Interaction Between Mutations in the *suppressor of Hairy wing* and *modifier of mdg4* Genes of *Drosophila melanogaster* Affecting the Phenotype of *gypsy*-Induced Mutations

Pavel Georgiev and Marina Kozycina

Institute of Gene Biology and Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow 117334, Russia

Manuscript received June 9, 1995
Accepted for publication October 7, 1995

ABSTRACT

The suppressor of Hairy-wing [su(Hw)] protein mediates the mutagenic effect of the *gypsy* retrotransposon by repressing the function of transcriptional enhancers located distally from the promoter with respect to the position of the su(Hw)-binding region. Mutations in a second gene, *modifier of mdg4*, also affect the *gypsy*-induced phenotype. Two major effects of the $mod(mdg4)^{1u1}$ mutation can be distinguished: the interference with insulation by the su(Hw)-binding region and direct inhibition of gene expression that is not dependent on the su(Hw)-binding region position. The $mod(mdg4)^{1u1}$ mutation partially suppresses ct^6, sc^{D1} and Hw^1 mutations, possibly by interfering with the insulation effect of the su(Hw)-binding region. An example of the second effect of $mod(mdg4)^{1u1}$ is a complete inactivation of *yellow* expression in combination with the y^2 allele. Phenotypic analyses of flies with combinations of $mod(mdg4)^{1u1}$ and different *su(Hw)* mutations, or with constructions carrying deletions of the acidic domains of the su(Hw) protein, suggest that the carboxy-terminal acidic domain is important for direct inhibition of *yellow* transcription in bristles, while the amino-terminal acidic domain is more essential for insulation.

INSERTION of the *gypsy* (*mdg4*) retrotransposon into various *Drosophila melanogaster* genes results in mutations with phenotypes that can be reversed by second site mutations in *the suppressor of Hairy-wing* [*su(Hw)*] gene (MODOLELL *et al.* 1983). This finding suggests a direct involvement of the su(Hw) protein in the generation of mutant phenotypes by *gypsy*, because the lack of a functional su(Hw) protein results in a reversion of the *gypsy*-induced phenotype. su(Hw) is a zinc finger protein that binds to a specific sequence, similar to the octamer motif, located in the 5′-transcribed untranslated region of *gypsy* (SPANA *et al.* 1988; MAZO *et al.* 1989; DORSETT 1990; SPANA and CORCES 1990). The necessary and sufficient requirement of the su(Hw) protein for *gypsy* mutagenesis has been demonstrated in the case of *hsp70*, *yellow* and *cut* alleles induced by this retrotransposon (HOLDRIDGE and DORSETT 1991; JACK *et al.* 1991; GEYER and CORCES 1992; SMITH and CORCES 1992). The temporal and spatial expression of the last two genes is controlled by tissue-specific transcriptional enhancers located in the intron and/or in the 5′ region of the respective locus (GEYER and CORCES 1987; LIU *et al.* 1991). In both cases, the insertion of the *gypsy* element interferes with the expression of the gene in those tissues regulated by enhancers located distally from the *gypsy* insertion site with respect to the promoter (JACK *et al.* 1991; GEYER and CORCES 1992). In the case of *yellow*, the phenotypic effect of *gypsy* can be reproduced when the su(Hw)-binding sequences are present in the original *gypsy* insertion site, suggesting that the su(Hw) protein alone is responsible for the induction of a mutant phenotype (SPANA and CORCES 1990; GEYER and CORCES 1992). This negative effect of *su(Hw)* on transcription is not enhancer specific, because insertion of the su(Hw)-binding site in different regions of the *yellow* gene is able to inhibit the function of any enhancer located distally from the su(Hw)-binding region with respect to the *yellow* promoter (GEYER and CORCES 1992).

Several structural domains of the su(Hw) protein have important roles in eliciting *gypsy*-induced mutant phenotypes (HARRISON *et al.* 1993). Two acidic regions are located in the amino- and carboxy-terminal ends of the su(Hw) protein. Deletion of any of these acidic domains has no major consequence on the mutagenic effect of the su(Hw) protein. Nevertheless, a deletion of both regions simultaneously renders the protein nonfunctional, suggesting that the acidic domains have a functional role, but each one can substitute the other in mediating the *yellow* mutant phenotype induced by an insertion of the *gypsy* element. Also, a region of su(Hw) homologous to the leucine zipper motif is necessary for the negative effect of the su(Hw) protein on enhancer function (HARRISON *et al.* 1993).

A mutation in the *modifier of mdg4* gene has been isolated from a strain in which the *Stalker* transposable element was mobilized at high frequency. The *mod-*

Corresponding author: Pavel Georgiev, Institute of Gene Biology, Russian Academy of Sciences, 34/5 Vavilov St., Moscow 117334, Russia. E-mail: georg&biogen.msk.su

(mdg4)^{1u1} mutation modifies the phenotype of several *gypsy*-induced mutations (GEORGIEV and GERASIMOVA 1989) and the action of the mod(mdg4) protein is also realized through *gypsy* su(Hw)-binding sites. Two additional *mod(mdg4)* alleles have been obtained by EMS treatment, and they display the same phenotypic effect on *gypsy*-induced mutations as the original *mod(mdg4)^{1u1}* allele (T. I. GERASIMOVA and V. G. CORCES, personnal communication). All three *mod(mdg4)* mutations cause strong reduction of a 2.2-kb transcript encoded by the *mod(mdg4)* gene (T. I. GERASIMOVA and V. G. CORCES, personnal communication). Thus the *mod(mdg4)^{1u1}* mutation is a hypomorph associated with reduced amounts of the mod(mdg4) protein.

Here we have studied the effect of combinations of the *mod(mdg4)^{1u1}* mutation with different *su(Hw)* alleles on the phenotype of *gypsy*-induced mutations in the *yellow, scute, achaete* and *cut* genes. A number of *su(Hw)* mutations have been molecularly characterized and fly strains with several artificial constructions carrying deletions of certain parts of the *su(Hw)* gene are available (HARRISON *et al.* 1993), opening the possibility of analyzing the molecular mechanisms of the *su(Hw)* and *mod(mdg4)* functions in different systems using genetic approaches. We have obtained genetic evidence indicating that the *mod(mdg4)^{1u1}* mutation has two effects. First, *mod(mdg4)^{1u1}* reduces the insulating effect of the su(Hw)-binding region and second, it induces a direct inhibition of *yellow* gene expression. The domains of the su(Hw) protein involved in the interaction with *mod(mdg4)^{1u1}* have been examined.

MATERIALS AND METHODS

Stocks: Flies were cultured at 25° in standard Drosophila wheatmeal, yeast, sugar and agar medium. All crosses were performed in standard glass vials with 5–10 males and 10–15 females per vial.

Mutations in the *su(Hw)* gene used in these studies are listed in Table 1 and their structure is schematically presented in Figure 1. Using *XX/Y; T(2;3)Xa/D,* where *XX* is an abbreviation of the attached *X* chromosomes *C(1)RM, y f* and *Xa* is an abbreviation of the translocation *T(2;3) ap^{Xa}ap^{Xa},* several derivative strains with different *su(Hw)* mutations have been produced; these strains, *XX/Y; su(Hw)*/Xa,* where *su(Hw)** is any of a series of *su(Hw)* mutations described in Table 1. A strain of the genotype *y^{2}sc^{D1}ct^6; Df(3R)GC14/TM6 TbHu,* where *Df(3R)GC14* is a deletion covering the region where the *mod(mdg4)* gene is located, was also used in these studies.

Isolation of mutant strains: Several combinations of different *su(Hw)* mutations with *mod(mdg4)^{1u1}* were obtained using *Stubble* (*Sb,* 3–58.22) to ascertain the recombination between chromosomes with *su(Hw)* and *mod(mdg4)^{1u1}* mutations according to the following scheme: F_0 ♀ *XX/Y; Sb mod(mdg4)/mod(mdg4)* × ♂ *y^{2}sc^{1}ct^6/Y; su(Hw)/su;(Hw)* and F_1 ♀ *XX/Y; Sb mod(mdg4)/su(Hw)* × ♂ *y^{2}sc^{1}ct^6/Y; mod(mdg4)/mod(mdg4).*

Males were screened during the F_2 generation for reversion of the ct^6 phenotype [*mod(mdg4)/mod(mdg4)*] or for the absence of the *Sb* mutation [*su(Hw)/su(Hw)$^+$*], *i.e.,* ♂ *y^{2}sc^{1}ct^6/Y; su(Hw)mod(mdg4)/mod(mdg4).* Strains of the genotype *XX/Y/y^{2}sc^{1}ct^6; su(Hw)mod(mdg4)/Xa* were then isolated. The strain *y^{2}sc^{D1}ct^6; su(Hw)^2 Df(3R)GC14/TM6 TbHu* was obtained following a similar strategy.

