DNA position-specific repression of transcription by a *Drosophila* zinc finger protein

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Expression of the *yellow* (*y*) gene of *Drosophila melanogaster* is controlled by a series of tissue-specific transcriptional enhancers located in the 5′ region and intron of the gene. Insertion of the *gypsy* retrotransposon in the y^2 allele at −700 bp from the start of transcription results in a spatially restricted phenotype: Mutant tissues are those in which *yellow* expression is controlled by enhancers located upstream from the insertion site, but all other structures whose enhancers are downstream of the insertion site are normally pigmented. This observation can be reproduced by inserting just a 430-bp fragment containing the *suppressor of Hairy-wing* [*su(Hw)*]-binding region of *gypsy* into the same position where this element is inserted in y^2, suggesting that the *su(Hw)*-binding region is sufficient to confer the mutant phenotype. Insertion of this sequence into various positions in the *y* gene gives rise to phenotypes that can be rationalized assuming that the presence of the *su(Hw)* protein inhibits the action of those tissue-specific enhancers that are located more distally from the *su(Hw)*-binding region with respect to the promoter. These results are discussed in light of current models that explain long-range effects of enhancers on gene expression.

[*Key Words*: *Drosophila*; *yellow* gene; *su(Hw)*; zinc finger protein; transcriptional enhancers]

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The *yellow* (*y*) gene is required for pigmentation of cuticle structures of both larvae and adults, thereby providing a visual assay for its transcriptional activity. The temporal and spatial expression of *y* is controlled by tissue-specific transcriptional enhancers located in the 5′ region and intron of this gene (Geyer and Corces 1987). Insertion of the *gypsy* element at −700 bp from the transcription start site causes a tissue-specific alteration of *y* gene expression. Insertion at this position causes inactivation of enhancers placed upstream of *gypsy* that are responsible for *y* expression in the wings and body cuticle, producing flies in which these two tissues show abnormal pigmentation (Geyer et al. 1986). All other pigmented tissues with coloration controlled by enhancers located downstream from the *gypsy* insertion site are wild type.

The mutant phenotype caused by insertion of *gypsy* into the *y* gene requires the product of a second unlinked modifier, the *suppressor of Hairy-wing* [*su(Hw)*] gene. Mutations in this locus reverse the phenotype of *gypsy*-induced alleles in several genes besides *y* such as *Hairy-wing* (*Hw*), *scute* (*sc*), *forked* (*f*), *lozenge* (*lz*) (Modolell et al. 1983). The *su(Hw)* gene encodes a DNA-binding protein with structural similarities to eukaryotic transcription factors (Parkhurst et al. 1988). This protein is involved in the regulation of *gypsy* expression through its interaction with specific sequences of this retrotransposon (Parkhurst and Corces 1986; Spana et al. 1988). The *su(Hw)* protein binds to a 27-bp sequence containing an octamer motif flanked by two A/T tracts that provide a bend in the DNA necessary for the interaction (Spana and Corces 1990). The *su(Hw)*-binding region in *gypsy* contains 12 of these 27-bp sequences tandemly repeated, suggesting that 12 *su(Hw)* molecules may interact with the *gypsy* element, assuming that the protein binds as a monomer. This interaction is directly responsible for the mutagenic effect of *gypsy*, because deletions or other alterations in the *su(Hw)*-binding region of *gypsy* result in a decrease or abolishment of the mutagenic effect of this element (Geyer et al. 1988b; Peifer and Bender 1988; Flavell et al. 1990; Smith and Corces 1992). This effect has been studied in detail in the case of a *gypsy*-induced mutation in the *y* locus. Progressive deletions of the *su(Hw)*-binding region of the *gypsy* element inserted in *y* have a corresponding decrease in the mutagenic effect of this element, that is, fewer 27-bp binding sites present in *gypsy* result in a milder *y* phenotype (Smith and Corces 1992). These results suggest a correlation between the number of *su(Hw)* molecules bound to the *gypsy* element and the strength of the effect on the expression of the adjacent gene.

Here, we present evidence indicating that the presence of the *su(Hw)* protein bound to *gypsy* sequences is not only necessary but also sufficient to explain the muta-

genic effect of this retrotransposon when inserted in the 5′ region of the *y* gene. Furthermore, this effect is directional, that is, only those transcriptional enhancers located distal to the *su(Hw)*-binding site with respect to the *y* promoter are affected by the presence of bound *su(Hw)* protein. These results suggest that the inactivating effects of *gypsy* are the result of the interaction of the *su(Hw)* protein with tissue-specific transcription factors bound to distal transcriptional enhancers and offer new insights into the mechanisms by which enhancer elements interact with the promoter.

