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Dominant Effects of *suppressor of Hairy-wing* Mutations on *gypsy*-Induced Alleles of *forked* and *cut* in *Drosophila melanogaster*

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ABSTRACT

Mutations induced by the *gypsy* retrotransposon in the *forked* (*f*) and *cut* (*ct*) loci render their expression under the control of the *suppressor of Hairy-wing* [*su(Hw)*] gene. This action is usually recessive, but *su(Hw)* acts as a dominant on the alleles f^k, ct^k and ct^{MRpN30}. Molecular analysis of the *gypsy* element present in f^k indicates that this allele is caused by the insertion of a modified *gypsy* in which the region normally containing twelve copies of the octamer-like repeat that interacts with the *su(Hw)* product is altered. Analysis of the *gypsy* element responsible for the ct^k and ct^{MRpN30} mutations also reveals a correlation between the dominant action of *su(Hw)* and disruption of the octamer region. We propose that these disruptions alter the affinity and interaction of *su(Hw)* protein with gypsy DNA, thereby sensitizing the mutant phenotype to fluctuations in *su(Hw)* product.

THE insertion of transposable elements is responsible for most spontaneous mutations in *Drosophila melanogaster* (RUBIN 1983; LEVIS, O'HARE and RUBIN 1984). Transposable elements may cause mutant phenotypes by a variety of mechanisms, depending on the location of the insertion site with respect to different structural and functional domains of the affected gene. For example, insertion of the *gypsy* retrotransposon in the 5′ region of the *yellow* or *cut* loci affects the rate of transcription of these genes in a tissue-specific fashion (CORCES and GEYER 1991; JACK *et al.* 1991), whereas insertion of the copia element in an intron of the *white* gene results in aberrant RNA processing (ZACHAR *et al.* 1985; MOUNT, GREEN and RUBIN 1988; PENG and MOUNT 1990). In many cases, the phenotype of transposable element-induced mutations can be suppressed or enhanced by second site mutations at various modifier loci.

The *gypsy* retrotransposon induces mutant alleles in genes, such as *yellow* (*y*), *Hairy-wing* (*Hw*), *scute* (*sc*), *cut* (*ct*), *forked* (*f*), *lozenge* (*lz*), whose phenotypes are reversed by second-site mutations at the *suppressor of Hairy-wing* locus [*su(Hw)*] (MODOLELL, BENDER and MESELSON 1983; PARKHURST and CORCES 1986a; RUTLEDGE *et al.* 1988). The y^2 mutant phenotype is caused by a *gypsy* insertion 700 bp upstream of the *yellow* transcription start site (CHIA *et al.*, 1986; GEYER, SPANA and CORCES 1986; PARKHURST and CORCES 1986a). This allele has been used as a model to demonstrate that the y^2 phenotype observed in a wild-type *su(Hw)* background results from interaction of the *su(Hw)* product with specific sequences located in the 5′ transcribed untranslated portion of the *gypsy* retrotransposon (CORCES and GEYER 1991). The *su(Hw)* gene encodes a protein containing two acidic domains and 12 copies of the DNA-binding zinc finger motif (PARKHURST *et al.* 1988). This protein is involved in the regulation of *gypsy* expression through interaction with specific sequences of this retrotransposon (PARKHURST and CORCES 1986a; SPANA, HARRISON and CORCES 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 twelve of these 27-bp sequences, tandemly repeated, suggesting that at least 12 *su(Hw)* molecules, assuming that the protein binds as a monomer, interact with the *gypsy* element. This interaction is directly responsible for the mutant effect of *gypsy*, since deletions or other alterations in the *su(Hw)* binding site present in *gypsy* result in a decrease or abolishment of the mutant effect of this element (GEYER, GREEN and CORCES 1988; PEIFER and BENDER 1988; FLAVELL *et al.* 1990; SMITH and CORCES 1992). Progressive deletions of the *su(Hw)* binding region of the *gypsy* element inserted in *yellow* have a corresponding effect on the mutant action of this element, *i.e.*, fewer 27-bp binding sites present in *gypsy* result in a milder *yellow* 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. The presence of the *su(Hw)* protein bound to *gypsy* sequences is not only necessary, but is also sufficient to elicit the mutant phenotype, since the insertion of *su(Hw)* binding sites in the 5′ region of the *yellow* and *hsp70* genes interferes with their expression and gives rise to the same phenotype as the insertion of a complete *gypsy* element (SPANA and CORCES 1990; HOLDRIDGE and DORSETT

1991; P. GEYER and V. CORCES, submitted for publication).

The work described here presents evidence that the dominant action of *su(Hw)* on specific alleles of the *cut* (*ct*) and *forked* (*f*) loci is correlated with alterations in the *su(Hw)* binding region of the *gypsy* element inserted in these alleles. These results give further insights into the mechanisms by which the *gypsy* retrotransposon causes mutant phenotypes.

MATERIALS AND METHODS

Fly stocks: Flies were raised at 22° and 65% relative humidity on yeasted cornmeal dextrose media. The f^k*tuh-1*^{h}, *tuh-3* stock was obtained from D. KUHN (University of Central Florida, Orlando). The ct^{MRpN30} strain was obtained as a partial revertant of ct^{MR2} (TCHURIKOV *et al.* 1989). The ct^k stock was obtained from J. JACK (Sloan Kettering Institute, New York).