Strains containing a *gypsy*-induced *y* mutation and homozygous for *mod(mdg4)^{1u1}* were obtained using *FM4/y$^+$sc^{1}Bx^2; mod(mdg4)/mod(mdg4);* F_1 ♀ *FM4/y$^+$sc^{1}Bx^2; mod(mdg4)/mod(mdg4)* × ♂ *y*/Y; mod(mdg4)/mod(mdg4),* F_2 ♀ *FM4/y*; mod(mdg4)/mod(mdg4)* × ♂ *y*/Y; mod(mdg4)/mod(mdg4),* F_3 ♀ *y*/y*; mod(mdg4)/mod(mdg4)* × ♂ *y*/Y; mod(mdg4)/mod(mdg4).*

To make compound strains with sex-linked *gypsy*-induced mutations, *mod(mdg4)^{1u1}* and *su(Hw)* alleles, the following crosses were performed. Males with a tested *gypsy*-induced mutation were mated to *XX* females carrying a *D* (*Drop*) mutation. After this step, crosses were carried out according to the following scheme: F_1 ♀ *XX/Y; su(Hw)mod(mdg4)/Xa* × ♂ *X*/Y; D/+,* F_2 ♀ *XX/Y; su(Hw)mod(mdg4)/Xa* × ♂ *X*/Y; su(Hw)mod(mdg4)/D,* and F_3 ♀ *XX/Y; su(Hw)mod(mdg4)/Xa* × ♂ *X*/Y; su(Hw)mod(mdg4)/su(Hw)mod(mdg4).*

The phenotype of *gypsy*-induced mutations was then analyzed in F_3 individuals.

Combinations of *mod(mdg4)^{1u1}* with different *su(Hw)* mutations and homozygous for *Hw^1* were obtained according to the following scheme: F_1 ♀ *y^{1}Hw^1/FM4; TM6B/D* × ♂ *y^{2}sc^{D1}ct^{6}v/Y; su(Hw)mod(mdg4)/Xa,* F_2 ♀ *y^{1}Hw^1/y^{2}sc^{D1}ct^{6}v; su(Hw)mod(mdg4)/TM6B* × ♂ *y^{1}Hw^1/Y; su(Hw)mod(mdg4)/TM6B,* and F_3 ♀ *y^{1}Hw^1/y^{1}Hw^1; su(Hw)mod(mdg4)/TM6B* × ♂ *y^{1}Hw^1/Y; su(Hw)mod(mdg4)/TM6B.*

Mutant phenotypes were then analyzed in homozygous females with an appropriate combination of mutations.

Transposition of a *P* transposon containing the *yellow* gene and *su(Hw)* binding region, *P(y)*, to new genomic positions: Only *P(y)* constructions located in the *X* chromosome were used for this purpose. After introduction of a transposase source, *P[ry+Δ2-3](99B)* (ROBERTSON *et al.* 1988) (abbreviated *Δ2-3*), strains with transpositions of *P(y)* to autosomes were obtained using the following strategy: F_1 ♀ *P(y)/FM4; D/+* × ♂ *y^{2}w/Y; Sb Δ2-3/TM3,* F_2 ♀ *y^{1}ac; mod(mdg4)/mod(mdg4)* × ♂ *P(y)/Y; D/Sb Δ2-3,* F_3 Selection of males *y^{1}ac/Y; D/mod(mdg4)* with *P(y),* F_{4a} ♀ *XX/Y; mod(mdg4)/mod(mdg4)* × ♂ *y^{1}ac/Y; D/mod(mdg4); P(y)/+,* F_{4b} ♀ *XX/Y* × ♂ *y^{1}ac/Y; D/mod(mdg4); P(y)/+,* and F_{5b} ♀ *XX/Y* × ♂ *y^{1}ac/Y; D/+; P(y)/+.*

The phenotype of *P(y); mod(mdg4)/mod(mdg4)* and *P(y); D/+* flies was then examined and chromosme-containing insertions were isolated.

RESULTS

All effects of mutations in the *mod(mg4)* gene take place through the su(Hw) protein: We have studied the interaction between *mod(mdg4)^{1u1}* and several *gypsy*-induced mutations. Flies homozygous for a strong *su(Hw)* mutation show a complete suppression of the *gypsy*-induced mutant phenotype (MODOLELL *et al.* 1983). In combination with a strong *su(Hw)* mutation, *mod(mdg4)^{1u1}* does not change the phenotype. A number of such examples can be found in the experiments described below. Thus, the *mod(mdg4)^{1u1}* mutation changes the phenotype of *gypsy*-induced mutations only in the presence of a functional su(Hw) protein. The same result was obtained with heterozygous *su(Hw)^2 mod(mdg4)^{1u1}/su(Hw)^2 Df(3R)GC14* flies.

Influence of the *mod(mdg4)^{1u1}* mutation on the phenotype of *y^2* derivatives: As demonstrated previously (GEORGIEV and GERASIMOVA 1989), *mod(mdg4)^{1u1}* in combina-

TABLE 1

Alleles and constructions used in this work

Allele	Phenotype of the allele and the cause of its formation	Reference
ct^{6}	Cut wing allele, *gypsy* insertion in the regulatory region of *cut* between the wing margin-specific enhancer and the promoter	JACK (1985)
Hw^{1}	*gypsy* insertion near the midpoint of the *achaete* structural gene	BALCELLS *et al.* (1988)
mod(mdg4)^{1u1}	Hypomorphic allele, insertion of *Stalker* in the intron of the gene	GERASIMOVA *et al.* (unpublished)[a]
sc^{D1}	Mild allele, *gypsy* insertion downstream to the *scute* gene	CAMPUZANO *et al.* (1985)
sd^{3B}	Mild allele, *gypsy* insertion between the *yellow* and *acheate* genes	CAMPUSANO *et al.* (1985)
su(Hw)^{v}	Amorphic allele, deletion of *su(Hw)* locus	PARKHURST *et al.* (1988)
su(Hw)^{e1} *su(Hw)^{e3}*	Strong alleles, nonfunctional su(Hw) protein	HARRISON *et al.* (1993)
su(Hw)^{2}	Strong allele, *jockey* insertion in the first intron	PARKHURST *et al.* (1988)
su(Hw)^{f3}	Mild allele, *jockey* insertion in the first intron	HARRISON (1991)
su(Hw)^{E8}	Strong allele, point substitution in the 7th zinc finger motif	HARRISON *et al.* (1993)
su(Hw)^{e2}	Weak allele, point substitution in the 7th zinc finger motif	HARRISON *et al.* (1993)
su(Hw)^{e7}	Mild allele, premature termination of the protein product, loss of 223 amino acids	HARRISON *et al.* (1993)
su(Hw)^{j}	Weak allele, premature termination of the protein product, loss of 149 amino acids	HARRISON *et al.* (1993)
y^{2}	*gypsy* insertion 700 bp upstream to the *yellow* gene promoter	PARKHURST and CORCES (1986)
y^{2PR1}	Partial *y^{2}* reversion, insertion of *jockey* in the su(Hw)-binding region	GEYER *et al.* (1988)
y^{2PR2}	Partial *y^{2}* reversion, insertion of *hobo* in the su(Hw)-binding region	GEYER *et al.* (1988)
y^{59b}	Deletion of the *yellow* promoter and su(Hw)-binding region	GEYER *et al.* (1990)
y$^{1\#8}$	Deletion of the *yellow* promoter	GEYER *et al.* (1990)
su(Hw)^{NoAD}	Deletion of the amino- and carboxy-terminal acidic domains	HARRISON *et al.* (1993)
su(Hw)$^{\Delta 100}$	Deletion of the amino-terminal acidic domain	HARRISON *et al.* (1993)
su(Hw)$^{\Delta 283}$	Deletion of the leucine zipper domain	HARRISON *et al.* (1993)
y$^{pD\text{-}786}$	*yellow* gene and *gypsy* insertion (−700) with a partially deleted su(Hw)-binding region	SMITH and CORES (1992)
y^{-1868}, *y^{-800}*, *y^{-700}*, *y^{+660}*, *y^{+1310}*, *y^{+2490}*	The su(Hw)-binding region is inserted in different sites of the *yellow* gene, (the numbers denote position in the relation to the *yellow* cap site)	GEYER and CORCES (1992)
Df(3R)GC14	γ ray-induced deficiency in 3R (93D6-7 to 93D9-10)	LINDSLEY and ZIMM (1992)

[a] T. GERASIMOVA, D. GDULA, D. GERASIMOV, O. SIMONOVA and V. G. CORCES.

tion with the *y^{2}* mutation suppresses the *yellow* gene expression in bristles (see also Table 2). Two partial reversions of the *y^{2}* mutation (*y^{2PR1}* and *y^{2PR2}*) increase the body and wing pigmentation to 3+ (Table 1; Figure 1). They are induced by an insertion of either the *hobo* or *jockey* elements into the su(Hw)-binding region of *gypsy* (GEYER *et al.* 1988). The combination of *y^{2PR1}* or *y^{2PR2}* mutations with *mod(mdg4)^{1u1}*, or *mod(mdg4)^{1u1}/Df(3R)GC14*, leads to a complete loss of pigmentation in the body, wings, bristles and hairs, as is the case for the *y^{2}* mutation (Table 2). Thus, the *mod(mdg4)^{1u1}* mutation blocks *yellow* expression not only in bristles but also in the body and wings. A partial inactivation of the su(Hw)-binding region caused by insertion of

