hobo Induced Rearrangements in the *yellow* Locus Influence the Insulation Effect of the *gypsy* su(Hw)-Binding Region in *Drosophila melanogaster*

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ABSTRACT

The su(Hw) protein is responsible for the insulation mediated by the su(Hw)-binding region present in the *gypsy* retrotransposon. In the y^2 mutant, su(Hw) protein partially inhibits *yellow* transcription by repressing the function of transcriptional enhancers located distally from the *yellow* promoter with respect to *gypsy*. y^2 mutation derivatives have been induced by the insertion of two *hobo* copies on the both sides of *gypsy*: into the *yellow* intron and into the 5′ regulatory region upstream of the wing and body enhancers. The *hobo* elements have the same structure and orientation, opposite to the direction of *yellow* transcription. In the sequence context, where two copies of *hobo* are separated by the su(Hw)-binding region, *hobo*-dependent rearrangements are frequently associated with duplications of the region between the *hobo* elements. Duplication of the su(Hw)-binding region strongly inhibits the insulation of the *yellow* promoter separated from the body and wing enhancers by *gypsy*. These results provide a better insight into mechanisms by which the su(Hw)-binding region affects the enhancer function.

INSERTIONS of *gypsy* (*mdg4*) retrotransposons into various *Drosophila melanogaster* genes result 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 effect has been extensively studied by using the *yellow* (*y*) gene (Corces and Geyer 1991). The *gypsy*-induced y^2 allele displays a tissue-specific mutant phenotype characterized by the loss of pigmentation in the wings and in the body cuticle, whereas all other tissues of the larvae and adult flies show the wild-type coloration (Nash and Yarkin 1974). In this mutation, *gypsy* was inserted at −700 bp from the transcription start site of the *yellow* gene. The enhancers controlling *yellow* expression in the wings and in the body cuticle are located upstream of the *gypsy* insertion site (Geyer *et al.* 1986; Parkhurst and Corces 1986; Geyer and Corces 1987; Martin *et al.* 1989). The region of *gypsy* responsible for its mutagenic effect is the binding site for the su(Hw) protein (Parkhurst *et al.* 1988; Spana *et al.* 1988; Mazo *et al.* 1989; Dorsett 1990; Spana and Corces 1990). Thus, it has properties characteristic of a chromatin insulator: only enhancers located distally from the promoter are affected (Corces and Geyer 1991; Holdridge and Dorsett 1991; Jack *et al.* 1991; Geyer and Corces 1992; Roseman *et al.* 1993; Cai and Levine 1995; Scott and Geyer 1995). The second gene that affects *gypsy*-induced phenotypes, *modifier of mdg4* [*mod(mdg4)*], encodes a protein that interacts with su(Hw). Mutations in *mod(mdg4)* enhance the phenotype of the y^2 by inactivating *yellow* transcription (Georgiev and Gerasimova 1989; Georgiev and Corces 1995), either due to changes in the chromatin structure that interferes with the function of all enhancers of the *yellow* gene (Gerasimova *et al.* 1995; Gerasimova and Corces 1996) or by direct inhibition of the *yellow* promoter (Georgiev and Kozycina 1996).

In this article, we describe the genetic instability induced by *hobo* transposable elements in derivatives of the y^2 mutation. *hobo* is a small transposon (3 kb in size) with short inverted repeats (Streck *et al.* 1986). The largest *hobo* element encodes a transposase that is specific for the members of the *hobo* family (Blackman *et al.* 1989; Calvi *et al.* 1991). The first derivative of the y^2 allele was induced by the insertion of two *hobo* elements: in the intron of the *yellow* gene and in the 5′ regulatory region downstream to the body and wing enhancers. Both *hobo* elements had the same direction and identical restriction maps. In contrast to previous observations (Calvi *et al.* 1991; Ho *et al.* 1993; Sheen *et al.* 1993), duplications of the region between *hobo* elements occurred with a high frequency. The duplications included the regulatory region of the *yellow* gene and *gypsy* sequences. Flies with such duplications showed the wild-type level of pigmentation of the body and wings, which seemed to be due to the normal expression

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of the *yellow* gene controlled by the *yellow* transcriptional enhancers, although they remained flanked by the su(Hw)-binding region.

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. Additional information about the genetic markers can be found in Lindsley and Zimm (1992).

The following strains were synthesized in the previous work (Georgiev and Kozycina 1996): *XX/Y; Xa/D, XX/Y; su(Hw)^2/Xa, XX/Y; su(Hw)^v/Xa, XX/Y; mod(mdg4)^{1u1}/mod(mdg4)^{1u1}*, where *XX* is an abbreviation for *C(I)RM, y f*; *Xa* is an abbreviation of the translocation *T(2;3) ap^{Xa}ap^{Xa}*.

Genetic crosses: The *y^{2}sc^{D1}w^{aG}* strain contains about 20 *hobo* copies. The *C(1)RM, yf* strain has no *hobo* elements. Crosses of *y^{2}sc^{D1}w^{aG}* males with *C(1)RM,yf* females activate the transposition of *hobo*. To study *hobo*-mediated rearrangements in the *y* alleles, dysgenic *y*sc^{D1}w^{aG}* males (*y** - *hobo*-induced *y* allele) were individually crossed to 6-8 *C(1)RM,yf* females. The males with a new y phenotype were mated to *C(1)RM,yf* females, and the phenotype was examined in the next generation. Only the similar events obtained from independent males were referred to as independent events. The stocks with new *y* alleles were established, but in general they retained some level of instability, and the males with the new y phenotype appeared with a low frequency, $\sim 1 \times 10^{-3}$.

The phenotypic analysis was performed at 25° in 3–5-day-old males. The results were compared with those obtained in control flies with a known phenotype (Georgiev *et al.* 1992). The degree to which the *y* alleles differed from the wild type was determined visually. The wild-type expression was estimated at 5 points, whereas the absence of *yellow* expression was indicated by 0.

To study the influence of the *su(Hw)^2/su(Hw)^v* heterozygote or the *mod(mdg4)^{1u1}/mod(mdg4)^{1u1}* homozygote on the expression of the *y* alleles, the following crosses were carried out. Males with a *y* allele to be tested were crossed to *C(1)RM,yf* females carrying the *Drop* (*D*) mutation as a dominant marker. F_1 *y; D/+* males were crossed to *C(1)RM,yf; su(Hw)^2/Xa* or *C(1)RM,yf; mod(mdg4)^{1u1}/mod(mdg4)^{1u1}* females. F_2 *y; D/su(Hw)^2* or *y; D/mod(mdg4)^{1u1}* males were crossed to *C(1)RM,yf su(Hw)^v/Xa* or *C(1)RM,yf; mod(mdg4)^{1u1}/mod(mdg4)^{1u1}* females. Analysis of the phenotype of *y; su(Hw)^2/su(Hw)^v* or *y; mod(mdg4)^{1u1}/mod(mdg4)^{1u1}* males was performed at 25° in the F_3 or F_4 generation. The results were compared with those obtained in control flies.

Molecular methods: For Southern blot hybridization, DNA from adult flies was isolated using the protocol described by Ashburner (1989). Treatment of DNA with restriction endonucleases, blotting, fixation, and hybridization with radioactive probes prepared by random primer extension was performed as described in the protocols for the Hybond-N$^+$ nylon membrane (Amersham, Arlington Heights, IL) and in the laboratory manual (Sambrook *et al.* 1989). Phage with cloned regions of the *yellow* locus were obtained from J. Modolell (Campuzano *et al.* 1985) and V. Corces (Geyer *et al.* 1986). The probes were made from gel-isolated fragments after an appropriate restriction digestion of plasmid subclones.

For Northern blot hybridization, total RNA was extracted at the pupal stages by using the sodium dodecyl sulfate (SDS)-phenol technique (Spradling and Mahowald 1979). The samples were homogenized in 10 ml of 10 mm Tris-HCl (pH 7.4), 100 mm NaCl, 1 mm EDTA, 0.5% SDS, and the homogenate was extracted several times with phenol-chloroform with subsequent chloroform extraction. Poly(A)$^+$ RNA was then isolated by chromotography on oligo(dT)-cellulose and fractionated by electrophoresis, transferred to Nytran membranes (Schleicher and Schuell, Keene, NH), and incubated with ^{32}P-labeled probes. The DNA fragment used as a hybridization probe to detect the *yellow* transcript was obtained by digestion of the cDNA clone of the *yellow* gene with *Hin*dIII and *Bgl*II restriction endonucleases. The *yellow* cDNA clone was obtained from P. Geyer.

Genomic DNA libraries were constructed using DNA partially digested with *Sau*3A. The digested DNA was ligated in the λ*Gem*11/*Bam*HI phage vector (Promega, Madison, WI). The recombinant DNA was packaged *in vitro* using a packaging extract from Promega, and the phage particles were plated using the *Escherichia coli* strain LE392 at a density of 3000 pfu/plate. The plaques were blotted onto Hybond-N nylon membranes according to the supplier protocol (Amersham). These membranes were hybridized with ^{32}P-labeled DNA probes to select the desired plaques; 30,000–40,000 plaques from each recombinant DNA library were screened. Positive plaques were picked up from the plates and rescreened to obtain pure clones.

In other cases, DNA samples were restricted with *Bam*HI endonuclease and subjected to agarose gel electrophoresis. Bands of corresponding size were cut from the gel, and DNA was extracted by electroelution. After that, the DNA was ligated to the arms of the λ*Gem*11/*Bam*HI phage vector (Promega).

Subcloning and purification of the plasmid DNA and mapping of restriction sites were performed by standard techniques (Sambrook *et al.* 1989).