Results

The su(Hw)*-binding region from* gypsy *can elicit the same mutant phenotype as the complete element*

We have used the *y* gene as a model system to study the molecular basis of *gypsy* mutagenesis (for review, see Corces and Geyer 1991). Figure 1 shows a diagrammatic representation of the structure of this gene and the location of different tissue-specific enhancer elements relative to the insertion of the *gypsy* element in the y^2 mutation. This insertion is 700 bp upstream from the *y* start site of transcription and causes a phenotype showing mutant coloration in the wings and body cuticle of the adult, but the rest of the pigmented cuticular structures of the larvae and adult are wild type.

Because the *su(Hw)*-binding region of *gypsy* is necessary for *gypsy*-induced mutagenesis, we decided to determine whether these sequences are sufficient to evoke the same phenotype as the intact element. As a first experiment, we wished to test the effects of insertion of the 430-bp fragment containing the *su(Hw)*-binding region of *gypsy* (Spana and Corces 1990) into the *y* gene at the site of insertion of the *gypsy* element in the y^2 mutation. Because no convenient restriction sites were available, the *su(Hw)*-binding site was cloned into a derivative of a *y* gene carrying 51 bp of the *gypsy* long terminal repeat (LTR) at position −700 (see Materials and methods). The presence of LTR sequences at this position has no effect on *y* expression (Geyer et al. 1988a). This *y* gene contains sufficient 5′- and 3′-flanking sequences to rescue a *y* null allele completely (Geyer and Corces 1987). The final plasmid p-700R carries a fragment of the *y* gene, into which 12 copies of the *su(Hw)*-binding site are inserted in the opposite orientation relative to that of the y^2 mutation, cloned into the transformation vector Carnegie 20 (Rubin and Spradling 1983). This plasmid was injected into y^-; $rosy^-$ (ry^-) embryos, and transformants were selected by the ry^+ phenotype. The phenotype of this and other transformants is summarized in Figure 2. Flies transformed with p-700R show a phenotype indistinguishable from that of y^2, with mutant coloration in the adult cuticle of both males and females and in the wing blades (Fig. 3) but wild-type bristles (Fig. 4) and tarsal claws (Fig. 5), as well as larval cuticular structures (Fig. 6). Therefore, the *su(Hw)*-binding region is sufficient to elicit the same phenotype as the complete retrotransposon even when positioned in the opposite orientation.

To control for possible complications arising from the additional *gypsy* sequences present in p-700R, we tested two other constructs. In these cases, the *su(Hw)*-binding region was inserted into the 5′ region of *y*, 800 bp upstream of the transcription start site, in either orientation. In these plasmids, the *su(Hw)*-binding region is 100 bp upstream from the normal *gypsy* insertion site in y^2, but it is still located between the enhancers that control abdominal and wing pigmentation and the *y* promoter (Fig. 1). Flies transformed with either p-800 or p-800R show the same phenotype as y^2 and p-700R (Figs. 2 and 3).

To test whether the presence of the *su(Hw)* protein is

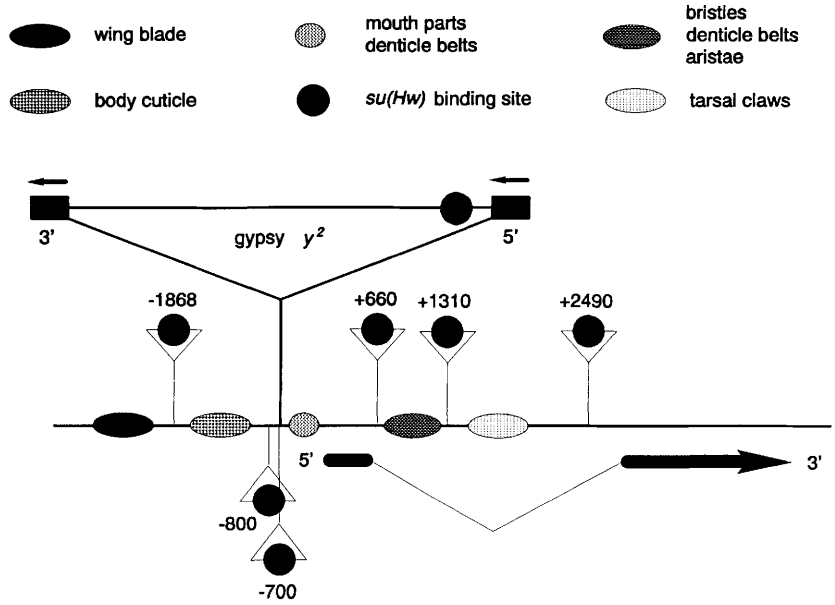

Figure 1. Structure of the *y* locus and transformation plasmids. The two exons of the *y* gene are represented by thick lines separated by one intron. The arrow indicates the direction of transcription. The *gypsy* element inserted at −700 bp from the transcription start site in the y^2 allele is also shown. Solid rectangles indicate the *gypsy* LTRs; arrows indicate the direction of transcription of this element. Solid circles represent the *su(Hw)* protein. Transcriptional enhancers are represented by ovoid structures, with various patterns as labeled, located in the intron and the 5′ region of the gene. The tissues in which *y* expression is controlled by each enhancer are indicated at the *top*. The insertion points of the *su(Hw)*-binding region in the different constructs described in the text are indicated by triangles representing the DNA sequence, with solid circles depicting the bound *su(Hw)* protein.

