The *su(Hw)* protein insulates expression of the *Drosophila melanogaster white* gene from chromosomal position-effects

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Mutations in the *suppressor of Hairy-wing* [*su(Hw)*] locus reverse the phenotype of a number of tissue-specific mutations caused by insertion of a gypsy retrotransposon. The *su(Hw)* gene encodes a zinc finger protein which binds to a 430 bp region of gypsy shown to be both necessary and sufficient for its mutagenic effects. *su(Hw)* protein causes mutations by inactivation of enhancer elements only when a *su(Hw)* binding region is located between these regulatory sequences and a promoter. To understand the molecular basis of enhancer inactivation, we tested the effects of *su(Hw)* protein on expression of the mini-*white* gene. We find that *su(Hw)* protein stabilizes mini-*white* gene expression from chromosomal position-effects in euchromatic locations by inactivating negative and positive regulatory elements present in flanking DNA. Furthermore, the *su(Hw)* protein partially protects transposon insertions from the negative effects of heterochromatin. To explain our current results, we propose that *su(Hw)* protein alters the organization of chromatin by creating a new boundary in a pre-existing domain of higher order chromatin structure. This separates enhancers and silencers distal to the *su(Hw)* binding region into an independent unit of gene activity, thereby causing their inactivation.

Key words: domain boundaries/*Drosophila*/heterochromatin/position-effects/*su(Hw)* protein

Introduction

Transposable elements are found in the genomes of a variety of organisms and are a major cause of spontaneous mutations in *Drosophila*. Mechanisms by which mobile elements evoke a mutant phenotype are varied and depend upon multiple factors, such as the position of insertion within a gene, the way the target gene is regulated and the properties of the individual element (Smith and Corces, 1991). In some cases, insertion of transposable elements causes tissue-specific mutations by interacting with *cis*-linked regulatory regions of the target gene. A better understanding of normal mechanisms of gene expression will result from the analysis of this class of control mutants.

In *Drosophila*, integration of the gypsy retrotransposable element is responsible for tissue-specific mutations at a number of loci, including *yellow*, *Ultrabithorax* and *cut*

(Modolell *et al.*, 1983). Gypsy is 7.5 kb in length, contains long terminal repeats (LTRs) of ~500 bp and encodes putative proteins homologous to retroviral proteins (Marlor *et al.*, 1986). Insertion of gypsy within a gene can cause a mutant phenotype even when it resides as much as 10–30 kb from a promoter (Peifer and Bender, 1986; Jack *et al.*, 1991). In several cases, gypsy-induced mutations are reversed by mutations at the second site modifier locus, the *suppressor of Hairy-wing* [*su(Hw)*] (Modolell *et al.*, 1983). *su(Hw)* mutations specifically reverse mutations caused by gypsy and do not affect mutations induced by any other transposable element (Rutledge *et al.*, 1988).

The *su(Hw)* gene encodes a 110 kDa protein that is present throughout *Drosophila* development in most tissues of the fly (Parkhurst *et al.*, 1988). This protein contains several motifs characteristic of eukaryotic transcription factors. These include a DNA binding domain containing 12 repeats of the zinc finger motif and two acidic regions and a leucine zipper, domains implicated in promoting protein–protein interactions. *su(Hw)* protein is localized in the nucleus and is present on third instar larval polytene chromosomes at 100–200 euchromatic sites (Spana *et al.*, 1988). The *su(Hw)* protein binds specifically to a region of the gypsy element located 3' of the 5' LTR. This region contains 12 copies of a sequence similar to the octameric motif, a mammalian enhancer element (5'PyPuTTGCATAC3'). The repeats are separated by A-T rich sequences. DNA footprinting experiments suggest association of more than one *su(Hw)* molecule with this region; as many as 12 molecules may associate if it binds as a monomer (Spana *et al.*, 1988; Mazo *et al.*, 1989). Analysis of complete and partial revertants of gypsy-induced mutations indicates that the entire *su(Hw)* binding region is critical for gypsy mutagenesis. Disruption of these sequences by secondary insertions, or deletion of a portion of this region alters the mutant phenotype (Geyer *et al.*, 1988; Peifer and Bender, 1988; Flavell *et al.*, 1990; Smith and Corces, 1992).