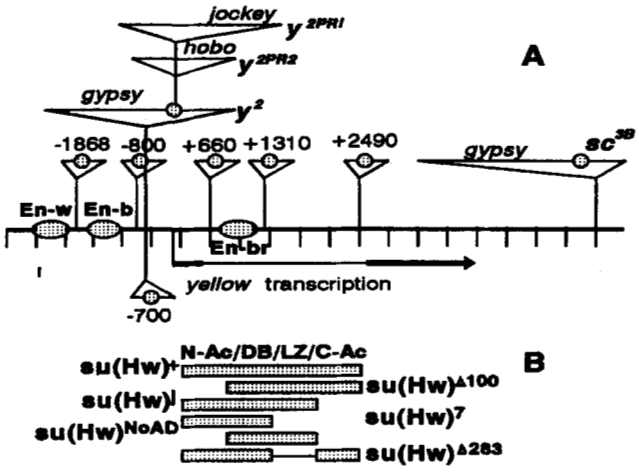

FIGURE 1.—Schematic representation of the *y* and *su(Hw)* mutations and constructions used in this work. (A) Structure of *y* alleles and *y* transformation plasmids. Two exons of the *yellow* gene are shown by thick lines. They are separated by one intron. The arrow indicates the direction of transcription. The circles indicate the su(Hw)-binding regions either in *gypsy* or in constructs used for transformation. Numbers indicate the location of the su(Hw)-binding regions with respect to the *yellow* promoter. Transcriptional enhancers are indicated by ovoid structures. En-w, wing blade enhancer; En-b, body cuticle enhancer; En-br, bristle enhancer. (B) Schematic presentation of *su(Hw)* mutations and constructions with deletions of functional domains of the su(Hw) protein. N-Ac, amino-terminal acidic domain; DB, zinc finger DNA-binding domain; LZ, leucine zipper domain; C-Ac, carboxy-terminal acidic domain.

a mobile element is compensated by the $mod(mdg4)^{1u1}$ mutation.

To understand whether the repressive effect of $mod(mdg4)^{1u1}$ depends on the number of su(Hw)-binding sites, we have used a strain with the $y^{pD\text{-}786}$ P transposon kindly provided by P. SMITH (SMITH and CORCES 1992). This transposon contains the *yellow* gene and a *gypsy* element with only four instead of 12 su(Hw)-binding sites. The phenotype of flies carrying this construction is close to that of y^{2PR1} and y^{2PR2}. However, the $mod(mdg4)^{1u1}$ mutation in combination with this construction displayed a very weak effect on *yellow* expression (Table 2): only the pigmentation of some bristles was slightly reduced, but the level of pigmentation of the body and wings was not changed. To exclude the possibility of position effects, we activated the transposition of the P transposon by crossing with the *Δ2-3(99B)* strain and isolated five strains with transpositions of the construction to novel sites in the genome. In all cases, $mod(mdg4)^{1u1}$ partially decreased the expression of the *yellow* gene only in the bristles. Thus, four su(Hw)-binding sites are not enough to provide for a strong inhibitory effect of the $mod(mdg4)^{1u1}$ mutation.

The role of the location of the su(Hw)-binding region in mediating the effect of the $mod(mdg4)^{1u1}$ mutation: Usually, the su(Hw)-binding region inactivates only enhancers located more distally with respect to the promoter (JACK *et al.* 1991; GEYER and CORCES 1992). In contrast, flies with a combination of $mod(mdg4)^{1u1}$ and y^2 do not express the *yellow* gene in bristles, although the bristle enhancer is located in the intron and it is not separated from the *yellow* promoter by the su(Hw)-binding region (GEORGIEV and GERASIMOVA 1989). To further analyze the role of the location of the *gypsy* su(Hw)-binding region relative to the *yellow* gene in eliciting the $mod(mdg4)^{1u1}$ effect, we used several strains carrying constructions with a su(Hw)-binding region inserted in different sites of the *yellow* locus (Figure 1) (GEYER and CORCES 1992). In the y^{-1868} strains, the su(Hw)-binding region is located 1868 bp upstream of the transcription start site and separates only the wing enhancer from the *yellow* promoter, resulting in a selective decrease of *yellow* expression in the wings. In two independently obtained strains with the y^{-1868} construction, the presence of the $mod(mdg4)^{1u1}$ mutation does not change the y^2 phenotype (Table 2). Nevertheless, we can not exclude that the action of the $mod(mdg4)^{1u1}$ mutation is blocked by surrounding sequences. To test the role of position effects in more detail, we activated the transposition of the y^{-1868} construction by crossing with the *Δ2-3(99B)* strain and isolated in each case a number of new derivative strains containing the same insertion either in the second or in the third chromosome. Eighteen independent derivatives were obtained that have the same phenotype as the original one and are completely suppressed by the $su(Hw)^2$ mutation. In all cases, the introduction of the $mod(mdg4)^{1u1}$ mutation does not lead to a strong effect on the y phenotype. Thus, the $mod(mdg4)^{1u1}$ mutation does not influence *yellow* expression if the su(Hw)-binding region is located between the body and wing enhancers.

In y^{-700} and y^{-800}, the su(Hw)-binding region isolates the body and wing enhancers from the *yellow* promoter, and therefore they mimic the *gypsy* effect in the y^2 mutation (GEYER and CORCES 1992). y^{-800} constructions from five independent strains and y^{-700} constructions from two independent strains were tested in combination with $mod(mdg4)^{1u1}$. The inhibitory action on bristle and hair pigmentation was strong in all cases tested, identical to that of the y^2 mutation, although in several strains position effect variegation was observed: the color of different bristles varied from black to *yellow* in the same fly (Table 2).

In the y^{+660} construction, the su(Hw)-binding region is located in the intron and separates the bristle enhancer from the *yellow* promoter (GEYER and CORCES 1992). Flies with this construction have y^- thorax and leg bristles, while the body and wings are normally pigmented. The introduction of the $mod(mdg4)^{1u1}$ mutation leads to a complete repression of the *yellow* gene expression only in the bristles in one strain, while a second one also shows partial repression in the body and wings. This difference may be explained by the influence of sequences surrounding the construction. After crosses

TABLE 2

Dependence of the effect of *mod(mdg4)^{1u1}* on the properties and location of the *gypsy* su(Hw)-binding region within the *yellow* locus

Mutations and constructions	*mod(mdg4)^{1u1}*	No. of tested inserts	Pigmentation					
					Bristles			
			Body	Wings	Th	L	Ab	W
y^2	+/+	—	1	1	5	5	5	5
	m/*m*^{a}	—	**0**	**0**	**0**	**0**	**0**	**0**
y^{2PR1} or y^{2PR2}	+/+	—	3	3	5	5	5	5
	m/*m*^{a}	—	**0**	**0**	**0**	**0**	**0**	**0**
ypD-786	+/+	6	4	4	5	5	5	5
	m/*m*	6	4	4	**3**	**3**	5	**4**
y^{-1868}	+/+	2	5	3	5	5	5	5
	m/*m*	2	5	3	**4–5**	**4–5**	5	5
y^{-800}	+/+	5	2	2	5	5	5	5
	m/*m*	3	2	2	**0**	**0**	**2**	**1**
	m/*m*	2	2	2	**1–4**	**1–4**	**1–4**	**1–4**
y^{-700}	+/+	2	1	5	5	5	5	5
	m/*m*	1	1	1	**0**	**0**	**1**	**0**
	m/*m*	1	1	1	**1–4**	**1–4**	**1–4**	**1–4**
y^{+660}	+/+	2	5	5	0	0	4–5	5
	m/*m*	1	**4**	**4**	0	0	**3**	**4**
	m/*m*	1	**2**	**2**	0	0	**0**	**0**
y^{+1310}	+/+	3	5	5	5	5	5	5
	m/*m*	3	5	5	**1**	**1**	**4**	**4**
y^{+2490}	+/+	3	5	5	5	5	5	5
	m/*m*	2	5	5	**1**	**1**	**4**	**4**
y^{+2490}	*m*/*m*	1	**3**	**3**	**0**	**0**	**3**	**3**
sc^{3B}	+/+	—	5	5	5	5	5	5
	m/*m*	—	5	5	**2**	**1**	**4**	**4**

Th, thoracal; Ab, abdominal; W, wing; L, leg bristles; su, su(Hw) allele; *m*, *mod(mdg4)^{1u1}*. Bold figures represent cases where the *mod(mdg4)^{1u1}* mutation changes *yellow* expression. The level of pigmentation of *yellow* alleles was determined visually in 3–5-day old adults. Flies from every cross were scored twice. The level of pigmentation was ranked on a scale from 0 to 5. A value of 0 corresponds to the pigmentation of y^- flies. A value of 5 corresponds to the pigmentation of y^+ flies. Flies with well characterized *y* alleles were used as controls to determine level of pigmentation (GEORGIEV *et al.* 1992).