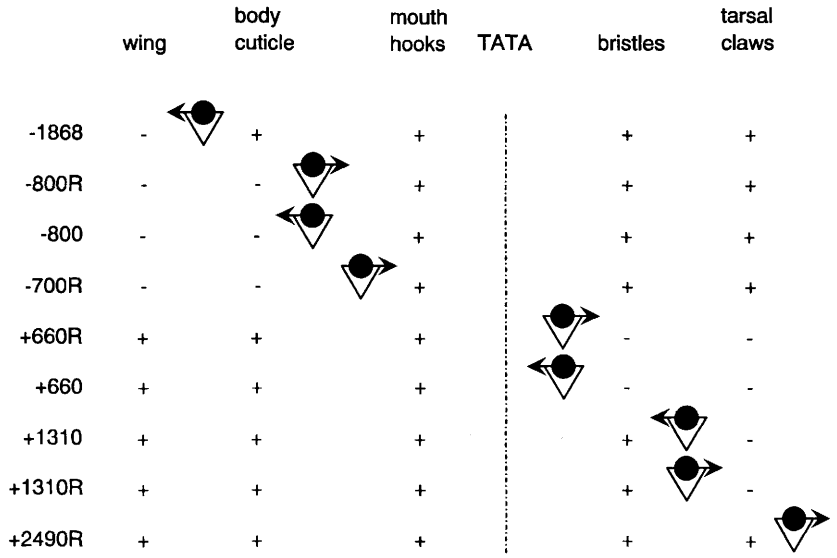

Figure 2. Summary of *y* phenotypes in transformed lines. (*Top*) The relative location with respect to the TATA box of different tissue-specific enhancers responsible for the expression of the *y* gene in various tissues. Numbers at *left* indicate the location of the insertion site of the *su(Hw)*-binding region into the *y* gene in the various plasmids used for germ-line transformation. Each lane summarizes information on transformed lines obtained with each plasmid. The position of the inserted sequences relative to various *y* enhancers is indicated diagrammatically by a triangle that represents the *su(Hw)*-binding region; the solid circles represent the *su(Hw)* protein; the arrow indicates the orientation of the inserted sequences relative to the *y* gene. The coloration of each tissue is indicated by + (wild type) or − (mutant) signs.

required to produce the observed phenotype in these transformants, we analyzed the effect of mutations in the *su(Hw)* locus on *y* expression. The appropriate crosses with transformed lines were carried out so that flies containing the p-800 or p-800R transposons were made homozygous for mutations in the *su(Hw)* gene. The alleles used in this experiment were $su(Hw)^V$, a null allele caused by a deletion of most of the *su(Hw)* gene, and $su(Hw)^f$, which is a hypomorph caused by a mutation in one of the zinc fingers (D. Harrison and C. Corces, in prep.). In the $su(Hw)^V/su(Hw)^f$ mutant background, insertion of the *su(Hw)*-binding region at −800 bp has