Studies using the *yellow* (*y*) gene as a model system have provided useful information concerning the molecular basis of tissue-specific mutagenesis by gypsy. The *yellow* gene is required for pigmentation of larval and adult cuticle; mutations change the normal cuticle color from black to yellow. Temporal and spatial expression of *yellow* is controlled by several independent tissue-specific transcriptional enhancer elements (Geyer and Corces, 1987). Insertion of the gypsy element causes the tissue-specific mutation *y2*. In this mutation, gypsy is integrated 700 bp upstream of the *yellow* transcription start site (Geyer *et al.*, 1986) causing inactivation of enhancer elements upstream of this site. These enhancers control *yellow* expression in the cuticle of the wing and body so the insertion produces flies mutant in coloration only in these tissues. Transgenic flies carrying *yellow* genes containing a gypsy element deleted for the entire *su(Hw)* binding region do not have

altered *yellow* gene expression, indicating directly that this region is necessary to confer a mutant phenotype (Smith and Corces, 1992). Transgenic flies carrying *yellow* genes containing only the *su(Hw)* binding region at -700 do display aberrant *yellow* expression and have a *y2* phenotype demonstrating that this region is sufficient for gypsy mutagenesis (Geyer and Corces, 1992).

The *su(Hw)* protein inactivates any enhancer element of the *yellow* gene only when the *su(Hw)* binding region is inserted between that enhancer element and the promoter (Geyer and Corces, 1992). Tissue-specific enhancers located both 5' and within the intron can be affected. Binding of *su(Hw)* protein within the intron of the *yellow* gene does not inhibit progression of the transcription complex, since in this position only those enhancer elements downstream of the binding region are blocked. The *su(Hw)* protein inactivates the heat shock enhancer of the *hsp70* gene in the same position dependent manner (Holdridge and Dorsett, 1991). Mutational effects of gypsy insertions at the *cut* and *Ultrabithorax* loci are entirely consistent with inactivation of enhancers when gypsy interposes between them and the promoter (Peifer and Bender, 1986; Simon *et al.*, 1990; Qian *et al.*, 1991; Jack *et al.*, 1991). This interpretation may explain why some genes are favorite targets for gypsy mutagenesis: these genes contain extensive regulatory regions where insertion of gypsy into large areas causes inactivation of the distal enhancers, regardless of distance.

The mechanism by which *su(Hw)* protein interferes with enhancer function is unclear. That inactivation requires insertion of a *su(Hw)* binding region between the affected enhancer and promoter suggests that interference with enhancer action is directional and not simply due to proximity of the *su(Hw)* protein and an enhancer. This directional specificity indicates that *su(Hw)* protein interacts with transcription factors of the affected tissue-specific enhancer elements and not with the transcription complex. We have considered three possible classes of model to explain the effect: (i) enhancers are entry points for transcription factors that track along the DNA until they reach a promoter and *su(Hw)* protein prevents the progression of these transcription factors; (ii) the looping of enhancer complexes to contact the promoter is prevented by an intervening array of *su(Hw)* protein which either captures the enhancer complex or disrupts the interaction of the enhancer transcription factors with a bridging complex; or (iii) the binding of *su(Hw)* protein to a region of DNA induces a change in chromatin structure which interferes with the function of enhancer factors (Geyer and Corces, 1992). To distinguish between these models, we have tested the effects of *su(Hw)* protein on *white* gene expression. The *white* gene encodes a protein required for eye pigmentation, allowing a visual assessment of its transcriptional activity. Our results demonstrate that *su(Hw)* protein stabilizes expression of transposons carrying the *white* gene when they are integrated into euchromatic sites. Our evidence suggests that invariant expression of these transposons arises from inactivation of positive and negative regulatory elements present within flanking DNA. In addition, we have found that *su(Hw)* protein partially blocks negative influences of heterochromatin on *white* expression. We propose that inactivation of enhancers and silencers occurs by creation of a boundary within an existing domain of chromatin structure. This boundary divides the previous unit of gene activity into two parts, separating the distal

control elements into a distinct functional domain so they are no longer able to activate their normal promoter.

Results

The su(Hw) protein inactivates the white eye enhancer when inserted between that enhancer and the promoter

There are several reasons why the *white* gene is a good model for analyzing the mechanism by which *su(Hw)* inactivates enhancers. First, the *white* gene is molecularly well characterized (Hazelrigg *et al.*, 1984; Levis *et al.*, 1985; Pirrotta *et al.*, 1985). Second, tissue-specific enhancer elements of the gene have been identified (Qian *et al.*, 1992). Third, the level of eye pigmentation is a sensitive indicator of the amount of *white* transcription. Fourth, *white* expression is cell autonomous, allowing assessment of expression in individual cells. Finally, none of the several transposable element-induced *white* mutations characterized result from gypsy insertion (Birchler *et al.*, 1989).