Genomic DNAs were subjected to PCR to amplify sequences from the *y* allele (Saiki *et al.* 1985; Mullis and Faloona 1987). The primers used in DNA amplification were as follows: from the *hobo* element, 5′GACTCGACTACCTACGAGACC3′ [h1, 313-293 according to the map of the *hobo* element described by Streck *et al.* (1986)]; from the *yellow* locus, 5′GAATGCTGCGTTTGTCTGTTTGG3′ [y1, 1181-1159 (Geyer *et al.* 1986)] and 5′TCTGTGGACCGTGGCGCGGTAAC3′ (y2, 2899-2877); from the *gypsy* mobile element, 5′CAACCTTGCA GAGGACTCCTTAG3′ [g1, 2674-2696 (Marlor *et al.* 1986)]. The products of amplification were fractionated by electrophoresis in 1–2% agarose gels in Tris-acetate (TAE) buffer.

DNA sequencing was performed according to the dideoxy chain-termination methodology (Sanger *et al.* 1977). The PCR product was directly sequenced using a Sequenase II DNA sequencing kit for PCR product (Amersham) according to the manufacturer's instructions.

RESULTS

The original *hobo*-induced *y* allele, *y^{dh1}*, contains the su(Hw)-binding region surrounded by two *hobo* elements: The original *y^{dh1}* allele spontaneously appeared in the *y^{2}sc^{D1}w^{aG}* strain. In the parental *y^2* allele (the yellow color of the body cuticle and wing blade), *yellow* transcription in the body and wings was blocked by the su(Hw)-binding region of *gypsy*. By contrast, *y^{dh1}* flies displayed a weak pigmentation of the wings and, in addition, the mutant color of bristles on the notum and legs (Table 1).

To understand the molecular basis, the *y^{dh1}* allele was cloned. A recombinant DNA library was probed with the *Sal*I-*Bgl*II and *Hin*dIII-*Bam*HI fragments from the *yellow* locus. Three recombinant phages hybridizing with

TABLE 1

Phenotypes of *hobo*-induced *yellow* alleles and the effect of the *su(Hw)* mutation

	Pigmentation					
			Bristles			
y alleles	Body	Wings	Th	L	W	Ab
y^{2}	1(5)	1(5)	5	5	5	5
y^{dh*}	1(5)	2(5)	1	2	5	5
y^{rh*}	5(5)	5(5)	1	2	5	5
y^{mh32}	4	4	1	2	5	5
y^{lh*}	3(5)	3(5)	1	2	5	5
y^{1h}	0	0	0	0	0	0
y^{2h15}	1(5)	1(3)	1	2	5	5
y^{2h12}	1(1)	1(2)	1	2	5	5
y^{2h131},y^{2h115}	1(1)	1(2)	1	2	5	5
y^{2h16}	1(2)	1(3)	2(5)	3(5)	5	5
y^{2h25}	1(2)	1(4)	2(5)	3(5)	5	5
y^{2h29}	1	1	5	5	5	5

Bristles are subdivided into thoracic (Th), leg (L), wing (W), and abdominal (Ab). The number in parentheses shows the effect of the $su(Hw)^{v}/su(Hw)^{2}$ mutations combination. For determination of the *yellow* phenotype, the levels of pigmentation in different tissues of adult flies were estimated visually in 3–5-day-old males developing at 25°.

y^{dh*} (y^{dh1}, y^{dh12}, y^{dh19}, y^{dh21}, y^{dh24}), y^{lh*} (y^{lh11}, y^{lh13}, y^{lh14}, y^{lh15}, y^{lh17}), y^{rh*} (y^{rh1}, y^{rh2}, y^{rh3}, y^{rh7}, y^{rh9}).

Classification of *y* alleles is given in other work (Georgiev *et al.* 1992). The *y* alleles with the similar phenotype have an identical combination of letters in the superscript. The letters in superscripts indicate the following: "*h*," the allele has been obtained in a system with an active *hobo* element; "2," the pigmentation of body and wings of flies with this allele is the same as in y^{2} flies; "*d*," "*l*," "*m*," "*r*," the pigmentation of wings corresponds to the 2+, 3+, 4+, and 5+ levels, respectively. The numbers in the superscript of the allele indicate the origin of the allele; for example, y^{2h115} allele is derivative of y^{lh11} allele.

both probes and five recombinant phages hybridizing with only one probe were obtained. The restriction analysis of the obtained phage clones did not reveal any changes in the *gypsy* sequences but showed the presence of two additional insertions in the *yellow* gene (Figure 1).

DNA sequencing of the insertions showed that both of them corresponded to a partially deleted 2.2-kb *hobo* mobile element. One *hobo* designated as *hobo-1* (*yellow*-proximal element) was inserted in the *yellow* intron at the position +875 to the *yellow* gene transcription start site (Geyer *et al.* 1986); that is, it was located in the region of the *yellow* bristle enhancer (Geyer and Corces 1987; Martin *et al.* 1989). Thus, the decrease of notum and leg bristle pigmentation might be a result of partial inactivation of the bristle enhancer element. The second *hobo*, *hobo-2* (distal element), was inserted at the position −2464, within the region of the wing enhancer of the *yellow* locus (Geyer and Corces 1987; Martin *et al.* 1989). Both *hobo* elements were flanked by 8-bp target site duplications, CTTTATAC and ATATCTAG, respectively. These *hobo* elements had identical structures and were inserted in the same orientation, opposite the direction of *yellow* transcription.

To elucidate the role of *hobo* mobile elements in the altered expression of *yellow*, we examined the phenotype of y^{dh1} in flies heterozygous for the $su(Hw)^{2}/su(Hw)^{v}$ mutations, which almost completely inactivate the *su(Hw)* gene (Harrison *et al.* 1993). The y^{dh1}; $su(Hw)^{2}/su(Hw)^{v}$ flies exhibited wild-type levels of pigmentation in the body cuticle and wing blade but a mutant coloration of the notum and leg bristles (Table 1). This suggests that in y^{dh1} the su(Hw) protein acts to block the body and wing enhancers. *hobo* insertions slightly activate the expression of *yellow* in the wings and repress it in the notum and leg bristles.

An interesting feature of the system was that two *hobo* elements were separated by a strong insulator, the su(Hw)-binding region of *gypsy*. Therefore, we decided to analyze the mutagenesis in the system and the nature of the mutations obtained. The two-step analysis of mutational changes resulting in new phenotypes was performed: (1) Southern blot hybridization with the fragments of the *yellow* locus (Figure 1) and (2) cloning of the changed fragments and their detailed mapping and analysis. The observed structural changes were compared with phenotypic changes.

***hobo*-Induced rearrangements are frequently associated with duplications of the region between two *hobo* elements:** In the F_2 generation from dysgenic crosses (see above), two main classes of mutant derivatives were obtained from the y^{dh1} strain (Tables 1 and 2): y^{1h} (complete inactivation of the *yellow* gene) and y^{rh} (normal pigmentation of the body and wings but a mutant phenotype in the notum and leg bristles).

Two examined y^{1h} alleles had a deletion of *yellow* and *gypsy* sequences located between the *hobo* elements (Figure 2A). The other three y^{1h} alleles were induced by deletions extending from *hobo-1* to the *yellow* regions located to the left or to the right of the *hobo* insertion (data not shown). As a result, *yellow* expression was completely inactivated.

DNAs from six independent y^{rh} alleles were probed with the fragments of the *yellow* gene. All bands characteristic of y^{dh1} DNA were detected. At the same time, additional hybridizing bands appeared that were the same in all DNA samples (Figure 2, B and C). It was suggested that the y^{rh} alleles were associated with the duplication of some parts of the *yellow* gene. We cloned DNA fragments of y^{rh1} corresponding to the two *Bam*HI bands obtained in the course of electrophoresis, which hybridized to the *yellow* probe. Detailed restriction maps of the cloned DNAs are shown in Figure 3. The y^{rh1} allele was derived by the duplication of the region between two *hobo* elements. All repeated elements in y^{rh1} and other mutations with the duplication are numerated in the *yellow*-proximal to the *yellow*-distal direction (*hobo-1*, *2*, and *3*, *gypsy-1* and *2*, etc.)