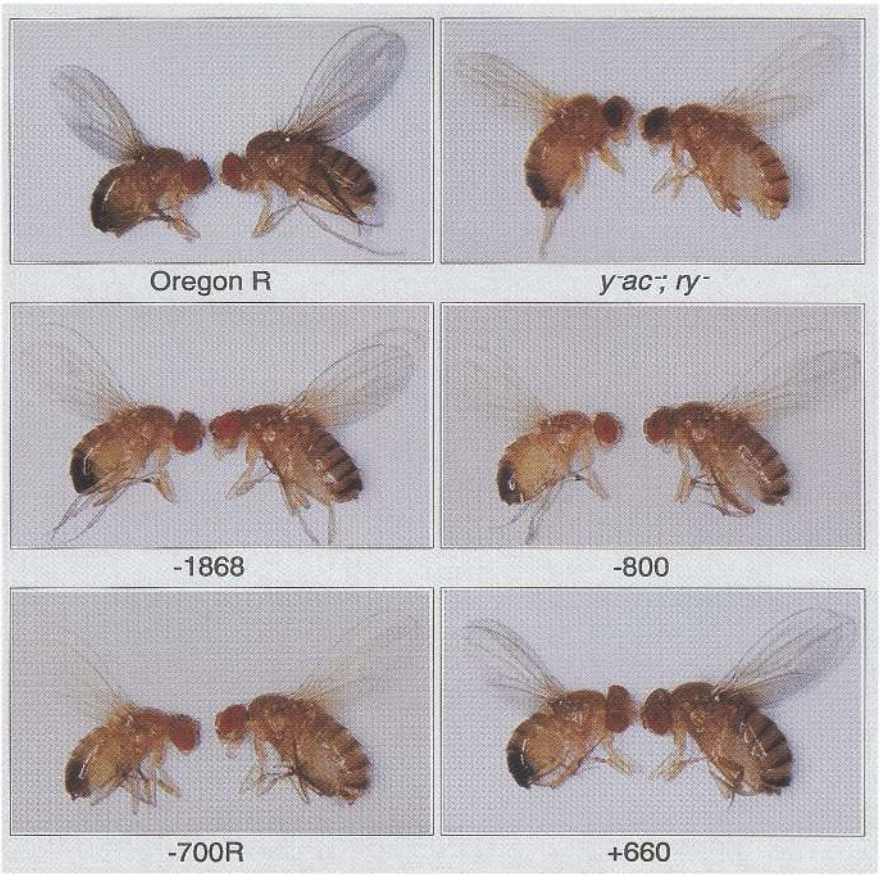

Figure 3. Wing and body cuticle phenotypes of transformed lines. Flies of the genotype y^- $ac^-;ry^{506}$ were transformed with plasmids containing the *y* gene and the *su(Hw)*-binding region inserted in different positions. The insertion site of the *su(Hw)* binding-region in the *y* gene in each transformed line is indicated under each panel. Oregon-R is wild type; y^- ac^-; ry^- is the parental stock used for germ-line transformation.

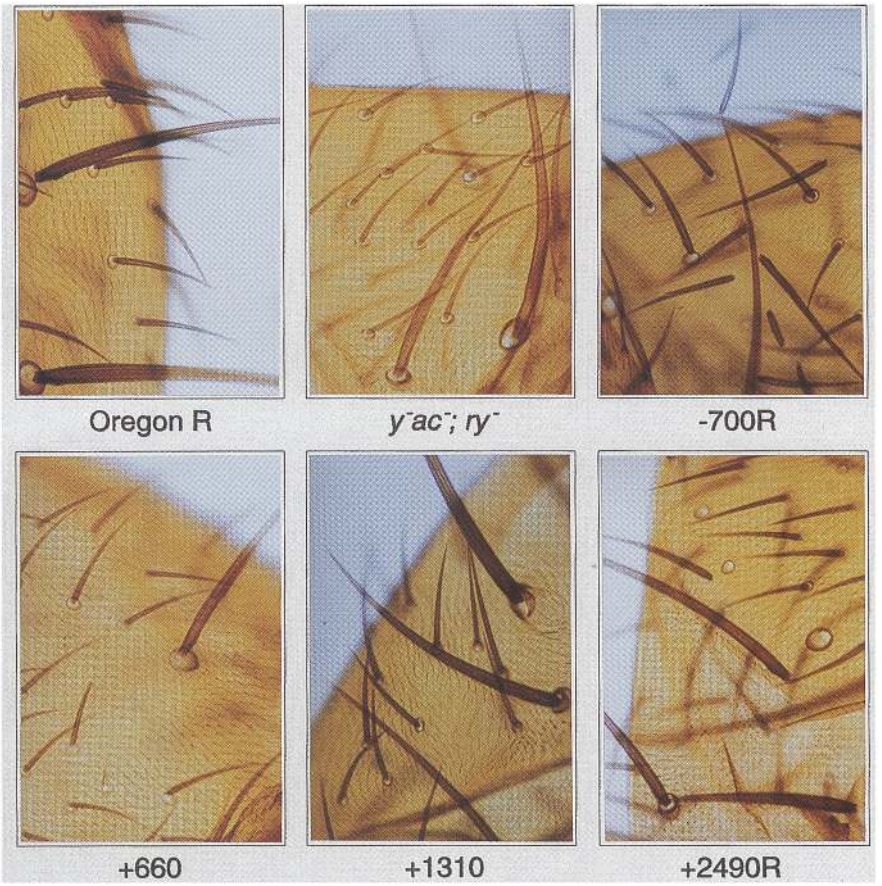

Figure 4. Coloration of the bristles in transformed lines. Numbers under each panel indicate the location of the *su(Hw)*-binding region inserted in the *y* gene in the respective transformed line. Oregon-R is wild type; y^- ac^-; ry^- is the parental stock used for germ-line transformation.

no phenotypic effect; that is, pigmentation in all cuticular structures is wild type (data not shown). This result indicates that the presence of the *su(Hw)* protein bound to its target sequence in the 5′ region of *y* is necessary to induce the observed *y* mutant phenotype.

These experiments support our conclusion from the analysis of p-700R transformants suggesting that the *su(Hw)*-binding region is sufficient to cause inactivation of the wing and body enhancers. Furthermore, they indicate that the precise location and orientation of the *su(Hw)*-binding sites is inconsequential in the generation of the mutant phenotype.