[a] The same results have been obtained with *Df(3R)GC14/mod(mdg4)^{1u1}* heterozygotes.

between y^{+660} and *Δ2-3(99B)*, 24 independent strains with transpositions of the y^{+660} construction to the second or third chromosome were obtained. These strains have the same phenotype as the original y^{+660} mutant and are completely suppressed by the *su(Hw)^2* mutation. The effect of the *mod(mdg4)^{1u1}* mutation on *yellow* expression depends on the position of the construction and varies over a wide range: from complete inactivation of *yellow* gene expression in the body, wings, and bristles to the absence of an inhibiting effect on *yellow* expression (not shown).

The su(Hw)-binding region is located in the *yellow* intron downstream of the bristle enhancer in the y^{+1310} and y^{+2490} constructions, resulting in a wild-type yellow phenotype (GEYER and CORCES 1992). As in the previous case, the inhibitory effect of the *mod(mdg4)^{1u1}* mutation depends on the position of the construction in the genome and changes from a partial decrease of *yellow* expression in bristles to a complete inactivation in bristles and insignificant in the body and wings.

The *gypsy* element is inserted downstream of the *yellow* locus in the sc^{3B} mutation (CAMPUZANO *et al.* 1985). The expression of *yellow* is not changed in the sc^{3B} strain. However, carrying the sc^{3B} mutation and homozygous for *mod(mdg4)^{1u1}* display a reduced bristle pigmentation of 1+ to 4+ (Table 3). Thus, the effect of the *mod(mdg4)^{1u1}* mutation strongly depends on the location of su(Hw)-binding sites relative to the *yellow* promoter as well as on the position of the construction in the genome.

Block of transvection by the *mod(mdg4)^{1u1}* mutation: The y^{59b} mutation is a null allele derived from y^2 by deletion of a region including the su(Hw)-binding region and the *yellow* promoter. Flies of the genotype $y2/y^{59b}$ show a y^+ phenotype as a result of *trans*-activation of the *yellow* promoter in the y^2 allele by *yellow* enhancers located on the y^{59b} chromosome (GEYER *et al.* 1990). Thus, the su(Hw)-binding region does not interfere with the activation of the *yellow* promoter if the *yellow* enhancers are located in the homologous chromosome. To better understand the mechanism of the action of the *mod(mdg4)^{1u1}* mutation, we examined its effect on

TABLE 3

Inhibition of transvection by the *mod(mdg4)^{1u1}* mutation

Genotype		Pigmentation	
yellow alleles	*mod(mdg4)^{1u1}* mutation	Body, wings	Bristles
y^{59b}/y^{59b}	+/+	0	0
y^{2}/y^{2}	+/+	1	5
y^{2}/y^{2}	*m/m*	0	0
y^{2}/y^{59b}	+/+	**5**	5
$y^{2}/y^{59b\,a}$	*m/m*	**0**	0
$y^{2PR1}/y^{2PR1\,b}$	+/+	2	5
$y^{2PR1}/y^{59b\,b}$	+/+	**5**	5
$y^{2PR1}/y^{59b\,b}$	*m/m*	**0**	0

Bold figures indicate examples of transvection and the effect of the *mod(mdg4)^{1u1}* mutation on the transvection phenomenon.

[a] The same results has been obtained with *Df(3R)GC14/mod(mdg4)^{1u1}* heterozygotes.

[b] Combination with y^{2PR2} mutation have the same phenotype. See Table 2 for a desciption of the *yellow* phenotype.

the y^{2}/y^{59b} complementation. y^{2}/y^{59b} flies homozygous for *mod(mdg4)^{1u1}* or *mod(mdg4)^{1u1}/Df(3R)GC14* heterozygotes show complete absence of *yellow* expression (y^{-} phenotype) (Table 3).

The same result was obtained with partial y^{2}-revertants y^{2PR1} and y^{2PR2}. Females of the genotypes y^{2PR1}/y^{59b} and y^{2PR2}/y^{59b} display a y^{+} phenotype but appear y^{-} in combination with the *mod(mdg4)^{1u1}* mutation (Table 3). We also tested the effect of *mod(mdg4)^{1u1}* mutation on the phenotype of $y^{2}/y^{1\#8}$ flies. $y^{1\#8}$ is a deletion of the *yellow* promoter and does not contain *gypsy* sequences (GEYER *et al.* 1990). In $y^{1\#8}/y^{2}$ heterozygotes, the body and wing enhancers of $y^{1\#8}$ transactivate *yellow* gene transcription in the y^{2} allele, resulting in a y^{+} phenotype (GEYER *et al.* 1990). The presence of *mod(mdg4)^{1u1}* completely inhibits *yellow* expression (null phenotype) in these flies.

Interaction of the *mod(mdg4)^{1u1}* mutation with different *su(Hw)* alleles in the control of *yellow* expression in *gypsy*-induced y mutations: We next studied the interaction between mutations in the *su(Hw)* and *mod(mdg4)* genes. The *su(Hw)^{v}* mutation is a deletion of the *su(Hw)* gene (HARRISON *et al.* 1993). As was mentioned above, this mutation in homozygotes, either alone (HARRISON *et al.* 1993) or in combination with *mod(mdg4)^{1u1}*, completely suppresses the y^{2} mutation (Table 4). *su(Hw)^{v}* is a recessive mutation and *su(Hw)$^{+}$/su(Hw)^{v}* does not influence the phenotype of either y^{2}, y^{2PR1} or y^{2PR2}. However, *su(Hw)^{v}/su(Hw)$^{+}$* heterozygotes show a complete suppression of the inhibitory effect of *mod(mdg4)^{1u1}* or *mod(mdg4)^{1u1}/Df(3R)GC14* mutations on *yellow* expression in bristles, indicating that this inhibition is very sensitive to the concentration of the su(Hw) protein. The pigmentation of the body and wings slightly exceeds that characteristic of the y^{2} allele (Table 4). Other strong *su(Hw)* mutations (HARRISON *et al.* 1993), which inactivate the protein product, *su(Hw)^{e1}* and *su(Hw)^{e3}*, or completely destroy its DNA binding activity, *su(Hw)^{E8}*, have the same properties (Table 4).

The sensitivity of the inhibitory effect of the *mod(mdg4)^{1u1}* mutation to the su(Hw) protein concentration was confirmed in experiments with two mutations, *su(Hw)^{2}* (strong mutation) and *su(Hw)^{f3}* (weak mutation). Both mutations are caused by the insertion of the *jockey* transposable element into the intron of the *su(Hw)* gene in different orientations (HARRISON 1991). *su(Hw)^{f3}* produces five times less su(Hw) protein than *su(Hw)$^{+}$*, whereas no su(Hw) protein has been detected by the Western blot analysis in *su(Hw)^{2}* (HARRISON *et al.* 1993). The *su(Hw)^{2}* mutation completely suppresses the y^{2} mutation and its partial revertants. In combination with *mod(mdg4)^{1u1}* homozygotes or *mod(mdg4)^{1u1}/Df(3R)GC14* heterozygotes, *su(Hw)^{2}/+* has a slight dominant effect, partially suppressing *mod(mdg4)^{1u1}* in bristles (Table 4). Similar results have been obtained with the y^{2PR1} and y^{2PR2} alleles (Table 4).

su(Hw)^{f3} is a mild mutation and suppresses the y^{2} mutant phenotype only partially. However, in combination with *mod(mdg4)^{1u1}*, *su(Hw)^{f3}* completely suppresses the mutant phenotype. The heterozygote *su(Hw)^{f3}/+* suppresses only very slightly the inhibitory effect of *mod(mdg4)^{1u1}* on *yellow* expression in bristles (Table 4). Thus, small differences in the amount of the su(Hw) protein are important for the *mod(mdg4)^{1u1}* effect. The su(Hw) protein concentration must be higher in *su(Hw)^{f3}/+* and lower in *su(Hw)^{v}/+*, although the differences are small (HARRISON 1991). Nevertheless, these differences are associated with visible changes in the inhibition of *yellow* expression. On the other hand, *mod(mdg4)^{1u1}* converts the mildest *su(Hw)^{f3}* mutation into a strong one.

Another well-characterized mild mutation, *su(Hw)^{e2}*, is a result of an amino acid substitution in the seventh zinc finger motif of the su(Hw) protein, leading to weaker interaction with the su(Hw)-binding region (HARRISON *et al.* 1993). However, the combination of *su(Hw)^{e2}* with *mod(mdg4)^{1u1}* leads to a complete suppression of the mutant y^{-} phenotype in flies. Moreover, the *su(Hw)^{e2}/+* heterozygote combined with the *mod(mdg4)^{1u1}* allele has the same effect as *su(Hw)^{v}* or other strong mutations, completely inactivating the su(Hw) protein. Thus, *mod(mdg4)^{1u1}* converts a mild *su(Hw)* mutation in the DNA-binding domain into a strong one (Table 4).

The role of the acidic domains of the su(Hw) protein in mediating the effect of *mod(mdg4)^{1u1}*: In the next series of experiments, we tried to identify the su(Hw) domains responsible for the inhibitory effect of the *mod(mdg4)^{1u1}* mutation. For this purpose, we used several previously characterized *su(Hw)* mutations (Figure 1).