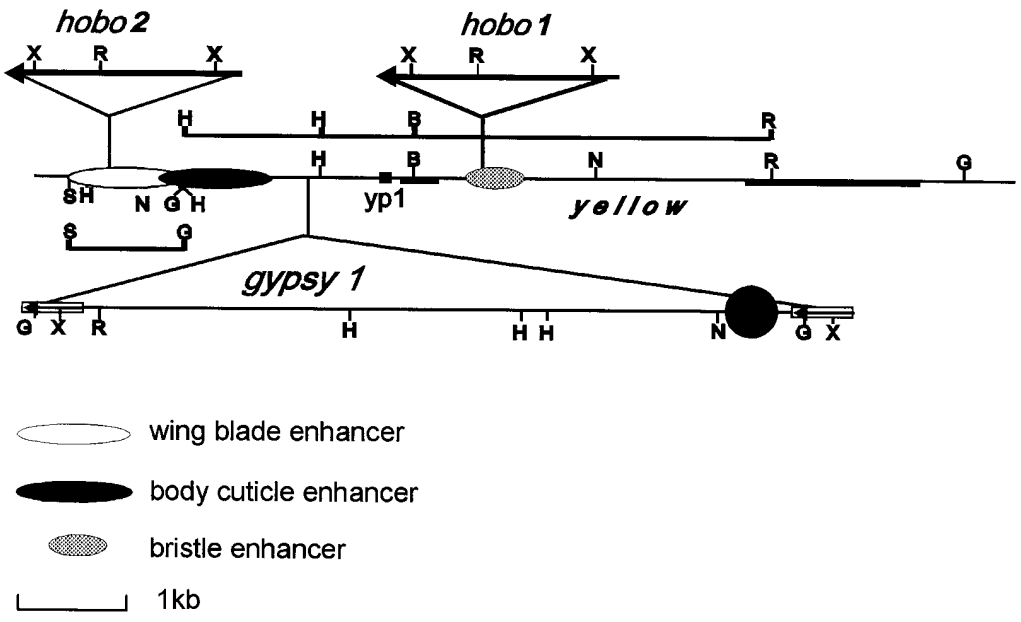

Figure 1.—The structure of the *yellow* locus in the y^{dh1} allele. Two exons of the *yellow* gene are shown by thick black lines separated by one intron. The *gypsy* element is inserted at −700 bp from the transcriptional start site, as in the y^{2} allele. The arrows in boxes indicate the *gypsy* LTRs and their direction. The black circle shows the su(Hw)-binding region. The transcriptional enhancers of the *yellow* gene are represented by ovoid structures. The thick arrows indicate *hobo* elements and their direction. R, *Eco*RI; H, *Hin*dIII; G, *Bgl*II; X, *Xho*I; B, *Bam*HI; S, *Sal*I; N, *Nco*I. The genomic DNA fragments *Sal*I - *Bgl*II, *Hin*dIII - *Hin*dIII, *Hin*dIII - *Bam*HI, and *Bam*HI - *Eco*RI, used for Southern blot hybridization, are indicated by black lines. yp1, *yellow* promoter 1.

The DNA of y^{rh7} flies that had been restricted with *Bam*HI or *Bgl*II differed from other y^{rh} alleles in Southern blot analysis (Figure 2, C and D). The y^{rh7} allele had a 6-kb deletion that occupied the region extending from the *hobo-3* element and partially included *gypsy-2*, which left the su(Hw)-binding region of the latter unchanged (Figure 3). PCR cloning of the deletion breakpoints showed that the sequences between the 5′ end of the *hobo*-3 element and 3428 bp in the *gypsy* sequence were deleted (according to the *gypsy* map presented by Marlor *et al.* 1986).

It may be concluded that the duplication of the *hobo*-flanked region is the main mutagenic event in the system.

Loss of insulation in the y^{rh} alleles: As has been shown above for y^{rh} alleles, the duplication of *hobo-2*, body and wing enhancers, *gypsy*, and *yellow* promoters led to the restoration of *yellow* expression. The $su(Hw)^{2}/su(Hw)^{v}$ heterozygote did not visually change the phenotype of y^{rh1}, y^{rh2}, y^{rh3}, y^{rh7} and y^{rh9} flies (Table 1). Thus, the duplication made it possible to somehow overcome the su(Hw)-dependent insulation of the *yellow* gene.

The y^{rh7} flies contain a deletion of distal *yellow* enhancers. Thus, in the presence of two su(Hw)-binding regions and two promoters, one pair of *yellow* enhancers, that is, the proximal body and wing enhancers, restored *yellow* transcription. The result could be explained either by the loss of insulation in a particular sequence context or by initiation of transcription from the distal promoter, *yellow* promoter 2, by the proximal enhancers not isolated from the latter by the su(Hw)-binding region.

To check whether the *yellow* promoter was properly activated in the system, the size and time of accumulation of *yellow* mRNA at the pupal stage were measured by Northern blot analysis (Figure 4). The RNAs isolated at three pupal stages from the y^{rh1} and *Oregon* strains had the same size, level, and time of expression, suggesting that the *yellow* gene in the mutant was transcribed from the normal promoter and was activated by its native enhancer elements.

Finally, we obtained one derivative allele, y^{mh32}, as a result of rearrangement between the *hobo* elements in the *yellow* and neighboring *achaete-scute* complex. The body and wing pigmentation of the y^{mh32} flies was close to wild type (Table 1). The origin of the mutation is quite different from mutations of the same class described above (M. Gause and P. Georgiev, unpublished results).

Briefly, the mutation was found to be induced by an additional inversion of the region between *hobo-2* and *hobo* located in the *scute* gene close to the *gypsy* su(Hw)-binding region (Figure 5). As a result, the body enhancer and part of the wing blade enhancer were flanked by two *gypsy* su(Hw)-binding regions and isolated from the *yellow* promoter 1. In this case, transcription of the *yellow* gene could start only from promoter 1, which is isolated from the enhancers by the su(Hw)-binding region. The decrease of pigmentation in the y^{mh32} flies may be explained by a deletion of the part of

TABLE 2

Mutagenesis in *hobo*-induced *yellow* alleles

Original *y* allele	Total number of flies scored	Main derivative alleles and a number of mutations obtained					Total frequency of mutagenesis
		y^{rh}	y^{lh}	y^{dh}	y^{2h}	y^{1h}	
y^{dh1}	2840	9		—	—	7	5.6×10^{-3}
y^{rh1}, y^{rh2}, y^{rh9}	8400	—	8	21	7	15	6.1×10^{-3}
y^{lh11}, y^{lh13}	1620	5			4	1	3.8×10^{-3}

The figures indicate the number of independent events, that is, the number of similar events obtained from different dysgenic F_1 males. Total frequency of mutagenesis means the ratio of the number of independent events to the total number of scored flies.

the wing enhancer (Figure 5). This result confirms the suggestion that the activation of *yellow* transcription in the body and wings really depends on the loss of insulation in the presence of two *gypsy* elements.

Genetic and molecular analysis of y^{rh} derivatives: To obtain y^{rh} derivative mutations, males from three independent strains, y^{rh1}, y^{rh2}, and y^{rh9}, were crossed to *C(1)RM,yf* females (Table 2). New alleles fell into four phenotypic classes: y^{1h} (complete inactivation of the *yellow* gene); y^{dh} (phenotype as in the parental y^{dh1} allele); y^{lh} (yellow notum and leg bristles and an intermediate level of body and wing pigmentation); and y^{2h} (the yellow color of the body and wings as seen in y^{2}).

DNAs from eleven randomly selected y^{1h} alleles did not hybridize to the probes from the *yellow* gene, indicating that these mutations represent deletions of se-

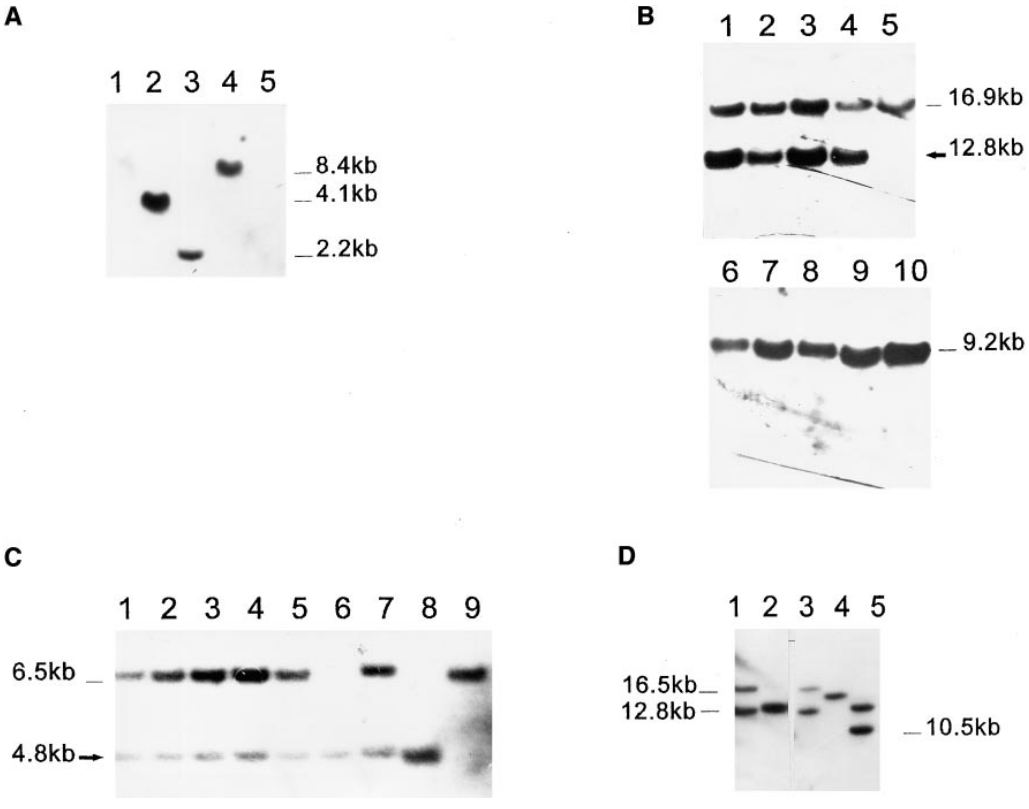

Figure 2.—Southern blot analysis of genomic DNAs from y^{dh} and its derivatives. (A) Southern blot analysis of genomic DNA from y^{1h1} (1), y^{1h2} (2), y^{1h3} (3), y^{dh1} (4), y^{1h4} (5) digested with *Bgl* II. The filter was hybridized with the *Hin*dIII-*Bam*HI fragment from the *yellow* locus. (B) Southern blot analysis of y^{rh} alleles. DNAs from y^{dh1} (5,10), y^{rh1} (1,6), y^{rh2} (2,7), y^{rh3} (3,8), and y^{rh9} (4,9) were digested with *Bam*HI (1-5) or *Bgl* II (6-10). The blots were probed with the *Hin*dIII-*Bam*HI fragment from the *yellow* locus. The bands corresponding to the duplicated region are indicated by arrows. (C) Southern blot analysis of y^{rh} alleles. DNAs from y^{rh1} (1), y^{rh2} (2), y^{rh3} (3), y^{rh4} (4), y^{rh5} (5), y^{rh7} (6,8), y^{rh9} (7), and y^{dh1} (9) were digested with *Bgl* II. The blots were probed with the *Sal* I-*Bgl* II fragment from the *yellow* locus. (D) Southern blot analysis of the y^{rh7} allele. DNAs of y^{rh1} (1, 3), y^{rh7} (2, 5), and y^{2h12} (4) were digested with *Bam*HI, and the blots were probed with the *Sal* I-*Bgl* II (1-2) and *Hin*dIII-*Bam*HI (3-5) fragments from the *yellow* locus.