The su(Hw)*-binding region has a directional effect on enhancer function*

From our results, we conclude that the presence of *su(Hw)*-binding sites in the 5′ region of *y* causes a spe-

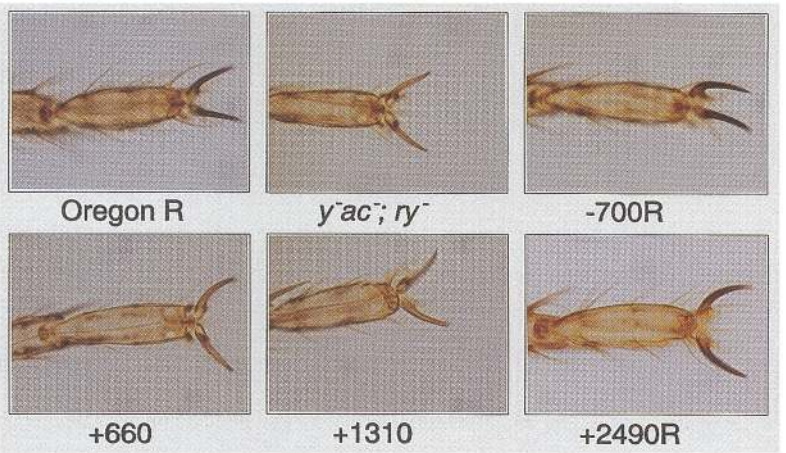

Figure 5. Coloration of the tarsal claws in transformed lines. The location of the *su(Hw)*-binding region inserted in the *y* gene in each transformed line is indicated by numbers under each panel. Oregon-R is wild type; y^- ac^-; ry^- is the parental stock used for germ-line transformation.

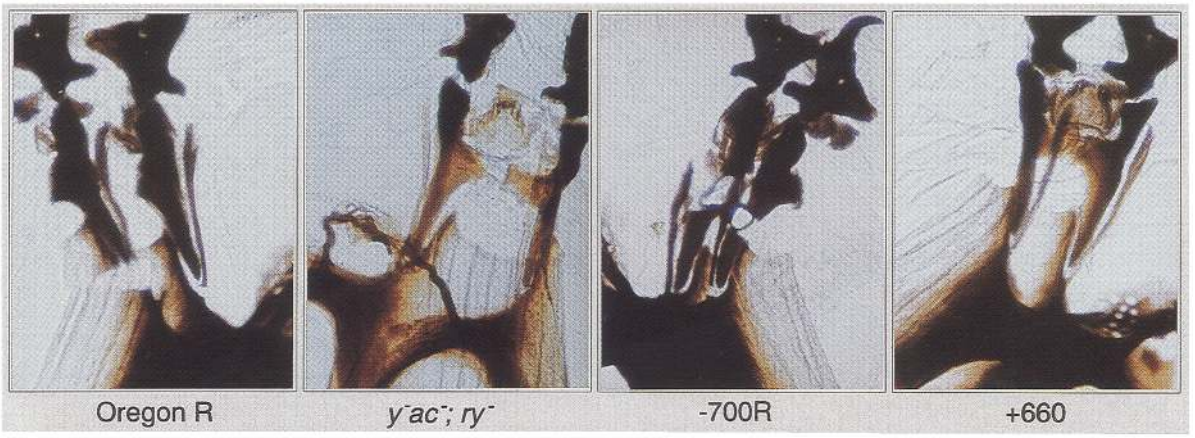

Figure 6. Phenotype of larval mouth hooks in transformed lines. Numbers under each panel indicate locations of the *su(Hw)*-binding region inserted in the *y* gene in the respective transformed line. Oregon-R is wild type; y^- ac^-; ry^- is the parental stock used for germ-line transformation.

cific phenotype characterized by mutant wings and abdominal cuticle, owing to the inability of the transcriptional enhancers responsible for *y* expression in these tissues to act on the promoter. One possible explanation of this result is that these two enhancers are nonfunctional because wing and body cuticle transcription factors interact with *su(Hw)* protein, whereas transcription factors bound to larval or bristle enhancers are incapable of interacting; therefore, expression in these tissues is normal. A second alternative is that the specificity is not determined by the nature of the transcription factors bound to the nonfunctional enhancers but, rather, by their location with respect to the *su(Hw)*-binding sites and the *y* promoter, that is, the wing and body cuticle enhancers are mutant in y^2 flies because they are located distal to the promoter with respect to the *su(Hw)*-binding sites.

To differentiate between these two possibilities, we constructed a plasmid containing the *su(Hw)*-binding region in the same orientation as in y^2, but located at −1868 bp from the transcription start site, that is, between the enhancers that control *y* expression in the wings and body cuticle (Fig. 1). Flies transformed with this plasmid (p-1868) show wild-type coloration of all larval and adult cuticular structures with the exception of the wing blades (Figs. 2 and 3, and data not shown). Transformants therefore contain a functional abdominal cuticle enhancer when its location in the gene is downstream from the *su(Hw)*-binding region insertion site. These results imply that the negative effect of the *su(Hw)* protein does not depend on the nature of the transcription factor bound to the enhancer but on its relative location with respect to the promoter and *su(Hw)*-binding sites.