The *su(Hw)^{NoAD}* mutation contains the *su(Hw)* gene encoding a protein lacking both acidic regions (HAR-

TABLE 4

Influence of combinations of *su(Hw)* and *mod(mdg4)^{1u1}* mutations on *yellow* expression in *gypsy*-induced *y* alleles

Genotype of the strain			*y* phenotype in y^2					*y* phenotype in y^{2PR1} and y^{2PR2}	
su(Hw) alleles	Genotype			Bristles					
	su(Hw)	*mod(mdg4)*	Body, wings	Th	L	Ab	W	Body, wings	Bristles
+	+/+	+/+	1	5	5	5	5	3	5
	+/+	*m/m*	**0**	**0**	**0**	**0**	**0**	**0**	**0**
v, E8	*su/su*	+/+	5	5	5	5	5	5	5
	su/su	*m/m*	5	5	5	5	5	5	5
	su/+	*m/m*[a]	**2**	5	5	5	5	**3**	5
2	*su/su*	+/+	5	5	5	5	5	5	5
	su/su	*m/m*[a]	5	5	5	5	5	5	5
	su/+	*m/m*[a]	**2**	**1–4**	**1–4**	**2–4**	**2–4**	**3**	**1–4**
f3	*su/su*	+/+	4	5	5	5	5		
	su/su	*m/m*	**5**	5	5	5	5		
	su/+	*m/m*	**2**	**1**	**1**	**2**	**2**		
e2	*su/su*	+/+	3	5	5	5	5		
	su/su	*m/m*	**5**	5	5	5	5		
	su/+	*m/m*	**2**	5	5	5	5		
NoAD	*su/su*	+/+	5	5	5	5	5		
	su/su	*m/m*	5	5	5	5	5		
	su/+	*m/m*	**2**	5	5	5	5		
j	*su/su*	+/+	1	5	5	5	5	3	5
	su/su	*m/m*	**2**	5	5	5	5	**4**	5
	su/+	*m/m*	1	5	5	5	5	**2**	5
Δ100	*su/su*	+/+	1	5	5	5	5	3	5
	su/su	*m/m*	**4**	**2**	**2**	**4**	5	**5**	**2–5**
	su/+	*m/m*	1	**1**	**1**	**2**	**2**	**3**	**1–3**
e7, Δ283	*su/su*	+/+	4	5	5	5	5		
	su/su	*m/m*	**5**	5	5	5	5		
	su/+	*m/m*	**2**	5	5	5	5		

Th, thoracal; Ab, abdominal; W, wing; L, leg bristles; *su*, *su(Hw)* allele; *m*, *mod(mdg4)^{1u1}*. Bold figures represent cases where the *mod(mdg4)^{1u1}* mutation changes *yellow* expression. For *y* phenotypes see Table 2.

[a] The same results have been obtained with *Df(3R)GC14/ mod(mdg4)^{1u1}* heterozygotes.

RISON *et al.* 1993). Flies of the genotype y^2; *su(Hw)^{NoAD}* display a wild-type phenotype. In combination with the *mod(mdg4)^{1u1}* mutation, *su(Hw)^{NoAD}* has the same effect as mutations that completely inactivate the su(Hw) protein (Table 4). Therefore, the su(Hw) acidic domains are responsible for the repression of *yellow* transcription by the *mod(mdg4)^{1u1}* mutation and a su(Hw) protein lacking both acidic domains loses all *mod(mdg4)^{1u1}* mediated functions.

To analyze the role of each acidic domain, the *su(Hw)^{j}* and *su(Hw)^{Δ100}* mutations were used to test the ability of the encoded proteins to interact with *mod(mdg4)^{1u1}*. *su(Hw)^{j}* results from the loss of the 149 carboxy-terminal amino acids of the su(Hw) protein. This mutation is weak and does not influence the phenotype of the y^2, y^{2PR1} or y^{2PR2} alleles. However, homozygous *su(Hw)^{j}* and even heterozygous *su(Hw)^{j}/su(Hw)$^{+}$* completely suppresses the inhibitory effect of *mod(mdg4)^{1u1}* on *yellow* expression in the bristles. On the other hand, homozygous *su(Hw)^{j}* in combination with *mod(mdg4)^{1u1}* only slightly enhances *yellow* expression in the body and wings. A similar effect was observed in the case of y^{2PR1} and y^{2PR2} alleles. Thus, the carboxy-terminal acidic domain seems to be important for *mod(mdg4)^{1u1}* mediated inhibition of *yellow* expression in bristles rather than in the body and wings (Table 4).

The *su(Hw)^{Δ100}* mutation has a deletion of the amino-terminal acidic domain of the su(Hw) protein. Two different strains with the *su(Hw)^{Δ100}* construction inserted into the second or third chromosome were used in this study. Like the *su(Hw)^{j}* allele, *su(Hw)^{Δ100}* does not suppress the y^2 mutant phenotype in homozygotes (HARRISON *et al.* 1993). The combination of homozygous *su(Hw)^{Δ100}* and *mod(mdg4)^{1u1}* mutations leads to a strong suppression of the y mutant phenotype in the body and wings but only to a partial suppression in bristles (Table 4). In the *su(Hw)^{Δ100}/+* heterozygote, *mod(mdg4)^{1u1}* strongly inhibits *yellow* expression in all areas. We can then conclude that in the case of *gypsy*-induced *y* mutations, the su(Hw) amino-terminal acidic domain is more important for the inhibition of *yellow* expression in the body and wings, while the carboxy-terminal domain is important for its inhibition in bristles. Thus, the function of the two acidic domains are

TABLE 5

Effects of *su(Hw)* mutations on transvection between *yellow* alleles in the presence of the *mod(mdg4)^{1u1}* mutation

Genotype		Pigmentation	
su(Hw) alleles	*yellow* alleles	Body, wings	Bristles
su(Hw)^j/su(Hw)^j	y^2/y^2	1	5
	y^2/y^{59b}	**4**	5
su(Hw)^j/+	y^2/y^2	1	5
	y^2/y^{59b}	**2**	5
su(Hw)$^{\Delta 100}$/su(Hw)$^{\Delta 100}$	y^2/y^2	4	2–5
	y^2/y^{59b}	**5**	2–5
su(Hw)^v/+	y^2/y^2	2	5
	y^2/y^{59b}	2	5
su(Hw)^2/+	y^2/y^2	2	1–4
	y^2/y^{59b}	2	1–4

Designations are as in Table 2. Bold figures represent cases where *su(Hw)* mutations restore positive transvection in the presence of the *mod(mdg4)^{1u1}* mutation.

distinguishable if analyzed in combination with *mod(mdg4)^{1u1}* mutation.

The *su(Hw)^{e7}* mutation leads to the loss of the carboxy-terminal acidic and leucine zipper domains (HARRISON *et al.* 1993). It suppresses strongly, but not completely, the y^2 mutant phenotype. In combination with *mod(mdg4)^{1u1}*, a complete suppression of the mutant phenotype takes place. The *su(Hw)$^{\Delta 283}$* construction is a deletion of the leucine zipper domain (HARRISON *et al.* 1993). Alone and in combination with the *mod(mdg4)^{1u1}* mutation, it behaves like *su(Hw)^{e7}*. Thus, the su(Hw) protein lacking the lecuine zipper domain does not inhibit *yellow* expression in y^2; *mod(mdg4)^{1u1}* flies.

The next series of experiments was designed to study the interaction between *su(Hw)* and *mod(mdg4)^{1u1}* mutations in y^{59b}/y^2 females. Heterozygote *su(Hw)^v/su(Hw)$^+$* and *su(Hw)^2/su(Hw)$^+$* with *mod(mdg4)^{1u1}* have the same effect on pigmentation of y^2/y^{59b} and y^2/y^2 females (Table 5). Thus, a decrease of the su(Hw) protein concentration in the *su(Hw)^v/su(Hw)$^+$* heterozygote does not suppress the negative effect of *mod(mdg4)^{1u1}* on transvection. The homozygous *su(Hw)^j* mutation strongly suppresses the inhibitory effect of *mod(mdg4)^{1u1}* in y^2/y^{59b} flies: the body and wing pigmentation increases to 4+, in contrast to y^2/y^2 (1+). In *su(Hw)^j/su(Hw)$^+$* heterozygotes, the suppression is weak (Table 5). As expected, y^2/y^{59b} females in combination with *su(Hw)$^{\Delta 100}$*; *mod(mdg4)^{1u1}* have wild-type pigmentation of the body and wings.