The *white* gene used in these studies is that of the CaSpeR vector (Pirrotta, 1988). This gene contains ~300 bp of 5' and 630 bp of 3' flanking DNA and has most of the first intron deleted. The CaSpeR vector has been widely used as a transformation vector. Transformants carrying the mini-*white* gene show a range of eye coloration from very pale yellow to dark red depending on the position of insertion into the genome. The extreme sensitivity of the mini-*white* gene to the chromosomal position-effects is because it lacks most of its normal regulatory sequences and acts like an enhancer trap. Genomic sequences located either 5' or 3' of the mini-*white* transposon can affect expression of this gene (Kellum and Schedl, 1991).

We first determined whether *su(Hw)* protein would block the eye enhancer of the *white* gene in the same way it blocks enhancers of other genes (Holdridge and Dorsett, 1991; Geyer and Corces, 1992). If so, inactivation of this enhancer would occur only when the *su(Hw)* binding region was located between the promoter and enhancer. One transposon was constructed that contained the *su(Hw)* binding region (B.R.) inserted directly upstream of the eye enhancer (Eye) which, in turn, was placed in front of the CaSpeR mini-*white* gene (B.R.:Eye > w). A second transposon was made which carried the binding region downstream of the enhancer (Eye:B.R. > w). Each plasmid also carried the intronless *yellow* gene which was used as a transformation marker (Geyer and Corces, 1987). These plasmids were injected into *y⁻ac⁻w⁻* embryos and transformants were selected by the *y⁺* phenotype. We examined the eye phenotype of several independent transformed lines. In each case, genomic Southern analysis was carried out to confirm the structure and number of transposons (data not shown). We obtained seven independent transformed lines which carried a single copy of the unblocked eye enhancer (B.R.:Eye > w). In each line, the flies had a nearly wild-type level of eye pigmentation (Figures 1 and 2). This supports the conclusion that *su(Hw)* protein does not affect enhancers from an upstream position (Holdridge and Dorsett, 1991; Geyer and Corces, 1992). In transgenic flies carrying the the *su(Hw)* binding region between the eye enhancer and the transcription start site (Eye:B.R. > w), we obtained nine independent lines; flies in these lines had a range of eye phenotypes from yellow to brown, instead of the predicted low level of eye

pigmentation (Figures 1 and 2). We considered two possible explanations for the observed variability in *white* gene expression. First, the *su(Hw)* protein may completely block the eye enhancer but this effect is masked by positive regulatory elements located at the 3' end of the *white* gene which activate transcription. Alternatively, *su(Hw)* protein may only be able to block the eye enhancer when the transposon is inserted into particular chromosomal locations, but not in others.

To address this issue, we placed a second *su(Hw)* binding region just downstream of the *white* transcription unit to eliminate influences of 3' genomic sequences on *white* expression. We tested two additional transposons which carried two binding regions for *su(Hw)* protein. In each transposon, one binding region was inserted 3' of the *white* gene whereas the second binding region was either upstream (B.R.:Eye>*w*>B.R.) or downstream (Eye:B.R.>*w*>B.R.) of the eye enhancer (Figures 1 and 2). Insertion of the 3' *su(Hw)* binding region did not affect the phenotype of flies carrying the B.R.:Eye>*w*>B.R. transposon. In 15 lines examined, 12 showed a high level of *white* expression. The three remaining lines showed variegated expression of the *white* gene. In 16 independent transgenic lines carrying the Eye:B.R.>*w*>B.R. transposon, all flies had yellow eye pigmentation. Thus, we conclude that *su(Hw)* protein causes inactivation of the *white* eye enhancer. Furthermore, these results suggest that the variable phenotype observed in lines carrying Eye:B.R.>*w* was caused by sequences located in the 3' genomic DNA. Flies carrying this transposon must also have the eye enhancer blocked. Thus, when flies carrying Eye:B.R.>*w* transposon show a high level of *white* gene expression it must result from insertion into a chromosomal position that contains an enhancer at the 3' end on the mini-*white* gene. Integration of this transposon into flanking DNA devoid of enhancers would produce flies with a low level of *white* expression. The constant eye phenotype seen in lines carrying the Eye:B.R.>*w*>B.R. transposon indicates that *su(Hw)* protein insulates the *white* gene from chromosomal position-effects.