The su(Hw) *protein can repress enhancers located downstream from the* y *promoter*

The experiments presented above indicate that the presence of the *su(Hw)* protein, bound to DNA in the 5′ region of *y*, can inhibit the action of enhancers located upstream from the *su(Hw)*-binding region. It was unclear whether the binding of this protein would have the same effects on enhancer elements located within the *y* transcription unit. However, mechanisms by which transcription factors associated with downstream enhancers to stimulate transcription from the *y* promoter should be the same as for enhancers located in the 5′ region; therefore, we would predict that the enhancers for the bristle and tarsal claws that reside in the intron of the *y* gene should be inactivated by the insertion of *su(Hw)*-binding sites.

To test this hypothesis, we constructed a plasmid (p+660) in which the *su(Hw)*-binding region from *gypsy* was inserted in the intron of the *y* gene at +660 bp from the transcription start site, separating the promoter from the bristle and tarsal claw enhancers (Figs. 1 and 2). Transformants containing this plasmid, with the *su(Hw)*-binding region in the same orientation as in y^2 or in the opposite orientation, show the same phenotype (Fig. 2). The cuticular larval structures and wings and body cuticle of the adults, all tissues in which *y* expression is controlled by enhancers located upstream from the *y* promoter, are wild-type in these transformants (Figs. 3 and 6). On the contrary, the bristles and tarsal claws of the adults show mutant *y* coloration (Figs. 4 and 5). Transformants carrying p+660 were made homozygous for a *su(Hw)* mutant to verify that the observed phenotype arose as a consequence of the binding of this protein. In a *su(Hw)* homozygous mutant background, flies carrying the transposon p+660 were wild type in coloration of all cuticle structures (data not shown). This endorses the conclusion that the presence of the *su(Hw)* protein bound to its target sequence interferes with the action of those enhancers located more distal with respect to the promoter than the *su(Hw)*-binding region.

Additional supporting information was obtained by analyzing the phenotypes of flies carrying the p+1310 plasmid. Two variants of this construct, in which the *su(Hw)*-binding region is inserted in either of the two

possible orientations at +1310 from the transcription start site, behave in the same fashion. In this location, the *su(Hw)*-binding region separates the bristle and tarsal claw enhancers. As predicted, transformants carrying these plasmids have wild-type wings and body cuticle, as well as larval structures (data not shown). In addition, the bristles of the adults are also wild type but the tarsal claws are mutant (Figs. 4 and 5). This confirms the hypothesis that the presence of DNA-bound *su(Hw)* interferes with the action of enhancers located more distal but not with those more proximal to the *y* promoter. In agreement with this conclusion, transformants containing plasmid p+2490, in which the *su(Hw)*-binding region has been inserted downstream from the tarsal claw enhancer (Figs. 1 and 2) show wild-type coloration in all larval and adult cuticular structures (Figs. 4 and 5, and data not shown).

Discussion

The results presented here indicate that the *su(Hw)*-binding region of *gypsy* is necessary and sufficient to elicit the same mutant phenotype as the complete element. The ability of the binding region to mediate *gypsy*-induced phenotypes suggests that the *su(Hw)* protein alone is responsible for the generation of these phenotypes. An important aspect of the repressive effect of *su(Hw)* on gene expression is the specificity of the inhibition. Binding of the *su(Hw)* protein in an intron or 5′ region of a gene does not have a generalized effect on gene expression. Rather, it affects transcription only in tissues in which the expression of the gene is controlled by transcriptional enhancers located distally with respect to the promoter from the *su(Hw)*-binding region. This is not the result of the temporal or spatial patterns of expression of this protein, because *su(Hw)* is present in all cells at all stages of *Drosophila* development (D. Harrison and V. Corces, in prep.). The specificity of *su(Hw)* effects indicates that this protein interacts with transcription factors bound to the affected tissue-specific enhancers. Understanding this mechanism might then shed light on how enhancers work to activate transcription from nearby promoters.