Effect of the *mod(mdg4)^{1u1}* mutation on the phenotype of *gypsy*-induced mutations in the *cut* locus: In contrast to the y^2 mutation, the combination of *mod(mdg4)^{1u1}* with several other *gypsy*-induced mutations leads to a partial suppression of their mutant phenotype. Well characterized among them is the *ct^6* mutation in the *cut* locus. The *ct^6* mutation is induced by a *gypsy* insertion between the *cut* promoter and an enhancer responsible for *cut* expression in the wing margins (JACK 1985; JACK *et al.* 1991). *su(Hw)* is known to block the interactions of the wing margin enhancer with the cut gene promoter (JACK *et al.* 1991; DORSETT 1993). In contrast to y^2, the *ct^6* mutation is strongly suppressed by *mod(mdg4)^{1u1}*: only 20% of flies with such genotype have one to five gaps randomly distributed through the wing margin (Table 6). Both strong *su(Hw)* mutations, such as *su(Hw)^v*, *su(Hw)^{E8}*, *su(Hw)^2* and *su(Hw)^{NoAD}*, and weak mutations, such as *su(Hw)^{e2}*, *su(Hw)^{f3}* and *su(Hw)^{e7}*, taken alone or in combination with *mod(mdg4)^{1u1}*, completely suppress the ct mutant phenotype. These *su(Hw)* mutations suppress the ct phenotype even in heterozygotes if combined with the homozygous *mod(mdg4)^{1u1}* mutation (Table 6).

su(Hw)^j and *su(Hw)$^{\Delta 100}$* slightly suppress the mutant phenotype of the *ct^6* mutation (flies with this genotype have many small gaps along the wing margin). In combination with *mod(mdg4)^{1u1}*, both *su(Hw)^j* and *su(Hw)$^{\Delta 100}$* completely suppress the mutant ct phenotype. They act even in the heterozygous state (Table 6). Thus, the inhibition of the wing margin enhancer by the su(Hw)-binding region in *ct^6* is much more sensitive than in the case of y^2 to changes in the concentration of su(Hw) protein, to the presence of both su(Hw) acidic domains and to the *mod(mdg4)^{1u1}* mutation.

Effect of the interaction between *mod(mdg4)^{1u1}* and different *su(Hw)* mutations on the phenotype of *gypsy* induced mutations in the *achaete-scute* complex: The *achaete-scute* gene complex (*AS-C*) consists of four genes (ALONSO and CABRERA 1988). The *achaete* (*ac*) gene, responsible for the development of hairs and dorsocentral bristles, is located 5 kb proximal to the *yellow* gene (CAMPUZANO *et al.* 1985). The *scute* locus is responsible for the development of all other bristles and located 40 kb proximal to the *yellow* locus (CAMPUZANO *et al.* 1985). The *sc^{D1}* mutation is caused by an insertion of *gypsy* 20 kb downstream of the *scute* locus (CAMPUZANO *et al.* 1985). Strong *su(Hw)* mutations, such as *su(Hw)^v*, *su(Hw)^{E8}*, *su(Hw)^2* and *su(Hw)^{NoAD}*, completely suppress the *sc^{D1}* mutant phenotype. Introduction of the *mod(mdg4)^{1u1}* mutation leads, as in the case of *ct^6*, to a partial suppression of the mutant phenotype (Table 6). All strong *su(Hw)* mutations in heterozygotes fail to affect the *sc^{D1}* mutant phenotype, but in combination with *mod(mdg4)^{1u1}*, they have a prominent suppressing effect on the mutant *sc* phenotype (Table 6). Weak *su(Hw)* mutations, such as *su(Hw)^{f3}* and *su(Hw)^{e2}*, have only a mild effect on the *sc^{D1}* mutant phenotype. However, combined with *mod(mdg4)^{1u1}* they are transformed into strong mutations resembling *su(Hw)^v*. The heterozygote *su(Hw)^{e2}/su(Hw)$^+$* in combination with *mod(mdg4)^{1u1}* completely suppresses the sc mutant phenotype as strong *su(Hw)* mutations.

The deletion of either C-terminal [*su(Hw)^j*] or N-terminal [*su(Hw)$^{\Delta 100}$*] acidic domains in the su(Hw) protein has no visible effect on *sc^{D1}* expression, whereas

TABLE 6

Influence of combination of *su(Hw)* and *mod(mdg4)^{lu1}* mutations on the phenotypic expression of *achaete*, *scute* and *cut* alleles

Genotype				sc^{D1}					Hw^{1}		
su(Hw) alleles	*su(Hw)*	mod	ct^{6}	AOR	PV	OC	ANP	SC	DC	W	SSA
$su(Hw)^{+}$	+/+	+/+	6	90	90	90	90	1	4	4	4
	+/+	m/m^{a}	n	50	50	+	90	1	2	1	2
$su(Hw)^{2}$	su/su	+/+	+	+	+	+	+	4	+	+	+
$su(Hw)^{v}$	su/su	m/m^{a}	+	+	+	+	+	4	+	+	+
	su/+	m/m^{a}	+	+	+	+	50	4	1	+	+
$su(Hw)^{e2}$	su/su	+/+	+	90	90	50	50	1	2	2	2
	su/su	m/m	+	+	+	+	+	4	+	+	+
	su/+	m/m	+	+	+	+	50	4	1	+	+
$su(Hw)^{f3}$	su/su	+/+	+	+	+	+	50	3	1	1	1
	su/su	m/m	+	+	+	+	+	4	+	+	+
	su/+	m/m	+	+	+	+	50	4	1	+	+
$su(Hw)^{NoAD}$	su/su	+/+	+	+	+	+	+	4	+	+	+
	su/su	m/m	+	+	+	+	+	4	+	+	+
	su/+	m/m	+	+	+	+	50	4	1	+	+
$su(Hw)^{j}$	su/su	+/+	pN	90	90	90	90	1	3	3	3
	su/su	m/m	+	90	90	10	50	1	2	1	2
	su/+	m/m	+	50	90	10	50	1	2	1	2
$su(Hw)^{\Delta 100}$	su/su	+/+	pN	90	90	90	90	1	2	2	3
	su/su	m/m	+	+	+	+	+	4	+	+	+
	su/+	m/m	+	+	10	+	50	4	1	1	1
$su(Hw)^{e7}$	su/su	+/+	+	90	90	+	50	1	2	1	2
	su/su	m/m	+	50	50	+	10	2	2	1	2
	su/+	m/m	+	50	50	+	10	1	2	1	2
$su(Hw)^{\Delta 283}$	su/su	+/+	+	90	90	10	10	2	2	1	+
	su/su	m/m	+	10	10	+	10	3	1	+	+

ct phenotype: +, wild type; n, the flies have small random gaps around the wing margin (this phenotype is present in 20% of ct^{6}; *mod(mdg4)^{lu1}/mod(mdg4)^{lu1}* flies, while others have a ct^{+} phenotype); pN, 100% of flies have 20–30 small gaps around the whole wing margin; 6, a strong *cut* wing phenotype, like ct^{6}. sc phenotype: figures indicate the percentage of flies with missing bristles; 10, 50, and 90% means the disappearance of bristles in >10, >50 and >90% of flies, respectively; +, these bristles are present in >90% of the flies; figures from 1 to 4 indicate the number of scutellar bristles. Hw phenotype: figures indicate the strength of the Hw phenotype in homozygous Hw^{1} females; 4, the phenotype of the Hw^{1} mutation (~100 extrachaetae on the wing, 15–20 additional bristles on the notum, 15–20 chaetae on the second segment of the antenna); 3, >50 extra chaetae on the wing, 10–15 additional bristles on the notum, 10–15 chaetae on the second segment of the antenna; 2, >10 extra chaetae on the wing, 5–10 additional bristles on the notum, 5–10 chaetae on the second segment of the antenna; 1, 1–10 extra chaetae on the wing, 1–5 additional bristles on the notum, 1–5 chaetae on the second segment of the antenna.

[a] The same results have been obtained with *Df(3R)GC14/mod(mdg4)^{lu1}* heterozygotes.

the deletion of both acidic domains in $su(Hw)^{NoAD}$ results in a complete suppression of the mutant phenotype. Thus, as in the case of y^{2}, the presence of one acidic domain is enough for the inhibitory action of the su(Hw) protein. However, in the presence of *mod(mdg4)^{lu1}*, the su(Hw) acidic domains differ in their functional significance: $su(Hw)^{\Delta 100}$ completely suppresses the sc^{D1} mutant phenotype while $su(Hw)^{j}$ fails to have any effect. $su(Hw)^{e7}$ and $su(Hw)^{\Delta 283}$ suppress partially, but in combination with *mod(mdg4)^{lu1}* almost completely, the sc^{D1} mutant phenotype (Table 6).