We next asked whether the increased distance of the eye enhancer from the promoter in the transposon Eye:B.R.>*w*>B.R. was responsible for the low level of *white* expression. If enhancer inactivation is due to binding of *su(Hw)* protein and not spacing, then eye pigmentation should be restored to a brownish-red color in the absence of *su(Hw)* protein. We examined the level of *white* gene expression in flies of 10 transformed lines carrying the Eye:B.R.>*w*>B.R. transposon and containing a homozygous mutation in the *su(Hw)* gene. In nine of the 10 lines, eye coloration was nearly wild-type in the absence of *su(Hw)* protein. Flies from a representative transgenic line in wild-type and mutant *su(Hw)* backgrounds are shown in Figure 3. One of the 10 lines manifested an interesting position-effect in a mutant *su(Hw)* background; pigmentation in the anterior portion of the eye was restored to brownish-red, but coloration in the posterior portion was white (Figure 3B). This position-effect is not observed in the presence of the *su(Hw)* protein where a uniform yellow color is seen. The lack of pigment in the posterior portion of the eye is most likely due to a repression of *white* expression arising from flanking genomic DNA, as this phenotype is similar to that studied by Hazelrigg and Petersen (1992). These repressing effects can be blocked by *su(Hw)* protein.

The *su(Hw)* protein insulates the enhancerless mini-*white* gene from chromosomal position-effects

The eye color of flies carrying the Eye:B.R.>*w*>B.R. was yellow, suggesting that the *white* gene was being transcribed at a low level in the eye. We wished to determine whether this level of transcription was due to low *white* expression from its basal promoter or a partial blockage of the eye enhancer. We constructed a plasmid carrying an enhancerless mini-*white* gene (CaSpeR) flanked by *su(Hw)* binding regions (B.R.>*w*>B.R.) and obtained 15 independent transformed lines carrying this transposon. In two lines, the eye phenotype was variegated and yellow. In the rest of the lines, all flies had a yellow eye color indistinguishable from that of flies carrying the Eye:B.R.>*w*>B.R. transposon (Figures 1 and 2). This suggests that the yellow color was due to basal *white* expression and that the eye enhancer is completely blocked by binding of the *su(Hw)* protein.

We also examined the eye phenotype of four transformed lines carrying the enhancerless mini-*white* gene surrounded by *su(Hw)* binding regions (B.R.>*w*>B.R.) in a mutant *su(Hw)* background. In this case, it is difficult to predict the resulting phenotype because the position of insertion could have a positive, negative or no effect. The eye phenotype in three lines was the same in wild-type and mutant *su(Hw)* backgrounds. However, in one line the eye color was lighter in a mutant *su(Hw)* background (Figure 3C). Thus, *white* expression was decreased in the absence of *su(Hw)* protein. It is unlikely that this repression is due to the interaction of a second protein binding in the absence of *su(Hw)*, as negative effects on *white* expression are observed in only a subset of lines. These results are consistent with an ability of *su(Hw)* protein to block repressing effects arising from the chromosomal site of insertion.

Dosage compensation is unaffected by the *su(Hw)* protein

In *Drosophila*, both X chromosomes are active in females. To offset the gene dosage difference in males, the levels of expression of nearly all X-linked genes are doubled. The wild-type *white* locus is on the X chromosome and is normally dosage compensated. In all of our transgenic lines, pigmentation levels in males were greater than in females (Figure 3). We conclude that the dosage compensation system operates on any chromosome and is not blocked by binding of *su(Hw)* protein. Sequences required for dosage compensation of the *white* gene are closely linked to the transcription start site, no further 5' than -216 bp (Pirrotta *et al.*, 1985). Dosage compensation should not be blocked by the binding of *su(Hw)* protein because the binding region was placed upstream of these sequences.

Repressing effects of heterochromatin are blocked by the *su(Hw)* protein

As indicated above, three of the transformed lines carrying the B.R.:Eye>*w*>B.R. plasmid had a variegated eye phenotype (Figure 4). Each transposon also carries the cell autonomous *yellow* gene as a marker for transformation. In each line in which *white* expression was variegated, so was *yellow* gene expression. As the observed position-effects were exerted on both genes, we reasoned that these transgenic lines most likely contained transposon insertions into a heterochromatic portion of the chromosome. Heterochromatin is the region of the chromosome which

Fig. 1. Eye phenotypes of transgenic flies carrying the mini-*white* transposons. Flies of the genotype *y⁻ac⁻w⁻* were transformed with the plasmids containing the *yellow* gene and the indicated modification of the mini-*white* gene of the CaSpeR vector. The *su(Hw)* binding region is shown as the shaded triangle, the *white* eye enhancer is indicated by the dotted circle and the position of the *white* transcription start site is shown by the arrow. Representative females are shown from five different lines of transformants. Each female is 3 days old and is heterozygous for the single insertion of the P element transposon.