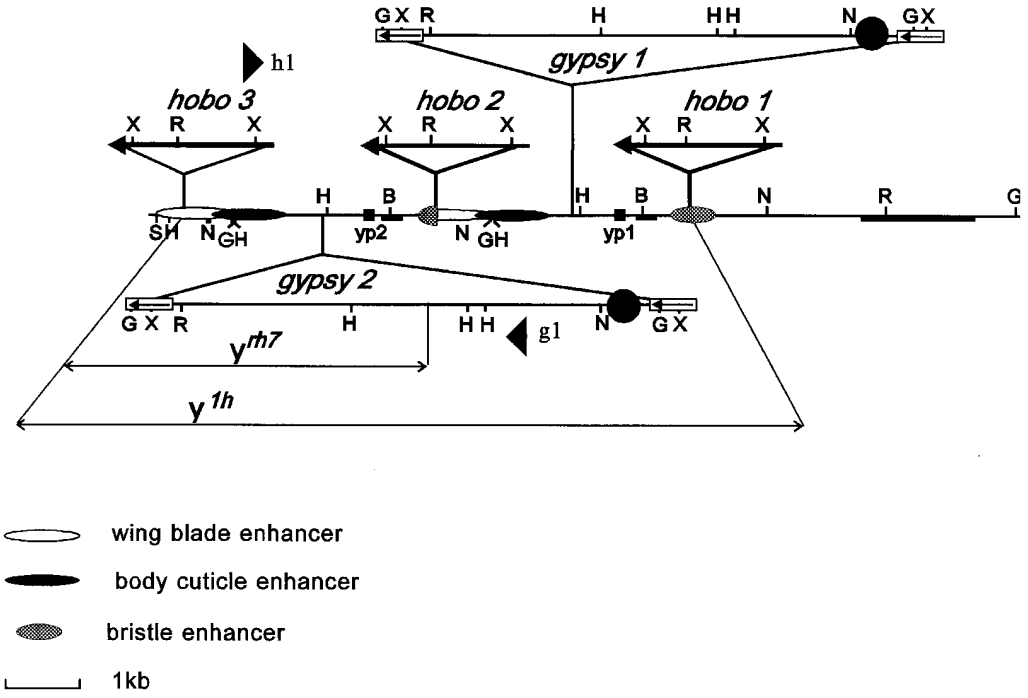

Figure 3.—The structure of the y^{rh1}, y^{rh7}, and y^{1h} alleles. The thin lines show the deletions in the y^{1h} and y^{rh7} alleles. Other designations are as in Figure 1. The breakpoints of deletion in the y^{rh7} allele were cloned by PCR between the primers in the *hobo* element (h1) and in the *gypsy* (g1). yp1, *yellow* promoter 1; yp2, *yellow* promoter 2.

quences between *hobo-1* and *hobo-3* (Figure 6). Ten derivative y^{dh} alleles had the same structure as the original y^{dh1} allele; that is, they were also induced by a recombination-mediated deletion of the sequences between *hobo-1* and *hobo-2*, between *hobo-2* and *hobo-3* elements, or between *gypsy-1* and *gypsy-2* (Figure 6). Two y^{1h} and three y^{dh} alleles appeared to result from complex inversions and additional duplications (data not shown). These alleles were not studied further.

Two other classes of mutations, y^{lh} and y^{2h}, were studied in more detail.

The nature of y^{lh} mutations: y^{lh} flies had yellow notum and leg bristles but an intermediate level of the body and wing pigmentation (Table 1). Four extensively studied y^{lh} DNAs restricted with *Bam*HI gave just one 24-kb band hybridizing to *yellow* gene probes (Figure 6). The 24-kb *Bam*HI fragment of y^{lh11} DNA was cloned. A detailed restriction map of the cloned y^{lh11} is shown in Figure 7A. The y^{lh11} mutation was caused by recombination between 5′-LTR of the *gypsy-2* and 3′-LTR of the *gypsy-1* and, as a result, the *hobo*-2 and *yellow* sequences located between the *gypsy* elements were deleted. According to Southern blot analysis, all y^{lh} alleles had the same structure, that is, they possessed two *gypsy* elements, lacking the intervening sequences. The $su(Hw)^2$ / $su(Hw)^v$ heterozygote suppressed the mutant phenotype of y^{lh} alleles (Table 1). This suggests that the su(Hw)-binding region partially but not completely blocks the body and wing enhancers in these alleles.

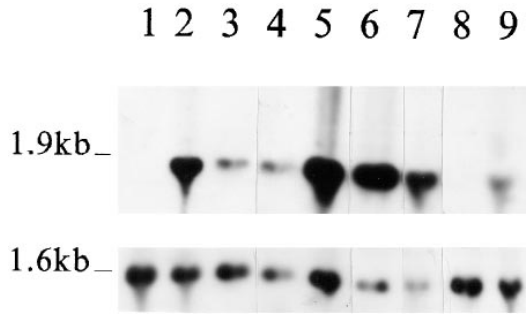

Figure 4.—Analysis of *yellow* transcripts in the mutant strains. Northern blot hybridization was performed with RNA isolated from y^{dh1}(1, 4, 8), y^{rh1}(3, 6, 9), and control Oregon flies (2, 5, 7). Poly(A)$^+$RNAs were isolated from 0–24-hr pupae (1-3), 48–72-hr pupae (4-6), and 72–96-hr pupae (7-9). ^{32}P-labeled DNA fragments containing the *yellow* and *ras2* genes of *D. melanogaster* were used as probes. The *yellow* probe hybridizes to 1.9-kb RNA, whereas *ras2* gives rise to a 1.6-kb transcript that is expressed at approximately constant levels during Drosophila development and is used as a marker for the amount of RNA.

To study the regulatory region responsible for the *yellow* activation in y^{lh} flies, we obtained the derivatives of the y^{lh11} and y^{lh13} alleles (Table 2). The major class of flies with a new mutation phenotype was y^{2h}. The mutant flies had no pigmentation of the body cuticle and the wing blade (Table 1).

Four mutant alleles were subjected to a molecular analysis: y^{2h111}, y^{2h112}, y^{2h115}, and y^{2h131}. Southern blot analysis showed deletions of 5 kb (y^{2h111}, y^{2h112}), 7 kb (y^{2h115}) and 8 kb (y^{2h131}) in the region flanking the *hobo*-2 element (Figure 7B). The deleted regions included the body and wing enhancers and a portion of the *gypsy* sequences (Figure 7A). In combination with the $su(Hw)^2$ / $su(Hw)^v$ heterozygote, y^{2h115} and y^{2h131} alleles exhibited only a slightly enhanced wing pigmentation. This result suggests that the body and wing blade enhancers can par-

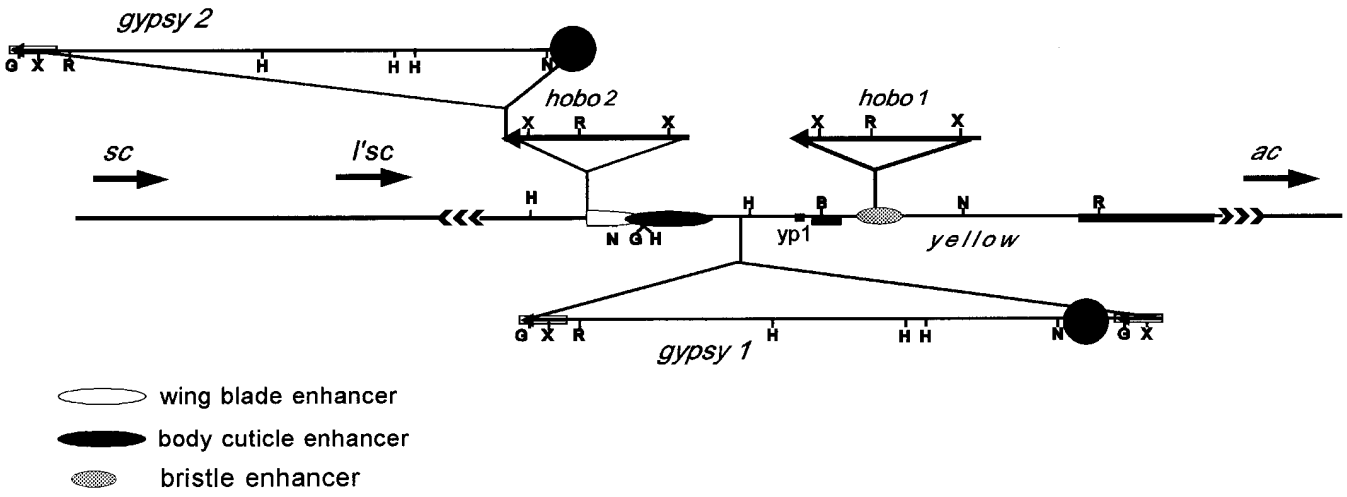

Figure 5.—The structure of the y^{mh32} allele. The arrows indicate direction of *achaete*, *scute*, and *l'scute* gene transcription. All other designations are as in Figure 1.

tially activate *yellow* expression in y^{lh} flies when separated from the promoter by two su(Hw)-binding regions.