The Hw^{1} mutation carries a *gypsy* insertion in the structural part of the *achaete* gene (BALCELLS *et al.* 1988). As a result, the *achaete* transcript is shortened from 1.1 to 0.9 kb, but the protein product is functionally active. *gypsy* insertion induces overexpression of the *achaete* gene, the effect being more pronounced in females. Homozygous females have ~90 extra chaete on the wing, extra bristles on the head, notum, scutellum and second segment of the antenna. Strong *su(Hw)* mutations completely suppress the mutant phenotype (LINDSLEY and ZIMM 1992). The *mod(mdg4)^{lu1}* mutation leads to a partial suppression of the Hw^{1} mutant phenotype. In general, the combination of *mod(mdg4)^{lu1}* with different *su(Hw)* mutations influences the Hw^{1} phenotype in the same way as in ct^{6} and sc^{D1}. Strong *su(Hw)* mutations have a dominant suppressing effect in combination with *mod(mdg4)^{lu1}*. Weak $su(Hw)^{f3}$ and $su(Hw)^{e2}$ mutations in combination with *mod(mdg4)^{lu1}* acquire properties of strong *su(Hw)* mutations. Deletions of either acidic domain only slightly suppress the Hw^{1} mutant phenotype. The combination of $su(Hw)^{\Delta 100}$ with *mod(mdg4)^{lu1}* leads to a complete suppression, while $su(Hw)^{j}$ does not change the action of *mod(mdg4)^{lu1}* on

the Hw^1 mutant phenotype. The $su(Hw)^{NoAD}$ construction, as other strong mutations, completely suppresses the Hw^1 mutant phenotype. The $su(Hw)^{e7}$ and the $su(Hw)^{\Delta 283}$ alleles partially suppress the Hw^1 mutant phenotype; $mod(mdg4)^{1u1}$ enhances the suppressing effect of $su(Hw)^{\Delta 283}$ (Table 6).

DISCUSSION

Two modes of action of the $mod(mdg4)^{1u1}$ mutation: The repressive effect of the su(Hw) protein on enhancer function shows an interesting directionality: only enhancers located distally from the promoter with respect to the position of the su(Hw)-binding region are affected by this protein (CORCES and GEYER 1991; JACK *et al.* 1991; GEYER and CORCES 1992). This directional effect offers some clues to the mechanism by which su(Hw) represses enhancer action. It suggests that su(Hw) acts either by interfering with DNA looping allowing transcription factors bound to the enhancers to interact with the transcription complex, or by interfering with the process of tracking of these factors toward the promoter, or by establishing chromatin domains of independent gene activity that insulate DNA sequences within a domain from neighboring regions (GEYER and CORCES 1992; ROSEMAN *et al.* 1993).

We have used the hypomorphic $mod(mdg4)^{1u1}$ mutation that has been induced by the insertion of the *Stalker* transposable element (GEORGIEV and GERASIMOVA 1989) to further understand the mechanisms by which su(Hw) affects gene expression. Two other *mod(mdg4)* mutations induced by EMS have the same effect on y^2, sc^{D1} and ct^6 mutations (T. I. GERASIMOVA and V. G. CORCES, personal comunication). The $mod(mdg4)^{1u1}$ mutation affects a 2.2-kb transcript encoded by the *mod(mg4)* gene. Also the $mod(mdg4)^{1u1}/Df(3R)GC14$ heterozygote interacts with $su(Hw)^2$ and *gypsy*-induced mutations in the same way as homozygote $mod(mdg4)^{1u1}/mod(mdg4)^{1u1}$. Thus, these results suggest that $mod(mdg4)^{1u1}$ is a hypomorphic loss-of-function mutation of the *mod(mg4)* gene.

The effects of the $mod(mdg4)^{1u1}$ mutation may be divided into two groups: disturbance of the insulating function of the su(Hw)-binding region and direct inhibition of target gene transcription. In most of the studied *gypsy*-induced mutations, one can observe the effect of $mod(mdg4)^{1u1}$ on su(Hw) insulation that results in a partial suppression of ct^6, sc^{D1} and Hw^1 mutations. On the other hand, $mod(mdg4)^{1u1}$ enhances the y^2 mutation to the *yellow*-null phenotype. As we shall discuss below, the loss of insulation may also take place in this case but it is compensated by direct inhibition of transcription.

The inhibition of transcription by the su(Hw) protein in the presence of $mod(mdg4)^{1u1}$: In the case of the y^2 mutation, the $mod(mdg4)^{1u1}$ mutation changes the action of the su(Hw)-binding region in such a way that it starts to inactivate the *yellow* transcription driven by enhancers not separated by the su(Hw)-binding region from the *yellow* promoter. The blocking of *yellow* expression in the body and wings by the $mod(mdg4)^{1u1}$ mutation is also a result of direct inhibition. For instance, in some y^{+660} and y^{+2490} constructions the su(Hw)-binding region inactivates *yellow* expression in the body and wings in the presence of $mod(mdg4)^{1u1}$, although the body and wing enhancers are not separated from the promoter by the su(Hw)-binding region in these constructions. *yellow* gene expression in bristles is inhibited by the $mod(mdg4)^{1u1}$ mutation even if the *gypsy* su(Hw)-binding region is located downstream from the 3′ end of the *yellow* gene as in the sc^{3B} mutation. However, the 5′ upstream −1868 position of the su(Hw)-binding region (y^{-1868}) is not favorable for the inhibitory action of $mod(mdg4)^{1u1}$. Thus, some yet unknown features of the DNA domain architecture seem to be important for $mod(mdg4)^{1u1}$-mediated inhibition of *yellow* expression.

We have recently described results demonstrating that the $mod(mdg4)^{1u1}$ mutation allows the su(Hw) protein to act in *trans* and to inhibit simultaneously transcription from two promoters located in homologous chromosomes (GEORGIEV and CORCES 1995). Here we have found that the su(Hw)-binding region in the y^2 mutation in the presence of $mod(mdg4)^{1u1}$ can also block the interaction between two enhancers and the *yellow* promoter in the y^2/y^{59b} combination. All these observations can be explained assuming that in the presence of $mod(mdg4)^{1u1}$ the su(Hw) protein directly inhibits the expression from the *yellow* promoter. A second explanation is that, in the presence of the $mod(mdg4)^{1u1}$ mutation, the su(Hw) protein can alter the chromatin structure and repress transcription of the *yellow* gene independently of the position of the su(Hw)-binding region. For example, in the dominant position effect described for the *brown* locus (DREESEN *et al.* 1991), heterochromatic sequences that inhibit expression of the *brown* gene in *cis* can also act in *trans* on the gene located in the other homolog. The absence of an effect of $mod(mdg4)^{1u1}$ on *yellow* gene transcription in a construction where the su(Hw)-binding region is inserted at position −1648 argues against the possibility that the $mod(mdg4)^{1u1}$ mutation affects chromatin structure. Although the su(Hw)-binding region in this construction is located between two enhancers of the *yellow* gene and blocks the wing enhancer, it does not interfere with the *yellow* expression in the presence of the $mod(mdg4)^{1u1}$ mutation. This result can hardly be explained in terms of changes of the chromatin structure in the *yellow* gene. Therefore, it is more probable that the inhibitory action of the $mod(mdg4)^{1u1}$ mutation takes place when the su(Hw) protein binding sites can interact with the promoter.

The data obtained in the present work suggest that the acidic domains of the su(Hw) protein are involved in direct inhibition of transcription in the presence of the $mod(mdg4)^{1u1}$ mutation. It was found previously that

acidic domains could act in conjunction and substitute for each other in mediating the insulating function of su(Hw) (HARRISON *et al.* 1993). However, in the presence of the *mod(mdg4)^{lu1}* mutation, the acidic domains have different functions in the inhibition of *yellow* transcription. The carboxy-terminal acidic domain of the su(Hw) protein plays a crucial role in the realization of the inhibitory action of *mod(mdg4)^{lu1}* on *yellow* expression in bristles and partially in the body and wings. Even some decrease in the number of carboxy-terminal acidic domains in *su(Hw)^{l}/ su(Hw)$^{+}$* heterozygotes completely suppresses the inhibitory action of the *mod-(mdg4)^{lu1}* mutation on *yellow* expression in bristles. In the presence of the *mod(mdg4)^{lu1}* mutation, the amino-terminal acidic domain has not a significant effect on *yellow* expression in bristles, but a su(Hw) protein with a deletion of this domain fails to block *yellow* transcription in the body and wings. It is not clear why this direct inhibitory effect cannot be observed in other tested *gypsy*-induced mutations. The specificity of transcription factors that interact with the *yellow* promoter and/or some features of the DNA domain architecture may be responsible for this observation.

Mechanism of alterations in the insulating properties of su(Hw): The insulating effect of the su(Hw)-binding region becomes much weaker in the presence of *mod-(mdg4)^{lu1}* mutation. This may result both in the activation of a suppressed gene (*ct^{6}, sc^{D1}*) and in the inhibition of an overexpressed gene (*Hw^{l}*). In the latter case, the su(Hw)-binding region probably isolates a silencer from the promoter. For the insulating function, the su(Hw) protein needs the acidic and leucine zipper domains (HARRISON *et al.* 1993). We have found that insulation is strongly but not completely inhibited by the *mod-(mdg4)^{lu1}* mutation. Thus, the mod(mdg4) protein may be directly involved in insulation through the formation of a complex with su(Hw). In the absence of the mod(mdg4) protein, insulation becomes completely dependent on the presence of the amino-terminal acidic domain.

Most *su(Hw)* mutations have dominant effects in the presence of the *mod(mdg4)^{lu1}* mutation. A strong *su(Hw)* mutation, even as heterozygous, suppresses the *mod-(mdg4)^{lu1}* effect on *yellow* expression in bristles and partially in the body and wings. A small additional amount of the su(Hw) protein in the *su(Hw)^{2}* and *su(Hw)^{f3}* mutations reduces this suppression. Many *su(Hw)* mutations in heterozygotes completely suppress insulation in combination with *mod(mdg4)^{lu1}*. Such dependence of all effects on the su(Hw) protein concentration in the presence of *mod(mdg4)^{lu1}* may be if the mod(mdg4) protein bound to su(Hw) enhances specific binding of the latter to the su(Hw)-binding region.