Enhancers are binding sites for one or more *trans*-acting factors that stimulate transcription from adjacent promoters in a distance- and orientation-independent manner. Several models have been proposed to account for their action (for review, see Serfling et al. 1985; Maniatis et al. 1987; Atchison 1988). In one of the more popular models, enhancers act as entry sites for transcription factors that interact with the transcription complex. This interaction takes place either by looping out the intervening DNA to bring enhancer-associated factors in direct contact with the promoter (looping model, Müller et al. 1989), or alternatively, these factors track along the DNA until they encounter the promoter (tracking model, de Villiers et al. 1982; Wasylyk et al. 1983; Kadesch and Berg 1986). Other models postulate that enhancers organize adjacent chromatin into a transcriptionally active conformation (Saragosti et al. 1980; Jongstra et al. 1984) or act by targeting adjacent genes to a particular nuclear locale (Jackson and Cook 1985; Cockerill and Garrard 1986). Most of the available evidence explaining mechanisms of enhancer action favor entry-site models, although different sets of results support alternative models. Evidence for the looping model has come from studies on transvection in *Drosophila* (Geyer et al. 1990), as well as results obtained by Müller et al. (1989), who developed a strategy to link two DNA ends noncovalently. The ends of the two fragments, one containing the SV40 enhancer and the second containing the rabbit β-globin gene, were biotinylated and coupled with streptavidin. Under these conditions, the SV40 enhancer was able to drive transcription of the β-globin gene in vitro. These results are difficult to explain in the context of a processive scanning model and support a looping mechanism that would facilitate the interaction of enhancer-bound transcription factors with the promoter, without the need for these factors to move across the streptavidin–protein bridge. Further support for a looping model can be drawn from experiments with interlocked circular DNAs that contain an enhancer in one circle and a reporter gene in the other; results from these experiments indicate that both a bacterial enhancer and an RNA polymerase I enhancer can stimulate transcription of an unlinked reporter gene (Dunaway and Dröge 1989; Wedel et al. 1990). One major prediction of looping models is that the interaction between an enhancer and its promoter should be insensitive to linearly placed obstructions in the interconnecting DNA. Evidence contradicting this prediction, thereby supporting a scanning mechanism, comes from results obtained by Courey et al. (1986), who found that the use of psoralen adducts to modify the DNA linking the SV40 enhancer to the human β-globin gene strongly inhibits globin transcription. These results suggest that the structure of the DNA connecting the enhancer to the promoter is important for gene expression and, therefore, argue against a looping model. The same conclusion can be drawn from results indicating that the insertion of the *lexA* operator between upstream activating sequences and the TATA box can block transcription from the *GAL1* promoter (Brent and Ptashne 1984).

Our results also suggest that the structure of the DNA connecting the enhancer to the promoter is important for proper function. We present evidence indicating that the *su(Hw)* protein inactivates enhancers of the *y* gene in a position-dependent manner, only when the *su(Hw)*-binding region is located between an enhancer and promoter. The same type of inhibitory effect has been observed when *su(Hw)*-binding sites are placed in the 5′ region of the *Drosophila hsp70* gene (Holdridge and Dorsett 1991). These results are supportive of tracking models for enhancer action assuming that the binding of the *su(Hw)* protein acts as an obstacle that interferes with sliding or tracking of transcription factors of distal enhancers that are moving toward the promoter. Nevertheless, deletion of a putative leucine zipper region present in *su(Hw)* results in a protein that is able to bind DNA in vivo but is incapable of causing a mutant phenotype,

that is, it cannot interact with transcription factors bound to upstream enhancers (D. Harrison and V. Corces, in prep.). These results suggest that the *su(Hw)* protein does not act as a passive road block for transcription factors; rather, it interacts actively with them either directly, through the leucine zipper region or, indirectly, through other proteins that bind this motif. A requirement for transcription factors to track along the DNA to reach the transcription complex in the promoter has been demonstrated recently for the expression of bacteriophage T4 late genes (Herendeen et al. 1992), and this type of mechanism could explain well the directionality in the inhibitory effects of *su(Hw)*.

The results presented here can also be interpreted in the framework of looping models for enhancer action if we assume that the affinity of enhancer-bound transcription factors for the *su(Hw)* protein is much higher than for proteins present in the transcription complex. If the transcription factors interact with *su(Hw)* through looping of the intervening sequences, the *su(Hw)* protein could act as a sink for upstream transcription factors by preventing their looping and interaction with the transcription complex. The specificity in this effect for distal enhancers could then be explained because these enhancers have to loop over the bound *su(Hw)* protein, whereas proximal enhancers do not. The explanation of the effects of the *su(Hw)* protein by looping mechanisms is not as intuitive when one considers the case of *gypsy*-induced mutations at other loci in which the enhancers are located at large distances from the *gypsy* insertion site and the promoter. Several *cut* mutations exist in which the presence of the *gypsy* element in various places in the 5′ region inhibits the action of a wing-specific enhancer located 80 kb upstream of the promoter (Jack et al. 1991). In addition, several mutations of the Bithorax complex have been shown to result from the insertion of *gypsy* elements within the third intron of the *Ultrabithorax* (*Ubx*) gene (*bithorax* mutations) and in the upstream regulatory region (*bithoraxoid* mutations) (Bender et al. 1983). In these cases, *gypsy* elements are inserted 25–35 kb from the *Ubx* promoter. Thus, similar long-range mutagenic effects on the activity of this gene again result from the insertion of *gypsy*, presumably owing to the inactivation of numerous enhancer elements within these regions of *Ubx* (Peifer and Bender 1986; Simon et al. 1990; Qian et al. 1991). Such long-range inhibitory effects of *su(Hw)* might seem at odds with looping mechanisms and supportive of tracking models, because it is difficult to see how the presence of the *su(Hw)* protein could affect the looping of such large DNA sequences that separate these enhancers from their respective promoters. Nevertheless, enhancer-bound transcription factors might loop out the intervening sequences and scan the DNA in search of the transcription complex. If the *su(Hw)* protein is bound to DNA sequences upstream from the promoter, these transcription factors might interact preferentially with *su(Hw)* than with the promoter, explaining the directionality in the mutagenic effect of the *su(Hw)*-binding region.