The nature of y^{2h} mutations: y^{2h} flies had the same level of wing and body pigmentation as y^{2} flies (Table 1). According to Southern blot analysis, the y^{2h12}, y^{2h15}, y^{2h16}, y^{2h25}, and y^{2h29} alleles had a deletion of the duplication and of the adjacent *yellow* sequences (Figure 7). There were some minor phenotypic differences between different alleles correlating with the size of the deletion.

The color of bristles in y^{2h12} and y^{2h15} flies is similar to that of the y^{dh1} allele. In the y^{2h15} allele, only sequences between *hobo*-2 and the proximal *Bgl* II site were deleted (about 600 bp). PCR cloning and sequencing showed that the sequences were deleted between −2463 and −1953 positions relative to the transcription start site of the *yellow* gene. The mutant *y* phenotype of the y^{2h15} allele was suppressed in the body and partially in the wings in the $su(Hw)^{2}/su(Hw)^{v}$ heterozygote (Table 1). This was expected because the previously defined body enhancer (from −1963 bp to −1266 bp) was present in its entirety, together with a portion of the wing blade enhancer (−2873 bp to −2463 bp).

In the y^{2h12} allele Southern blot hybridization showed a 2-kb deletion (Figures 2D and 8B). Thus, sequences from −2463 to −700, between *hobo*-2 and *gypsy* 3′-LTR, were deleted. In the y^{2h12} allele only a part of the wing blade enhancer between −2873 and −2463 was present. However, the $su(Hw)^{2}/su(Hw)^{v}$ heterozygote in combination with y^{2h12} allele still partially increased the pigmentation of the wings (Table 1) and the last segment of the abdomen in y^{2h12} flies (data not shown).

The other three alleles, y^{2h16}, y^{2h25}, and y^{2h29}, exhibited a more extensive pigmentation of the notum and leg bristles. The y^{2h16} allele had a deletion of about 5 kb long that spread from *hobo*-2 into the *gypsy* body sequences. The y^{2h25} allele had the largest deletion, from *hobo*-2 up to the border of the su(Hw)-binding region. PCR cloning and sequencing showed that only 1178 bp from the 5′ end of *gypsy* were present in the y^{2h25} allele, including the su(Hw)-binding region. The $su(Hw)^{2}/su(Hw)^{v}$ heterozygote in combination with y^{2h16} and y^{2h25}

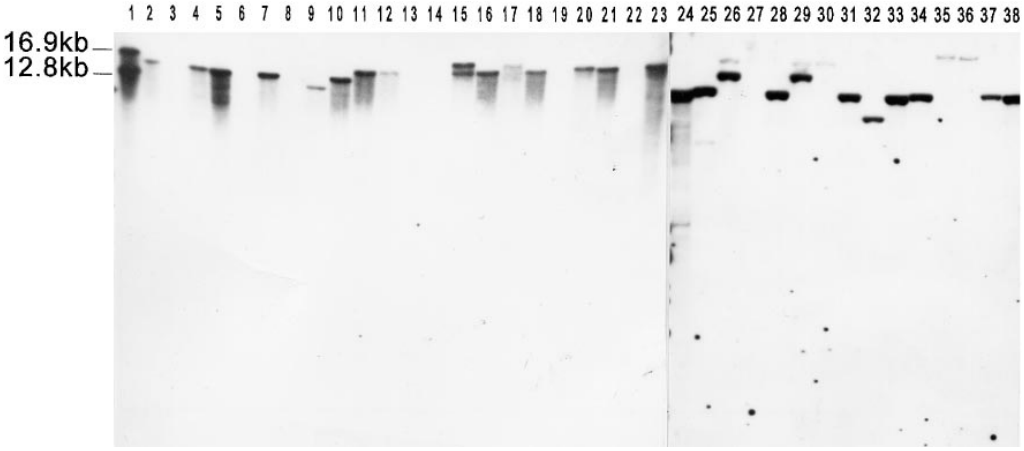

Figure 6.—Southern blot analysis of genomic DNAs from derivatives of the y^{rh1}, y^{rh2}, and y^{rh9} alleles. DNAs from the y^{1h} (3, 6, 8, 13, 14, 19, 22, 27, 30, 35, 36), y^{dh} (2, 4, 5, 7, 10, 16, 18, 20, 21, 24, 25, 28, 31, 33, 34, 37), y^{lh} (11, 12, 17, 26, 29), y^{2h} (9, 32), and y^{dh1} (23, 38) were digested with *Bam*HI, and the blots were probed with the *Hin*dIII-*Bam*HI fragment from the *yellow* locus.

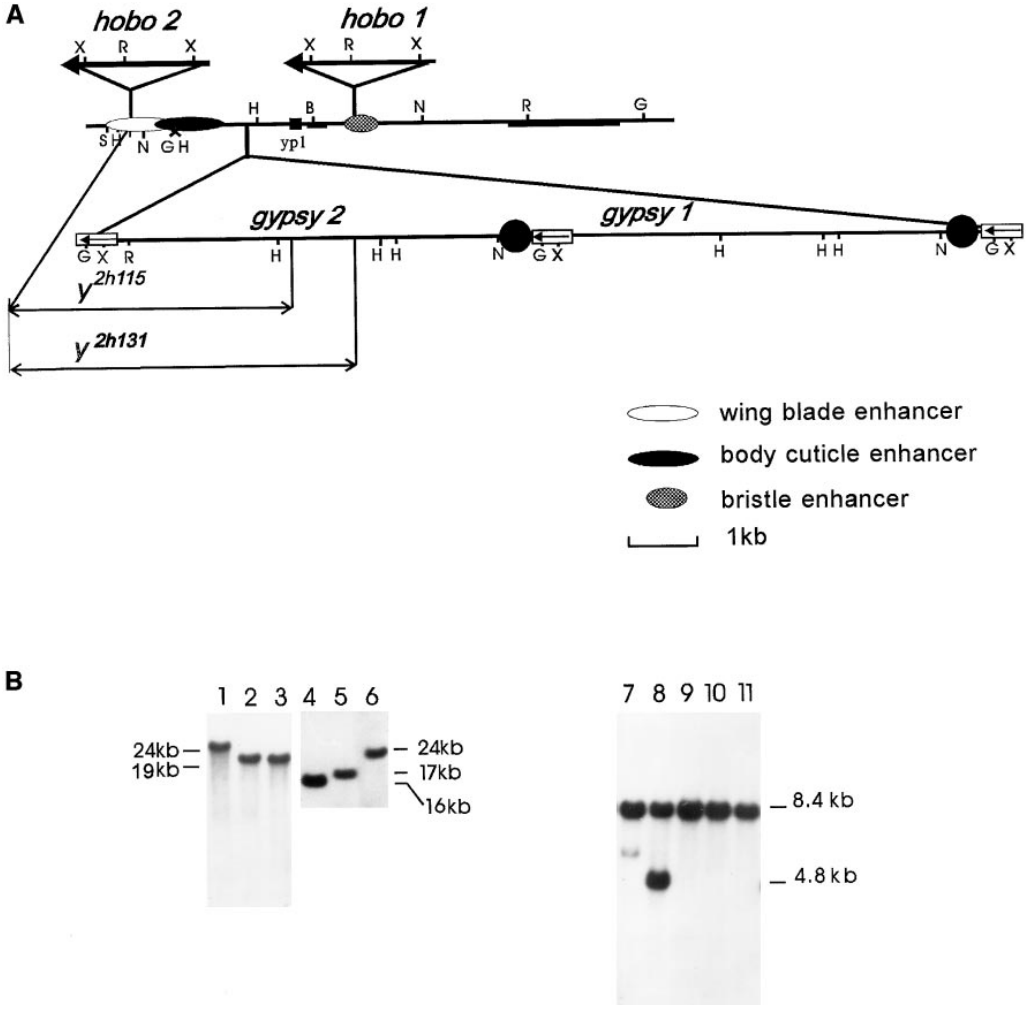

Figure 7.—The structure of the y^{lh11} and y^{lh13} alleles and their derivatives. (A) The structure of the y^{lnh11}, y^{2h115}, and y^{2h131} alleles. The arrows show the sizes of deletions in the y^{2h} alleles. All other designations are as in Figure 1. (B) Southern blot analysis of y^{lh11} derivatives. DNAs from y^{lh11} (1, 9), y^{2h111} (2), y^{2h112} (3), y^{2h131} (4, 10), y^{2h115} (5, 11), y^{lh13} (6), y^{dh1} (7), and y^{rh1} (8) were digested with *Bam*HI (1-6) or *Bgl*II (7-11). The blot was probed with the *Hin*dIII-*Bam*HI fragment from the *yellow* locus.

alleles significantly increased the pigmentation of the body, wings, and the tip of the abdomen and also completely suppressed the mutant *y* phenotype in bristles. This means, first, that about half of the wing enhancer can partially support *yellow* expression, not only in the wings but also in the body and in the tip of the abdomen. Second, both the *gypsy* body sequences and the su(Hw)-binding region participate in *hobo*-dependent repression of *yellow* transcription in the bristles.

This conclusion was supported by data on the structure of y^{2h29}, which had the same phenotype as y^{2} (Table 1). The Southern blot analysis, PCR cloning, and sequencing showed that the y^{2h29} allele had a deletion extending from *hobo*-2 to the *gypsy* 5′-LTR, thus removing the su(Hw)-binding region. In addition, an inversion between *hobo*-2 and another *hobo* located in an unidentified region of the genome removed the last part of the wing blade enhancer, from −2873 to −2463. Thus, in the absence of *gypsy*, the mutant bristle phenotype was completely reverted. Obviously, the $su(Hw)^{2}$/$su(Hw)^{v}$ heterozygote did not increase the pigmentation of y^{2h29} flies.