It is interesting to compare the results obtained with the *su(Hw)^{f3}* allele [caused by a 10-fold decrease in the su(Hw) protein concentration], with those obtained with *su(Hw)^{e2}* [which resuslts in a slight decrease of the DNA-binding capacity of the su(Hw) protein]. Both mutations are weak but behave like strong *su(Hw)* alleles and completely suppress all *gypsy*-induced mutations in the presence of *mod(mdg4)^{lu1}*. In this respect, they are similar, but in heterozygotes with *su(Hw)$^{+}$* only *su(Hw)^{e2}* completely suppresses the inhibitory effect of *mod(mdg4)^{lu1}* behaving again like strong *su(Hw)* mutations in which no functional su(Hw) protein is produced. Probably, the hypomorth *mod(mdg4)^{lu1}* mutation prevents the binding of the su(Hw)e2 protein to the su(Hw)-binding region. This result is in agreement with the suggestion that the role of *mod(mdg4)* is to stabilize specific su(Hw) protein interactions with the su(Hw)-binding region.

The work reported here gives insights into the mechanisms of the interaction between the *su(Hw)* and *mod(mdg4)* mutations in the regulation of gene expression. Additional molecular studies of the interaction between these two proteins will help to further understand these mechanisms.

We thank Dr. VICTOR CORCES for discussions and help with the manuscript; Drs. D. GDULA and P. SMITH for fly strains containing *su(Hw)* and *yellow* gene constructions. This research was supported by grants from the Russian Basic Research Fund, the Russian State Program "Frontiers in Genetics," and an Award from the Fogarty International Center to P.G.

LITERATURE CITED

ALONSO, M. C., and C. V. CABRERA, 1988 The achaete-scute gene complex of *Drosophila melanogaster* comprises four homologous genes. EMBO J. **7:** 2581–2591.

BALCELLS, L., J. MODOLELL and M. RUIZ-GOMEZ, 1988 A unitary basis for different *Hairy-wing* mutations of *Drosophila melanogaster*. EMBO J. **7:** 3899–3906.

CAMPUZANO, S., L. CARRAMOLINO, C. CABRERA, M. RUIZ-GOMEZ, R. VILLARES *et al.*, 1985 Molecular genetics of the *achaete-scute* gene complex of *D. melanogaster*. Cell **44:** 327–338.

CORCES, V. G., and P. K. GEYER, 1991 Interactions of retrotransposons with the host genome: the case of the *gypsy* element of Drosophila. Trends Genet. **7:** 86–90.

DORSETT, D., 1990 Potentiation of a polyadenylation site by a downstream protein-DNA interaction. Proc. Natl. Acad. Sci. USA **87:** 4373–4377.

DORSETT, D., 1993 Distance-independent inactivation of an enhancer by the suppressor of Hairy-wing DNA-binding protein of Drosophila. Genetics **134:** 1135–1144.

DORSETT, D., G. A. VIGLIANTI, B. J. RUTLEDGE and M. MESELSON, 1989 Alteration of *hsp82* gene expression by the *gypsy* transposon and suppressor genes in *Drosophila melanogaster*. Genes Dev. **3:** 454–468.

DREESEN, T. D., S. HENIKOFF and K. LOUGHNEY, 1991 A pairing-sensitive element that mediates trans-inactivation is associated with the Drosophila *brown* gene. Genes Dev. **5:** 331–340.

GEORGIEV P. G., and V. G. CORCES, 1995 The su(Hw) protein bound to gypsy sequences in one chromosome can repress enhancer-promoter interactions in the paired gene located in the other homolog. Proc. Natl. Acad. Sci. USA. **92:** 5184–5188.

GEORGIEV, P. G., and T. I. GERASIMOVA, 1989 Novel genes influencing the expression of the *yellow* locus and *mdg4* (*gypsy*) in *Drosophila melanogaster*. Mol. Gen. Genet. **220:** 121–126.

GEORGIEV P. G., V. A. YELAGIN, E. M. BUFF and N. P. KOLYAGIN, 1992 Properties of super-unstable mutations in the *yellow* locus of *Drosophila melanogaster*. Genetica (in Russia) **28:** 98–107.

GEYER, P. K., and V. G. CORCES, 1987 Separate regulatory elements are responsible for the complex pattern of tissue-specific and developmental transcription of the *yellow* locus in *Drosophila melanogaster*. Genes Dev. **1:** 996–1004.

GEYER, P. K., and V. G. CORCES, 1992 DNA position-specific repression of transcription by a Drosophila zinc finger protein. Genes Dev. **6:** 1865–1873.

GEYER, P. K., M. M. GREEN and V. G. CORCES, 1988 Mutant gene phenotypes mediated by a *Drosophila melanogaster* retrotransposon require sequences homologous to mammalian enhancers. Proc. Natl. Acad. Sci. USA **85:** 8593–8597.

GEYER, P. K., M. M. GREEN and V. G. CORCES, 1990 Tissue-specific transcriptional enhancers may act in trans on the gene located in the homologous chromosome: the molecular basis of transvection in Drosophila. EMBO J. **9:** 2247–2256.

HARRISON, 1991 Analysis of *suppressor of Hairy-wing*, a gene encoding a putative transcription factor of Drosophila. Ph.D. Thesis, The Johns Hopkins University, Baltimore, MD.

HARRISON, D. A., D. A. GDULA, R. S. COYNE and V. G. CORCES, 1993 A leucine zipper domain of the suppressor of Hairy-wing protein mediates its repressive effect on enhancer function. Genes Dev. **7:** 1966–1978.

HOLDRIDGE, C., and D. DORSETT, 1991 Repression of *hsp70* heat shock gene transcription by the suppressor of Hairy-wing protein of *Drosophila melanogaster*. Mol. Cell. Biol. **11:** 1894–1900.

JACK, J. W., 1985 Molecular organization of the *cut* locus of *Drosophila melanogaster*. Cell **42:** 869–876.

JACK, J., D. DORSETT, Y. DELOTTO and S. LIU, 1991 Expression of the *cut* locus in the Drosophila wing margin is required for cell type specification and is regulated by a distant enhancer. Development **113:** 735–747.

LINDSLEY, D. L., and G. G. ZIMM, 1992 *The Genome of Drosophila melanogaster*. Academic Press, New York.

LIU, S., E. MCCLEOD and J. JACK, 1991 Four distinct regulatory regions of the *cut* locus and thier effect on cell type specification in Drosophila. Genetics **127:** 151–159.

MAZO, A. M., L. J. MIZROKHI, A. A. KARAVANOV, Y. A. SEDKOV, A. A. KRICHEVSKAYA *et al.*, 1989 Suppression in Drosophila: *su(Hw)* and *su(f)* gene products interact with a region *gypsy* (*mdg4*) regulating its transcriptional activity. EMBO J. **8:** 903–911.

MODOLELL, J., W. BENDER and M. MESELSON, 1983 *Drosophila melanogaster* mutations suppressible by the *suppressor of Hairy- wing* are insertions of a 7.3-kilobase mobile element. Proc. Natl. Acad. Sci. USA **80:** 1678–1682.

PARKHURST, S., and V. G. CORCES, 1986 Interactions among the *gypsy* element and the *yellow* and *suppressor of Hairy-wing* loci in *Drosophila melanogaster*. Mol. Cell. Biol. **6:** 47–53.

PARKHURST, S. M., D. A. HARRISON, M. P. REMINGTON, C. SPANA, R. L. KELLEY *et al.*, 1988 The Drosophila *su(Hw)* gene, which controls the phenotypic effect of the *gypsy* transposable element, encodes a putative DNA-binding protein. Genes Dev. **2:** 1205–1215.

ROBERTSON, H. M., C. R. PRESTON, R. M. PHILLIPS, D. JOHNSON-SCHLITZ, W. K. BENZ *et al.*, 1988 A stable genomic source of P element transposase in *Drosophila melanogaster*. Genetics **118:** 461–470.

ROSEMAN, R. R., V. PIRROTTA and P. K. GEYER, 1993 The su(Hw) protein insulates expression of the *Drosophila melanogaster white* gene from chromosomal position-effects. EMBO J. **12:** 435–442.

SMITH, P. A., and V. G. CORCES, 1992 The suppressor of Hairy-wing binding region is required for *gypsy* mutagenesis. Mol. Gen. Genet. **233:** 65–70.

SPANA, C., and V. G. CORCES, 1990 DNA bending is a determinant of binding specificity for a Drosophila zinc finger protein. Genes Dev. **4:** 1505–1515.

SPANA, C., D. A. HARRISON and V. G. CORCES, 1988 The *Drosophila melanogaster* suppressor of Hairy-wing protein binds to specific sequences of the *gypsy* retrotransposon. Genes Dev. **2:** 1414–1423.

Communicating editor: J. A. BIRCHLER