An alternative explanation for the inhibitory effect of *su(Hw)* is that binding of this protein induces changes in the adjacent chromatin that could interfere with the binding of transcription factors to the respective enhancers. This hypothesis would have to assume that this altered chromatin structure only spreads distally to explain the specificity in the action of *su(Hw)*. Precedents for this type of effect have been described previously in *Drosophila*. The specialized chromatin structure (scs) sequences establish chromatin domains of independent gene activity by insulating the gene regulatory sequences in adjacent genomic DNA (Kellum and Schedl 1991). This insulating effect is similar to that of *su(Hw)* in the sense that scs sequences inhibit the positive or negative action of sequences located outside of the boundary, that is, more distal with respect to the promoter than the scs sequences. The precise mechanism by which scs sequences exert this effect and whether the *su(Hw)*-binding region acts in a similar way is not yet clear.

The mechanism by which *su(Hw)* affects distal enhancers, whether by altering the chromatin structure or direct interference with the tracking or looping of transcription factors, is not certain at this time. Experiments are now in progress to distinguish between these possibilities.

Materials and methods

DNA constructions

The *y* gene from plasmid pD-2873 was used in these studies. This gene contains the coding region and 2.8 kb of 5′- and 0.13 kb of 3′-flanking DNA and completely restores pigmentation to *y* null flies (Geyer and Corces 1987). The *su(Hw)*-binding region (Spana and Corces 1990) was placed at various positions within this *y* gene. In most cases (p-1868, p-800, p+660, p+1310, and p+2490), the *y* gene was digested with a restriction enzyme, repaired with the Klenow fragment of DNA polymerase I, and ligated to a blunt-ended fragment of the *gypsy* element containing sequences between nucleotides 647 and 1077 (nucleotide position is as described in Marlor et al. 1986). Plasmid p-700 was constructed using a *y* gene fragment containing the same amount of 5′- and 3′-flanking DNA but with a solo *gypsy* LTR at position −700. This DNA was digested with *Hpa*I and *Xba*I, resulting in the loss of all of the LTR except for 51 bp (between nucleotides 431 and 482). The *su(Hw)*-binding region was then inserted in this position as described above. The direction of insertion of the *su(Hw)*-binding region in each construct was determined by DNA sequencing. Constructs in which the *su(Hw)*-binding region is inserted in the opposite orientation relative to its position in the *gypsy* element found in the y^2 mutation are designated with the letter R following the name of the plasmid (Figs. 2–6). The *su(Hw)*-binding region is inserted only in the opposite orientation at position −700. Insertions of the *su(Hw)*-binding region in both orientations were tested at positions −800, +1310, and +2490. Each *y* gene containing a *su(Hw)*-binding region was cloned into the *Sal*I site of the Carnegie 20 plasmid (Rubin and Spradling 1983). Plasmid DNA isolation and DNA enzymology were carried out by standard procedures (Maniatis et al. 1982).

Germ-line transformation

Germ-line transformation was carried out as described by Rubin

and Spradling (1982). The host strain used in these experiments has a deletion of a portion of the X chromosome containing the *y* and *achaete* (*ac*) loci in addition to the ry^{506} mutation. DNA concentrations used in these experiments were 400 mg/ml of the Carnegie 20–*y* construct and 100 mg/ml of the "wings-clipped" helper plasmid pπ25.7 (Karess and Rubin 1984). Transformants were recognized by the ry^+ phenotype and used to establish stocks. Additional transformant lines were obtained by mobilizing a single insertion by crossing in the *Sb* ry^{506} P[$ry^+\Delta$2-3] (99B) chromosome described by Robertson et al. (1988). Only lines with single insertions were analyzed, and at least three lines per construct were obtained. The number of insertions within each line and the structure of the transposon following integration were determined by DNA Southern analysis. Transformants were crossed with y^2 sc^1 w^{67} ct^6 f^1; bx^{34e} $su(Hw)^V$/TM6, $su(Hw)^f$, *Ubx* flies to determine whether the effects of the insertion were reversible in a *su(Hw)* mutant background.

Cuticle preparations

Preparation of the cuticles was done as described by Baker et al. (1978). Briefly, flies were placed in 10% potassium hydroxide in a 98°C water bath for 2 min. The flies were rinsed in water and flattened to expel soft tissue. The carcasses were heated for an additional minute in 10% potassium hydroxide, boiled for 5 min in water, and dehydrated by passing through 95% ethanol, absolute ethanol, and xylene. The carcasses were then mounted in Permount.

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