		RANGE OF PHENOTYPES				
		Yellow	Orange	Brown	Red	Variegated
B.R.: Eye > w		0	0	5	2	0
Eye : B.R. > w		1	2	6	0	0
B.R.: Eye > w > B.R.		0	0	11	1	3
Eye : B.R. > w > B.R.		16	0	0	0	0
B.R. > w > B.R.		13	0	0	0	2

Fig. 2. Summary of phenotypes of transgenic fly lines obtained by germline transformation. The phenotypes of the lines carrying the various transposons are listed. The number refers to how many lines show that eye color. A low level of *white* expression produces the yellow eye phenotype; whereas wild-type expression gives a red eye color. Variegated eye phenotypes result from insertion of the transposon into regions of heterochromatin. Symbols are the same as used in Figure 1.

Fig. 3. Effects of *su(Hw)* mutations on transformants. The eye phenotype is shown of transformants carrying a mini-*white* gene with either a blocked eye enhancer (A and B) or no enhancer (C) in a wild-type [*su(Hw)*⁺/*su(Hw)*⁺] or homozygous mutant [*su(Hw)*^v/*su(Hw)*^f] background. The genotype of each pair of flies is shown below the photograph. In each case, a female and male are shown. In (A) the male fly is on the left and the female fly is on the right. In (B) and (C) the male fly is on the right and the female fly is on the left. Note that pigmentation of the eye in males is higher than in females. In 9/10 lines carrying Eye:B.R. > w > B.R. homozygous *su(Hw)* mutant background, the *white* eye enhancer is no longer inactive and eye color is restored to a near wild-type level as shown in (A). In one line (B) a chromosomal position-effect was observed in the absence of *su(Hw)* protein. The white color of the posterior of the eye most likely results from repressing effects in flanking DNA. In a wild-type *su(Hw)* background, pigmentation of the eye is uniform. Additional repressing effects of genomic DNA can be observed in (C) when transformants carrying B.R. > w > B.R. are homozygous mutant for *su(Hw)*.

Fig. 4. Eye phenotype of transgenic flies carrying transposon insertions into heterochromatin. Shown is the eye phenotype of flies from a single transformed line carrying an insertion of the plasmid B.R.:Eye>w>B.R. into a heterochromatic region of the chromosome. The genotypes and temperatures at which each fly was raised are shown below the photograph. The amount of variegation is increased in the absence of a Y chromosome (compare B and C), at lower temperature (compare B and D) and by reducing the level of *su(Hw)* protein present (compare B with E and F).

remains highly compacted during interphase. Placing euchromatic genes next to heterochromatin results in inactivation of gene expression in some cells but not others, causing a mosaic or variegated phenotype. These negative effects on gene expression may result from a spreading of heterochromatin into the euchromatic DNA (Henikoff, 1990). To determine whether the variegating transformed lines carried insertions into heterochromatin, we tested the effects of temperature and the Y chromosome on the eye phenotype. Heterochromatinization is increased at low temperatures and in the absence of the Y chromosome (Spofford, 1967). Flies from each variegating transformed line were grown at 18°C. In each line, growth at the lower temperature increased the amount of variegation (Figure 4). In a second set of experiments, females from the variegating lines were crossed with attached XY males to generate XO males. Comparison of these males with XY males demonstrated that the level of variegation increased in the absence of the Y chromosome (Figure 4). These results are consistent with the transposons being inserted near a heterochromatic region of the chromosome. *In situ* localization of the transposon in one line demonstrated that this line carried an insertion near the centromeric region of the second chromosome (data not shown).

The influence of *su(Hw)* protein on heterochromatic position-effects was determined by making these variegating transformed flies homozygous for mutations in the *su(Hw)* locus. For each line, we found that the lack of *su(Hw)* protein in flies homozygous for *su(Hw)* mutations drastically increased the amount of variegation in the eye (Figure 4). Interestingly, effects of *su(Hw)* mutations on heterochromatinization were dominant. In other words, flies carrying the variegating *white* transposon that were heterozygous for a mutation at the *su(Hw)* locus displayed an intermediate level of variegation (Figure 4). We conclude that the *su(Hw)* protein partially blocks negative effects of neighboring heterochromatin in a dosage-sensitive manner.