The presented results suggest that the body and wing enhancers have a modular organization and partially overlapping functions, in contrast to previous data (Geyer and Corces 1987; Martin *et al.* 1989). In addition, we show that the region of the wing enhancer located distally from −2463 is responsible for *yellow* activation not only in the wings but also in the tip of the abdomen.

The effect of the $mod(mdg4)^{1u1}$ mutation on the phenotype of the *hobo*-induced *y* alleles: The mod(mdg4) protein is a second component involved in insulation by the su(Hw)-binding region. Previously, the $mod(mdg4)^{1u1}$ mutation was shown to repress *yellow* expression in y^{2} mutants (Table 3). These flies had yellow color of the body cuticle, wing blades, and all kinds of bristles, including both wing and abdominal ones. As has been shown above, the insertion of a *hobo* mobile element, and especially the duplication of *gypsy* and *yellow* sequences, strongly influence the insulation properties of the su(Hw)-binding region.

To achieve a better understanding of the role of the mod(mdg4) protein, we studied the effect of the $mod(mdg4)^{1u1}$ mutation on the phenotypes of the *hobo*-induced alleles: the flies with all tested *y* alleles in combination with $mod(mdg4)^{1u1}$ displayed the yellow color of the bristles (Table 3). In y^{lh} flies, the $mod(mdg4)^{1u1}$ mutation

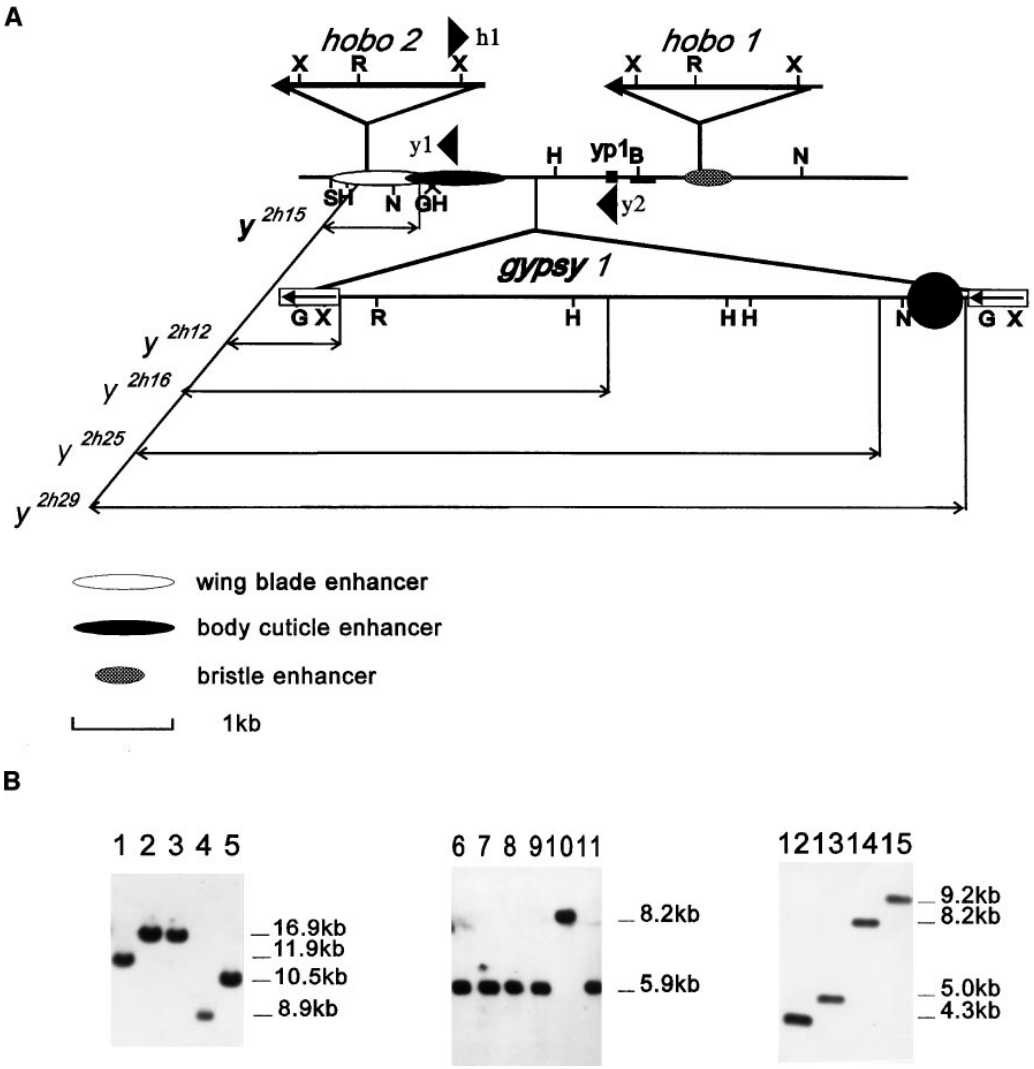

Figure 8.—The structure of the y^{2h} alleles, derivatives of y^{rh}. (A) Structure of the y^{2h} alleles. The arrows show the size of deletions. All other designations are as in Figure 1. The breakpoints of deletion in the y^{2h15} and y^{2h25} alleles were cloned by PCR between the primers in the *hobo* element (h1) and in the *yellow* gene (y1 and y2). (B) Southern blot analysis of the y^{2h} alleles. DNAs from y^{2h16} (1,14), y^{dh1} (2,6, 15), y^{dh2} (3), y^{2h25} (4,7,13), y^{2h29} (5,10,12), y^{2h12} (8), y^{2h15} (11), and y^{2h21} (9) were digested with *Bam*HI (1-5) or *Nco*I (6-11) or *Eco*RI (12-15). The blots were probed with the *Hin*dIII-*Bam*HI fragment from the *yellow* locus.

only partially decreased the level of body and wing blade pigmentation. Unexpectedly, in y^{rh} flies, the *mod (mdg4)*^{1u1} mutation failed to influence the normal wing and body pigmentation, although the pigmentation of bristles was reduced in all cases.

It was shown previously that the tip of the abdomen in y^{2} *mod(mdg4)*^{1u1} males had darker pigmented dots on the cuticle against a background of the mutant-colored cuticle characteristic of y^{2} flies (Gerasimova *et al.* 1995). y^{dh} or y^{lh} *mod(mdg4)*^{1u1} males exhibited the same variegation in the abdomen tip pigmentation (data not shown). However, *mod(mdg4)*^{1u1} failed to change the pigmentation in the tip of the abdomen in males with y^{2h} alleles. Thus, in the presence of the *mod(mdg4)*^{1u1} mutation, the body and wing enhancers are important for the variegated phenotype of pigmentation in the abdomen tip.

DISCUSSION

***hobo*-Mediated rearrangements in the presence of the su(Hw)-binding region are frequently associated with duplications:** Previous genetic and molecular studies showed that *hobo* elements were capable of mediating frequent chromosome rearrangements (Blackman *et al.* 1987; Hatzopoulos *et al.* 1987; Johnson-Schlitz and Lim 1987; Yannopoulos *et al.* 1987; Lim 1988; Ho *et al.* 1993; Sheen *et al.* 1993). It was suggested by Lim (1988) that homologous intrachromosomal recombination between *hobo* elements was responsible for such rearrangements. The *hobo*-mediated rearrangements were largely confined to individual chromosome arms (Laverty and Lim 1982; Blackman *et al.* 1987; Johnson-Schlitz and Lim 1987; Lim 1988), suggesting that they were produced mainly as the result of intramolecular recombination. They were dependent on the orientation of *hobo* (Lim 1988; Lim and Simmons 1994; Eggleston *et al.* 1996). When preexisting elements were in the same orientation in a chromosome, the outcome was a deletion of the intervening material and the presence of a single *hobo* at the deletion breakpoint. Lim (1988) and Lim and Simmons (1994) proposed a model in which *hobo* elements induced chromosome restructuring via homologous pairing and recombination between the elements at ectopic sites in the genome.

In the y^{dh1} allele, both *hobo* elements are inserted in

TABLE 3

Influence of the *mod(mdg4)*^{1u1} mutation on the *hobo*-induced *yellow* alleles

	Pigmentation					
			Bristles			
y alleles	Body	Wings	Th	L	W	Ab
y^2	1(0)	1(0)	5(0)	5(0)	5(0)	5(0)
y^{dh*}	1(0)	2(0)	1(0)	2(0)	5(1)	5(1)
y^{rh*}	5	5	1(0)	2(0)	5(1)	5(2)
y^{lh*}	3(1)	3(2)	1(0)	2(0)	5(1)	5(1)
y^{2h*}	1(0)	1(0)	1(0)	2(0)	5(0)	5(0)
y^{2h16}, y^{2h25}	1(0)	1(0)	2(0)	3(0)	5(0)	5(0)

Designations are as in Table 1. The numbers in parentheses show the effect of the homozygous *mod(mdh4)*^{1u1} mutation on the phenotype of the *y* alleles.

y^{dh*} (y^{dh1}, y^{dh12}, y^{dh19}, y^{dh24}), y^{rh} (y^{rh1}, y^{rh2}, y^{rh7}), (y^{lh*}, y^{lh11}, y^{lh13}, y^{lh17}), y^{2h*} (y^{2h12}, y^{2h115}, y^{2h131}).

the same relative orientation; therefore, the deletion of sequences between them should lead to a complete inactivation of the *yellow* gene. Unexpectedly, frequent *hobo*-mediated rearrangements leading to the y^{rh} phenotype were associated with a duplication of the genomic region between two *hobo* elements. The latter event seems to involve unequal recombination between sister chromatids. Previously, only a few reversible tandem duplications were observed in the studies of Uc unstable chromosomes (Lim 1979). In our system, if the region contained su(Hw)-binding sites, triplications and even quadruplications of the *yellow* region flanked by *hobo* elements took place (M. Gause and P. Georgiev, unpublished results).

