4)^{u1}	1680 (122)	7.3	1770 (118)	6.7	0.49
ESY(S)W (2)	4130 (23)	0.6	2640 (7)	0.3	0.08
mod(mdg4)^{u1}/mod(mdg4)^{u1}	1590 (93)	5.8	1380 (91)	6.6	0.40
ESY(S)W (3)	3800 (15)	0.4	2870 (9)	0.3	0.58
mod(mdg4)^{u1}/mod(mdg4)^{u1}	1170 (46)	3.9	1230 (62)	5.0	0.19

Designations are as in Table 1. ND, not determined. The probability value (*P*) was determined with a 2 × 2 contingency test comparing frequencies of *P* transpositions before and after excision of the Su(Hw) insulator.

role in the repression of *P* transpositions. According to the accepted structural models (Gerasimova and Corces 2001; West *et al.* 2002), the putative interaction between BTB domains of Mod(mdg4)-67.2 is required for generation by the Su(Hw) insulators of looped chromatin domains that preclude interactions between regulatory elements residing in distinct domains. From this viewpoint, a plausible explanation of the inability of paired, closely spaced Su(Hw) insulators to block enhancer-promoter communication is that they would preferentially interact with each other. This local interaction precludes the paired Su(Hw) insulators from interacting with other Su(Hw) insulators, which is necessary to separate the enhancer from the promoter.

Here we show that, in contrast to the neutralization of the enhancer blocking, pairing between two Su(Hw) insulators located between the ends of the *P* transposon does not significantly neutralize the repression of *P* transpositions. Thus, it is most likely that Mod(mdg4)-67.2 directly blocks the interaction between protein complexes bound to the ends of the *P* element. As shown for the BTB-containing PZLF and Bcl6 proteins, a charge pocket, formed by apposition of the two monomers, represents a molecular structure involved in

recruitment of transcriptional repression complexes (Melnick *et al.* 2002). It is possible that the Mod(mdg4)-67.2 dimers either directly interact with transposase complexes or recruit other proteins that interfere with the formation of the transposase complexes at the ends of the *P* transposon. Alternatively, Mod(mdg4)-67.2 might interact with proteins bound to the promoter region of the *P* transposon. Since the transposase binds to the site overlapping the promoter (Kaufman *et al.* 1989; Mullins *et al.* 1989), the assumed interaction between the promoter complex and Mod(mdg4)-67.2 might interfere with the transposase binding to the *P* transposon. Further molecular study is required for understanding the molecular basis of the described phenomenon.

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