Discussion

Insertion of the gypsy transposable element at a number of loci causes mutations that are reversed by second-site mutations at the *su(Hw)* locus. Previously, we proposed that the *su(Hw)* protein mediates the mutagenic effects of gypsy by inactivating only those regulatory elements which reside upstream of the *su(Hw)* binding region (Corces and Geyer, 1991; Geyer and Corces, 1992). Accumulating molecular information concerning the regulatory regions of genes affected by gypsy insertions is consistent with this hypothesis (Peifer and Bender, 1986; Simon *et al.*, 1990; Jack *et al.*, 1991; Qian *et al.*, 1991). Results described in this paper support and extend this model. In our current studies, we used the mini-*white* gene of the CaSpeR vector as a model system to test effects of *su(Hw)* protein. This system has allowed us to address the question of whether the *su(Hw)* protein is a general inactivator of enhancer and silencer function. Our results showed that *su(Hw)* protein blocks the *white* eye enhancer only when its binding region is inserted between the enhancer and promoter. This same position dependence of enhancer inactivation of a *su(Hw)* binding region has been previously established for other genes (Holdridge and Dorsett, 1991; Jack *et al.*, 1991; Geyer and Corces, 1992). Our experiments also demonstrated that

su(Hw) binding regions protect the mini-*white* gene of the CaSpeR vector from chromosomal position-effects. The phenotype of transformed flies carrying an unmodified CaSpeR vector is influenced by its chromosomal insertion site because this gene lacks the eye enhancer and relies on surrounding regulatory elements to control its expression. This has been demonstrated previously by Kellum and Schedl (1991). In their studies, the CaSpeR mini-*white* gene was insulated from euchromatic but not heterochromatic chromosomal position-effects by *scs* and *scs'* regions of the 87A7 heat shock locus only when these sequences were inserted both 5' and 3' of this gene. A single insertion at the 5' end was not sufficient to stabilize expression in different genomic locations even though single *scs* regions block the enhancer elements present in several other genes (Kellum and Schedl, 1992). These results suggest that the expression of the mini-*white* gene can be influenced by regulatory sequences either 5' or 3' of the gene. Our results showing position independent expression of the mini-*white* gene flanked by *su(Hw)* binding regions of transposons inserted into euchromatic sites supports the contention that *su(Hw)* is a general blocker of regulatory elements. It is likely that the stabilization of *white* gene expression arises from inactivation of silencer elements as well as enhancers present within the flanking DNA. Consistent with this proposal is our observation that expression of the *white* gene in two such transposons is repressed when placed in a *su(Hw)* mutant background.

su(Hw) protein partially blocks position-effect variegation. This variegation is currently understood to be caused by the variable spread of chromatin condensation that occurs in the vicinity of heterochromatic regions. Genes that become inserted near heterochromatin become inactivated in some cells but not in others due to the spread of heterochromatinization. Considerable evidence indicates that heterochromatin formation involves the assembly of multimeric protein complexes such that varying the concentration of any one component has strong effects on the extent of heterochromatinization (Locke *et al.*, 1988). Mutations in genes encoding proteins involved in the establishment and maintenance of heterochromatin often show dosage-sensitive effects on position-effect variegation (Eissenberg, 1989). It is interesting that flies heterozygous for *su(Hw)* mutations have detectable effects on the level of heterochromatin-induced variegation of *white* transposons, whereas a 50% decrease in the amount of *su(Hw)* protein does not generally alleviate the mutational effect of gypsy insertions nor does it affect the block of eye enhancer on the *white* gene expression in euchromatic positions. Thus, it is possible that *su(Hw)* protein is normally involved in the spreading of heterochromatin and its effects on position-effect variegation result from alterations in chromatin structure. This conclusion is strengthened by preliminary results which demonstrate that mutations in the *su(Hw)* gene enhance position-effect variegation of the inversion *In(1) w[m4]* (R.R.Roseman and P.K.Geyer, unpublished). Since loss of function *su(Hw)* mutations enhance variegation in our experiments, it is interesting that one Enhancer of position-effect variegation, *En(var)c101*, has been mapped to 3-54, very close to the location of *su(Hw)*; 3-54.8 (Lindsley and Zimm, 1992). We are testing the possibility that these two mutations are allelic. In addition, we are examining the effects of mutations in *su(Hw)* on several variegating translocations.