Such changes in the behavior of *hobo* elements may be explained by the existence of a special chromatin structure in the insertion area. For example, the su(Hw)-binding region, which acts as a strong insulator, may prevent ectopic intrachromosomal pairing between *hobo* elements. However, we prefer an alternative explanation, that *hobo*-mediated duplications are common events, but in the previous systems used for the study of *hobo*-mediated rearrangements, the duplications of the region between *hobo* elements did not change the phenotype and thus were not detected. In fact, we observed *hobo*-induced duplications in some other mutations (E. Bezborodova, M. Gause and P. Georgiev, unpublished results). Additional experiments are needed to check this possibility.

The role of *hobo* in *yellow* expression: *hobo* insertions into the *y* locus led to the reduction of notum and leg bristle pigmentation. This may be explained by the fact that one *hobo* element inserted into the intron of the *yellow* gene, exactly in the region of the bristle enhancer. No alleles associated with the excision of the *hobo* element were obtained, although deletions of *gypsy* sequences partially restored the bristle pigmentation. The *su(Hw)* mutations suppressed the mutant bristle phenotype, particularly those of *yellow* alleles that had a deletion of some parts of *gypsy*, but not of its su(Hw)-binding region. Recently, we have also found that *gypsy* sequences other than the su(Hw)-binding region can influence the expression of the *yellow* gene (P. Georgiev and T. Belenkaya, unpublished results). Thus, *gypsy* sequences, su(Hw)-binding region and the *hobo* insertion have additive negative effects on *yellow* expression in bristles.

A new insight in the enhancer/promoter insulation by the su(Hw)-binding region: An insulator is a sequence that prevents activation or repression from extending across it to the promoter. Only few direct examples of insulators have been reported. Kellum and Schedl (1991) showed that the *hsp70* locus of *Drosophila melanogaster* was bordered by two sequences, *scs* and *scs′*, that protected it from the effects of neighboring chromatin. The core 0.5-kb scs′ element binds the boundary-element-associated factor, which is responsible for the insulation function (Zhoa *et al.* 1995). Chung *et al.* (1993) identified a component of the β-*globin* gene cluster that prevented the action of the enhancer on the promoter. The su(Hw)-binding region is the most extensively studied insulator element that exhibits remarkable directionality (Corces and Geyer 1991; Holdridge and Dorsett 1991; Jack *et al.* 1991; Geyer and Corces 1992; Roseman *et al.* 1993; Cai and Levine 1995; Scott and Geyer 1995).

Our results show that two *hobo* mobile elements inserted at the *yellow* intron and the 5′ regulatory region (y^{dh} alleles) allow the body and wing enhancers to partially overcome the insulation effect of the su(Hw)-binding region and to slightly activate the *yellow* promoter. A possible explanation is that ectopic pairing between *hobo* elements may interfere with su(Hw) insulation. A role for pairing between the homologous elements in the partial suppression of su(Hw)-mediated insulation is supported by our analysis of y^{lh} alleles. In these alleles, the *yellow* promoter is isolated from body and wing blade enhancers by two copies of *gypsy*. Previous studies suggested that the greater the number of su(Hw)-binding sites, the more effective the insulation (Hoover *et al.* 1992; Smith and Corces 1992). However, the duplication of the su(Hw)-binding region in the y^{lh} has an opposite effect: the body and wing blade enhancers partially activate the *yellow* promoter. Thus, it is possible that the pairing between *gypsy* sequences or interaction between su(Hw)-binding regions partially neutralize the enhancer-blocking effect.

Duplication of *gypsy* and the *yellow* sequences located between two *hobo* elements in the y^{rh} alleles restored the insulated *yellow* expression. This phenomenon may be explained in several different ways. One possibility is that the duplicated *yellow* promoter in the y^{rh} alleles is not isolated by the su(Hw)-binding region from the

wing and body enhancers located downstream. The *yellow* transcription may pass the *hobo*, *gypsy*, and *yellow* gene sequences. In this case, mRNA of normal size may arise from splicing between the first distal exon located between *gypsy-2* and *hobo-2* and the second exon of the *yellow* gene. However, it is difficult to explain in this way the absence of other mRNAs expected to appear in the course of alternative splicing, for example, between the proximal first exon and the second exon of the *yellow* gene.

Of particular importance are the data on the y^{mh32} allele obtained as a result of inversion between *hobo* elements located in the *yellow* and *scute* loci (M. Gause and P. Georgiev, unpublished results). In this allele the *yellow* expression is activated by the wing blade and body enhancers located between two *gypsy* elements in the absence of the second noninsulated promoter. This supports an alternative explanation for the phenotype of y^{rh} alleles: that ectopic intrachromosomal pairing between two *gypsy* elements or interactions between su(Hw) proteins bound to two different su(Hw)-binding regions suppress the insulation and permit the enhancers located between two *gypsy* elements to activate *yellow* transcription. The possibility of ectopic intrachromosomal pairing between *gypsy* elements is supported by the high level of recombination between *gypsy* sequences: y^{lh} alleles arise as a result of recombination between *gypsy* LTRs. y^{dh} derivatives from y^{rh} may also be generated by recombination between *gypsy* sequences as well as between *hobo* elements.

The prevailing model concerning the mechanism of insulator function proposes that insulators are chromatin boundaries (Geyer and Corces 1992; Harrison *et al.* 1993; Roseman *et al.* 1993; Schedl and Grosveld 1995; Gerasimova and Corces 1996). A domain assembled by boundaries prevents interactions between regulatory elements by promoting the folding of a higher-order chromatin structure in such a way as to increase the likelihood of interactions between regulatory elements within a domain, while decreasing these interactions between domains (Vazquez and Schedl 1994). A recent direct finding that blocked enhancers retain their full activity suggests that the effects of the su(Hw) protein on the enhancer function may be caused by the formation of a such domain boundary (Cai and Levine 1995; Scott and Geyer 1995). In view of this, two su(Hw)-binding regions may act as boundaries to define distinct chromosomal domains causing the suppression of insulation seen in y^{lh} alleles. Distal enhancers under certain conditions may "bypass" the domain flanked from both sides by su(Hw)-binding regions and activate the proximal *yellow* promoter. However, this model fails to explain the activation of *yellow* promoter by enhancers flanked from both sides by a su(Hw)-binding region in the y^{mh} and y^{rh} alleles.

Another type of model suggests that the su(Hw)-binding region functions as a flexible regulatory element modulating enhancer-promoter interactions within complex genetic loci (Cai and Levine 1995; Georgiev and Kozycina 1996). Geyer (1997) proposed that insulators assemble complexes that might trap an enhancer in a nonproductive interaction, because the insulator lacks promoter function and no transcription occurs as a result (Decoy model). Other authors postulate that an insulator binding protein interacts and interferes with higher eucaryotic proteins that facilitate interactions between the enhancer and promoter (Morcillo *et al.* 1996, 1997). The results obtained in the present work may be explained by either model. The ectopic intrachromosomal pairing between two *gypsy* elements or the interactions between su(Hw) proteins bound to two different su(Hw)-binding regions may prevent the organization of a nonproductive complex between su(Hw) protein and proteins, whose functions are either to activate transcription by enhancer binding or to facilitate the interaction between enhancer and promoter.

On the mechanism of *mod(mdg4)* gene action: The *mod(mdg4)* gene encodes a protein that interacts with the su(Hw) protein and contributes to the insulating function of the su(Hw)-binding region (Georgiev and Corces 1995; Gerasimova *et al.* 1995; Georgiev and Kozycina 1996). In the case of the y^2 mutation, the hypomorph $mod(mdg4)^{1u1}$ mutation changes the action of the su(Hw)-binding region in such a way that it inactivates *yellow* transcription driven by enhancers not separated by the su(Hw)-binding region from the *yellow* promoter. This observation may be explained by assuming that in the presence of the hypomorphic $mod(mdg4)^{1u1}$ mutation, the su(Hw) protein directly inhibits the expression from the *yellow* promoter (Georgiev and Kozycina 1996). An alternative explanation is that together the su(Hw) and mod(mdg4) proteins are able to affect chromatin structure (Gerasimova *et al.* 1995; Gerasimova and Corces 1996). According to this hypothesis, binding of the su(Hw) protein to its target sequence creates a bidirectional repressive effect, similar to the silencing caused by heterochromatin. Subsequent interactions between the mod(mdg4) and su(Hw) proteins transforms this nonspecific silencer into a polar insulator.

The role of the chromatin structure in the action of $mod(mdg4)^{1u1}$ is supported by the observation that y^2, $mod(mdg4)^{1u1}$ males have variegated *yellow* expression in the tip of the abdomen: dots of a darkly pigmented cuticle against the background of mutant-colored cuticle characteristic of y^2 flies (Gerasimova *et al.* 1995). However, we have found here that dots of a darkly pigmented cuticle were absent in males carrying a combination of $mod(mdg4)^{1u1}$ with *y* alleles that had a deletion of enhancer elements. Therefore, variegated pigmentation on the tip of the abdomen may be interpreted as a result of the ability of enhancer elements to partially overcome su(Hw)-binding insulation in $mod(mdg4)^{1u1}$ background.