How does the *su(Hw)* protein block effects of regulatory elements? Any hypothesis must consider that their inactivation requires a particular positioning of the *su(Hw)* binding region relative to the regulatory sequence and promoter, that inactivation can occur over long distances from both the enhancer and promoter and that these effects do not show promoter specificity as a variety of genes can be mutated by gypsy integration (Bender *et al.*, 1983; Modollel *et al.*, 1983; Holdridge and Dorsett, 1991; Jack *et al.*, 1991; Geyer and Corces, 1992). One proposed mechanism is that *su(Hw)* protein blocks the tracking of transcription factors along DNA to the promoter (Geyer and Corces, 1992). This model addresses the positioning conditions of inactivation but it requires that the actions of enhancer and silencer elements occur primarily by scanning mechanisms and not by looping of intervening DNA facilitated by protein-protein interactions. Although, some enhancer-directed activation of transcription may involve tracking of transcription factors (Courey *et al.*, 1986; Herendeen *et al.*, 1992), several other enhancer elements appear to use a looping mechanism (Dunaway and Droge, 1989; Muller *et al.*, 1989; Wedel *et al.*, 1990). A second model involves *su(Hw)* protein blocking a looping interaction between the enhancer and promoter. This could be envisioned as a capture of (i) the enhancer complex as it loops towards the promoter, or (ii) the promoter prior to its interaction with the more distant enhancer or (iii) adapter proteins that might normally mediate the interaction of enhancer and promoter complexes (Geyer and Corces, 1992). The observation that *su(Hw)* protein inactivates enhancers only when the binding region lies between an enhancer and promoter, and not when directly upstream of the enhancer, argues against direct interactions with adapter molecules. Furthermore, *su(Hw)* protein bound at the *yellow* locus in the configuration wing enhancer-*su(Hw)*-body enhancer does not prevent the function of the body enhancer as it does when it is bound between both enhancers and the promoter. This suggests that *su(Hw)* protein does not have a general interaction with promoter-associated complexes. In a third model, the binding of *su(Hw)* protein is postulated to cause changes in chromatin structure which, in turn, inactivate enhancers or silencers (Geyer and Corces, 1992). In this model, the proposed mechanism of repression by *su(Hw)* is similar to that involved in silencing of the mating-type loci in yeast (Brand *et al.*, 1985; Johnson *et al.*, 1990). However, changes in chromatin structure must occur only in one direction because inactivation by *su(Hw)* protein requires a particular positioning of the binding region relative to the enhancer and promoter. Thus, we propose a modification of this model and suggest that *su(Hw)* protein is involved in establishing a boundary in a higher order domain of chromatin structure. Eukaryotic chromosomes are believed to be organized into discrete and independent higher order structural domains (Kellum and Schedl, 1991). These domains serve two functions: chromatin compaction and definition of independent units of gene activity. Separation of units of gene expression may be important to direct enhancer action to the appropriate target gene, preventing random long range activation of inappropriate genes. The importance of independent domains of gene activity for normal gene expression was suggested by results from studies of the bithorax complex in *Drosophila*. Deletion of a DNA segment separating two independent control elements causes inappropriate activation of gene expression by an

element that is normally silent (Gyurkovics *et al.*, 1990). Proteins involved in establishing domain boundaries of chromatin structure should have the following properties. They should (i) protect a reporter gene from chromosomal position effects, (ii) inactivate regulatory elements in a directional manner when inserted between the affected control element and promoter and (iii) alter chromatin structure (Kellum and Schedl, 1991, 1992). The binding of *su(Hw)* protein to the 12 binding sites of the gypsy element appears to fit these criteria. *su(Hw)* protein blocks the eye enhancer of the *white* gene and protects it from positive and negative control elements in genomic DNA. *su(Hw)* protein inactivates four different cuticle enhancers in the *yellow* gene and one enhancer element in the heat shock gene (Holdridge and Dorsett, 1991; Geyer and Corces, 1992). In addition, *su(Hw)* may be involved in establishing at least one class of chromatin structure, heterochromatin. Mutations in the *su(Hw)* gene appear to increase the amount of heterochromatin suggesting that its product is involved in establishing heterochromatin boundaries. Characteristics of the *su(Hw)* binding region of gypsy are similar to those described for other putative boundary regions such as the *scs* and *scs'* regions of the *Drosophila* 87A7 heat shock locus (Kellum and Schedl, 1991, 1992), the 'locus activating regions' of the human B-globin domain (Grosveld *et al.*, 1987; Forrester *et al.*, 1987) and the A-elements from the chicken lysozyme domain (Stief *et al.*, 1989). In these examples, however, the identity of proteins which interact with these sequences is not known. Reiterated *su(Hw)* binding sites were not found in the *scs* and *scs'* regions indicating the blocking activity of these regions involve different proteins (Kellum and Schedl, 1992).