In this work, we found that the duplication of *gypsy* in y^{rh} and y^{lh} alleles completely or partially suppressed the inhibitory effect of the *mod(mdg4)*^{1u1} mutation on *yellow* expression in the body and wings. Ectopic intrachromosomal pairing between *gypsy* elements could alter the properties of the su(Hw)-binding region as an insulator and suppress the effect of the *mod(mdg4)*^{1u1} mutation. However, it is difficult to explain this fact by assuming that the su(Hw) protein creates a bidirectional repressive effect in the absence of the mod(mdg4) protein. As was shown before, multimerization of sequences only enhanced the possibility of formation of a higher order chromatin structure (Dorer and Henikoff 1994).

The absence of the *mod(mdg4)*^{1u1} effect on *yellow* transcription in the *yellow*-containing construction, where the su(Hw)-binding region is inserted at position −1648 (Georgiev and Kozycina 1996), does not support the possibility that the *mod(mdg4)*^{1u1} mutation changes the 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 (Geyer and Corces 1992), it does not repress *yellow* transcription 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 by the su(Hw) protein.

The role of the *mod(mdg4)*^{1u1} mutation with regard to the *gypsy* insulator was previously studied in transgenic embryos (Cai and Levine 1997). The su(Hw)-binding region was inserted between defined enhancers and placed among divergently transcribed reporter genes (*white* and *lacZ*) containing distinct core promoter sequences. The *mod(mdg4)*^{1u1} mutation caused the insulator to function as a promoter-specific silencer that selectively represses *white*, but not *lacZ*. The repression of *white* does not affect the expression of the closely linked *lacZ* gene, suggesting that the insulator does not propagate changes in chromatin structure (Cai and Levine 1997).

Thus, the results presented in this work and some previous data support the possibility that the inhibiting action of the *mod(mdg4)*^{1u1} mutation is realized through a direct interaction of the su(Hw) protein with the *yellow* promoter, rather than through the action on chromatin structure.

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LITERATURE CITED

Ashburner, M., 1989 *Drosophila: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Blackman, R. K., R. Grimaila, M. M. D. Koehler and W. M. Gelbart, 1987 Mobilization of *hobo* elements residing within the decapentaplegic gene complex: suggestions of a new hybrid dysgenesis system in *Drosophila melanogaster.* Cell **49:** 497–505.

Blackman, R. K., M. M. D. Koehler, R. Grimaila and W. M. Gelbart, 1989 Identification of a fully-functional hobo transposable element and its use for germ-line transformation of Drosophila. EMBO J. **8:** 211–217.

Cai, H. N., and M. Levine, 1995 Modulation of enhancer-promoter interactions by insulators in the Drosophila embryo. Nature **376:** 533–536.

Cai, H. N., and M. Levine, 1997 The *gypsy* insulator can function as a promoter-specific silencer in the Drosophila embryo. EMBO J. **16:** 1732–1741.

Calvi, B. R., T. J. Hong, S. D. Findley and W. M. Gelbart, 1991 Evidence for a common evolutionary origin of inverted repeat transposons in Drosophila and plants: *hobo,* Activator and Tam3. Cell **66:** 465–471.

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.

Chung, J. H., M. Whiteley and G. Felsenfeld, 1993 A 5′ element of the chicken β-globin domain serves as an insulator in human erythroid cells and protects against position effect in Drosophila. Cell **74:** 505–514.

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.

Dorer, D. R., and S. Henikoff, 1994 Expansions of transgene repeats cause heterochromatin formation and gene silencing in Drosophila. Cell **77:** 993–1002.

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

Eggleston, W. B., N. R. Rim and J. K. Lim, 1996 Molecular characterization of *hobo*-mediated inversions in *Drosophila melanogaster.* Genetics **144:** 647–656.

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., and M. Kozycina, 1996 Interaction between mutations in the *suppressor of Hairy wing* and *modifier of mdg4* genes of *Drosophila melanogaster* affecting the phenotype of *gypsy*-induced mutations. Genetics **142:** 425–436.

Georgiev, P. G., V. A. Yelagin, E. M. Buff and N. P. Kolyagin, 1992 Properties of super unstable mutations in the *Drosophila melanogaster yellow* locus. Genetica (in Russian) **28:** 98–107.

Gerasimova, T. I., and V. G. Corces, 1996 Boundary and insulator elements in chromosomes. Curr. Opin. Genet. Dev. **6:** 185–192.

Gerasimova, T. I., D. A. Gdula, D. V. Gerasimov, O. Simonova and V. G. Corces, 1995 A Drosophila protein that impacts directionality on a chromatin insulator is an enhancer of position-effect variegation. Cell **82:** 587–597.

Geyer, P. K., 1997 The role of insulator elements in defining domains of gene expression. Curr. Opin. Genet. Dev. **7:** 242–248.

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., C. Spana and V. G. Corces, 1986 On the molecular mechanism of gypsy-induced mutations at the *yellow* locus of *Drosophila melanogaster.* EMBO J. **5:** 2657–2662.

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.

Hatzopoulos, P., M. Monastirioti, G. Yannopoulos and C. Lovis, 1987 The instability of the TE-like mutation dp(2;2)GYL of *Drosophila melanogaster* is intimately associated with the *hobo* element. EMBO J. **6:** 3091–3096.

Ho, Y. T., S. M. Weber and J. K. Lim, 1993 Interacting *hobo* transposons in an inbred strain and interaction regulation in hybrids of *Drosophila melanogaster*. Genetics **134:** 895–908.

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.

Hoover, K. K., T. I. Gerasimova, A. J. Chien and V. G. Corces, 1992 Dominant effects of *suppressor of Hairy-wing* mutations on *gypsy*-induced alleles of *forked* and *cut* in *Drosophila melanogaster*. Genetics **132:** 691–697.

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.

Johnson-Schlitz, D., and J. K. Lim, 1987 Cytogenetics of *Notch* mutations arising in the unstable X chromosome Uc of *Drosophila melanogaster*. Genetics **115:** 701–709.

Kellum, R., and P. Schedl, 1991 A position-effect assay for boundaries of higher order chromosomal domains. Cell **64:** 941–950.

Laverty, T. R., and J. K. Lim, 1982 Site-specific instability in *Drosophila melanogaster*: evidence for transposition of destabilizing element. Genetics **101:** 461–476.

Lim, J. K., 1979 Site-specific instability in *Drosophila melanogaster*: the origin of the mutation and cytogenetic evidence for site specificity. Genetics **93:** 681–701.

Lim, J. K., 1988 Intrachromosomal rearrangements mediated by *hobo* transposons in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **85:** 9153–9157.

Lim, J. K., and M. J. Simmons, 1994 Gross chromosome rearrangements mediated by transposable elements in *Drosophila melanogaster*. BioEssays **16:** 269–275.

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

Marlor, R. L., S. M. Parkhurst and V. G. Corces, 1986 The *Drosophila melanogaster gypsy* transposable element encodes putative gene products homologous to retroviral proteins. Mol. Cell. Biol. **6:** 1129–1134.

Martin, M., Y. B. Meng and W. Chia, 1989 Regulatory elements involved in the tissue-specific expression of the *yellow* gene of Drosophila. Mol. Gen. Genet. **218:** 118–126.

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 of *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.

Morcillo, P., C. Rosen and D. Dorsett, 1996 Genes regulating the remote wing enhancer in the Drosophila *cut* locus. Genetics **144:** 1143–1154.

Morcillo, P., C. Rosen, M. K. Baylies and D. Dorsett, 1997 Chip, a widely expressed chromosomal protein required for segmentation and activity of a remote wing margin enhancer in Drosophila. Genes Dev. **11:** 2729–2740.

Mullis, K. B., and F. A. Faloona, 1987 Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods Enzymol. **155:** 335–350.

Nash, W. G., and R. J. Yarkin, 1974 Genetic regulation and pattern formation: a study of the *yellow* locus in *Drosophila melanogaster*. Genet. Res. **24:** 19–26.

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.

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.

Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn *et al.*, 1985 Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science **230:** 1350–1354.

Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Sanger, F., S. Nicklen and A. R. Coulson, 1977 DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

Schedl, P., and F. Grosveld, 1995 Domains and boundaries, pp. 172–196 in *Chromatin Structure and Gene Expression*, edited by S. C. R. Elgin. Oxford University Press, Oxford, UK.

Scott, K. S., and P. K. Geyer, 1995 Effects of the su(Hw) insulator protein on the expression of the divergently transcribed Drosophila yolk protein genes. EMBO J. **14:** 6258–6279.

Sheen, F.-M., J. K. Lim and M. J. Simmons, 1993 Genetic instability in *Drosophila melanogaster* mediated by *hobo* transposable elements. Genetics **133:** 315–334.

Smith, P. T., 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.

Spradling, A. C., and A. P. Mahowald, 1979 Identification and genetic localization of mRNA from ovarian follicle cells of *Drosophila melanogaster*. Cell **16:** 589–598.

Streck, R. D., J. E. Macgaffey and S. K. Beckendorf, 1986 The structure of *hobo* transposable elements and their insertion sites. EMBO J. **5:** 3615–3623.

Vazquez, J., and P. Schedl, 1994 Sequences required for enhancer blocking activity of scs are located within two nuclease-hypersensitive regions. EMBO J. **13:** 5984–5993.

Yannopoulos, G., N. Stamatis, M. Monastirioti, P. Hatzopoulos and C. Louis, 1987 *hobo* is responsible for the induction of hybrid dysgenesis by strains of *Drosophila melanogaster* bearing the male recombination factor *23.5MRF*. Cell **49:** 487–495.

Zhoa, K., C. M. Hart and U. K. Laemmli, 1995 Visualization of chromosomal domains with boundary element-associated factor BEAF-32. Cell **81:** 879–889.

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