What is the normal cellular role of *su(Hw)* in *Drosophila*? Are the effects of *su(Hw)* on gypsy element insertions a reflection of this role? Complete inactivation of enhancer function requires multiple *su(Hw)* binding sites within a gene (Geyer *et al.*, 1988; Peifer and Bender, 1988; Flavell *et al.*, 1990; Smith and Corces, 1992). Thus, unless similar sets of reiterated binding sites for this protein are identified outside of gypsy elements, *su(Hw)* protein may not normally play a role in the establishment of chromatin domains. However, domain boundaries are most likely established by the assembly of several proteins. *su(Hw)* protein may cooperate with other proteins to create a boundary. Reiteration of binding sites for any one protein involved in boundary formation may allow establishment of a boundary using a single protein. Thus, gypsy may have evolved a region containing reiterated *su(Hw)* binding sites to ensure protection of the 5' LTR from a silencing mechanism that represses the promoter in the 3' LTR. That *su(Hw)* protein is required for activation of gypsy expression (Parkhurst and Corces, 1986) argues that this protein may also activate transcription. The nature of the effects of *su(Hw)* protein, whether positive or negative, may depend upon the properties of other proteins with which it interacts.

The properties of the *su(Hw)* protein that we have described in this paper may have practical uses in the experimental analysis of expression of other genes. It should be possible to construct tissue-specific mutations in any gene by inserting the *su(Hw)* binding region between a tissue-specific enhancer and promoter. Thus, the requirement for the product of a gene in individual tissues could be assessed. If such mutations are expected to be detrimental, they could be first established in a homozygous mutant background for

su(Hw) where insertion of the binding region would be of no phenotypic consequence. In addition, introduction of any gene flanked by *su(Hw)* binding regions into the chromosome should confer chromosomal position independent expression of that gene. This would help reduce the number of germline transformants that are needed to be generated in an experiment.

Materials and methods

Construction of plasmids

Plasmids used in these studies were derivatives of the P element transformation vector, CaSpeR3. This vector carries a mini-white gene containing ~300 bp of 5' and 630 bp of 3' flanking DNA and most of the first intron deleted (Pirrotta, 1988). The polylinker region of this plasmid was modified so that it contained a portion of the 5' regulatory region of the white gene. The inserted white regulatory sequences (Eye) were derived from the interval between -1084 and -1465 from the transcription start site and contain the testes and eye enhancer (Qian *et al.*, 1992). This derivative plasmid is called CaSpeR 3-ET. The *su(Hw)* binding region (B.R.) of gypsy contains all 12 binding sites (between nucleotides 647 and 1077 as numbered in Marlor *et al.*, 1986). These sequences were blunt-end ligated into unique restriction sites either downstream or upstream of the eye enhancer region to form plasmids B.R.:Eye>w or Eye:B.R.>w respectively. Transposons carrying two *su(Hw)* binding regions were constructed in the following manner. One *su(Hw)* binding region was inserted by blunt-end ligation into pCaSpe w15 at the *EcoRI* restriction site located 630 bp downstream of the white coding region. Plasmids B.R.:Eye>w>B.R., Eye:B.R.>w>B.R. and B.R.>w>B.R. were created by directional cloning of the appropriate 5' regions of transposons carrying a single 5' binding region into the *su(Hw)*-pCaSpe w15 plasmid. Each of these *su(Hw)* binding region derivatives of CaSpeR were modified further by upstream insertion of the intronless yellow gene (Geyer and Corces, 1987). This yellow gene is 5.2 kb in length, contains 2.8 kb of 5' and 0.13 kb of 3' flanking DNA and confers pigmentation of all larval and adult cuticle structures except the hairs and bristles. This gene was used as the marker for transformation. Plasmid DNA isolation and DNA enzymology were carried out by standard procedures (Maniatis *et al.*, 1982).

Germline transformation

Germline 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 yellow and *achaete* loci (M.M.Green, unpublished) and the *w1118* mutation (Hazelrigg *et al.*, 1984). DNA concentrations used in these experiments were 400 µg of the yellow-white construct per ml and 100 µg of the 'wings-clipped' helper plasmid pπ25.7 per ml (Karess and Rubin, 1984). Transformants were recognized by the pigmentation of the wing and body cuticle. Generation of additional lines was accomplished by mobilizing the P element transposon using a chromosomal source of transposase [*Sb rosy*⁵⁰⁶ P(ry⁺ Δ2-3) (99B) chromosome] as described by Robertson *et al.* (1988). The number of insertions within each line, and the structure of the transposon following integration were determined by DNA Southern analysis. Described in this paper are transformed lines which carry only a single transposon insertion.

Transformants were crossed with y2 sc w67 ct6 fl; bx34e *su(Hw)*/TM6, *su(Hw)*f, *Ubx* flies to determine the effects of a *su(Hw)* mutant background on the P element insertion. The effects of the Y chromosome on transformed lines with a variegated eye phenotype were determined by crossing female transformants to males carrying an attached XY chromosome [XY y v/O C(1)A-Y]. All male progeny from this cross were XO. Males from this cross were compared with males in the parental transformed line.

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