Electron microscopy: Flies were fixed in 4% glutaraldehyde and 0.1 M sodium cacodylate, pH 7.0. Samples were then dried, coated and examined in a Joel scanning electron microscope.

Molecular analysis of mutants: Digestion of DNA with restriction enzymes, ligation of DNA fragments, Southern analysis and labeling of DNA by random priming were carried out by standard procedures (SAMBROOK, FRISCH and MANIATIS 1989; FEINBERG and VOGELSTEIN 1983). Genomic DNA from Drosophila adults was prepared as described previously (PARKHURST and CORCES 1985). Partial *Sau*3AI libraries from different Drosophila mutants were constructed using λ DASH (Stratagene, La Jolla, California) as a cloning vector. The cDNA library was generated using the cDNA Synthesis System Plus (Amersham, United Kingdom) and λ gt10 arms. Screening of the libraries followed standard procedures (SAMBROOK, FRISCH and MANIATIS 1989). The ct^{MRpN30} and ct^k libraries were screened using genomic fragments generously provided by N. TCHURIKOV and J. JACK. Dideoxy sequencing was performed using sequenase (U. S. Biochemical Corp., Cleveland, Ohio).

RESULTS

Interaction of *forked* alleles with mutations in *su(Hw)*: Three spontaneous *forked* alleles, f^1, f^5 and f^k, are caused by the insertion of the *gypsy* retrotransposon (MODOLELL, BENDER and MESELSON 1983; PARKHURST and CORCES 1985; MCLACHLAN 1986; RUTLEDGE *et al.* 1988). These three mutations display contrasting phenotypic sensitivity to *su(Hw)* product levels. While *su(Hw)* acts as a recessive to suppress the f^1 and f^5 bristle phenotype, it suppresses the f^k allele as a dominant (MODOLELL, BENDER and MESELSON 1983). Figure 1 shows scanning electron micrographs of the scutellar bristles of f^k flies and in various combinations with *su(Hw)* mutations. The presence of the *gypsy* element in f^k results in scutellar bristles that are gnarled and bent (Figure 1B). This phenotype is slightly weaker than that of f^1 (compare figure 1B with figure 1B in PARKHURST and CORCES 1986b). In combination with one copy of $su(Hw)^V$, a null allele of *su(Hw)* (HARRISON, MORTON and CORCES 1992), the mutant phenotype becomes clearly weaker, although it is still different from wild type (Figure 1C). Heterozygous combinations of $su(Hw)^V$ with $su(Hw)^f$, a weak allele of *su(Hw)* that does not show the female sterile phenotype typical of mutations in this gene, further reverse the *gypsy*-induced phenotype (Figure 1D). The effect of one dose of the *su(Hw)* gene is only observed with f^k but not with f^1 or f^5 (data not shown), suggesting a difference in the basis of the gypsy-induced phenotype in these two classes of alleles. This discrepancy could be due to a different location of the gypsy insertion site in f^k or to alterations in the structure of the element present in this allele.

The structure of the *forked* locus in the f^k allele: The *forked* locus has been previously cloned by transposon tagging using the *gypsy* retrotransposon as a probe to isolate *forked* sequences from the f^1 allele (PARKHURST and CORCES 1985; MCLACHLAN 1986). In addition, the *gypsy* element present in the f^1 allele has been completely sequenced (MARLOR, PARKHURST and CORCES 1986). The *forked* locus encodes a major 2.5-kb transcript whose structure has been determined by sequence analysis of genomic and cDNA clones, and is contained within a 7-kb *Xho*I-*Sal*I fragment (K. HOOVER, A. CHIEN and V. CORCES, in preparation). This genomic fragment is capable of rescuing the *forked* phenotype via *P* element-mediated germline transformation. The structure of the 2.5-kb transcript is indicated in Figure 2.

To determine the reason for the different response observed in the f^1 and f^k alleles with regard to their interaction with the *su(Hw)* locus, we cloned and analyzed the *forked* locus of the f^k mutant. Genomic southern analysis localized an insertional aberration in the f^k mutant when compared with the wild type locus (data not shown). A genomic library was then prepared from the f^k mutant stock and screened with a 5.4-kb *Eco*RI *forked* genomic fragment (PARKHURST and CORCES 1985). Restriction analysis of positive clones showed the presence of a copy of the *gypsy* element in close proximity to that present in the f^1 allele (PARKHURST and CORCES 1985). Figure 2 shows that the *gypsy* elements present in f^1 and f^k are located within the second intron of the 2.5-kb RNA. The precise insertion site of the *gypsy* element in the *forked* locus in these two alleles was determined by DNA sequence analysis of genomic clones obtained from flies carrying these two mutations. These results indicate that the *gypsy* element in f^k is inserted 150 bp downstream from the *gypsy* insertion site in f^1.

Both f^1 and f^k are caused by *gypsy* insertions in close proximity into the same intron and with the same orientation (Figure 2). However, while *su(Hw)* functions as a recessive to suppress the f^1 phenotype, the extent of bristle forking observed in f^k is directly proportional to *su(Hw)* gene dosage. Reducing the *su(Hw)* copy number to half by crossing f^k with the

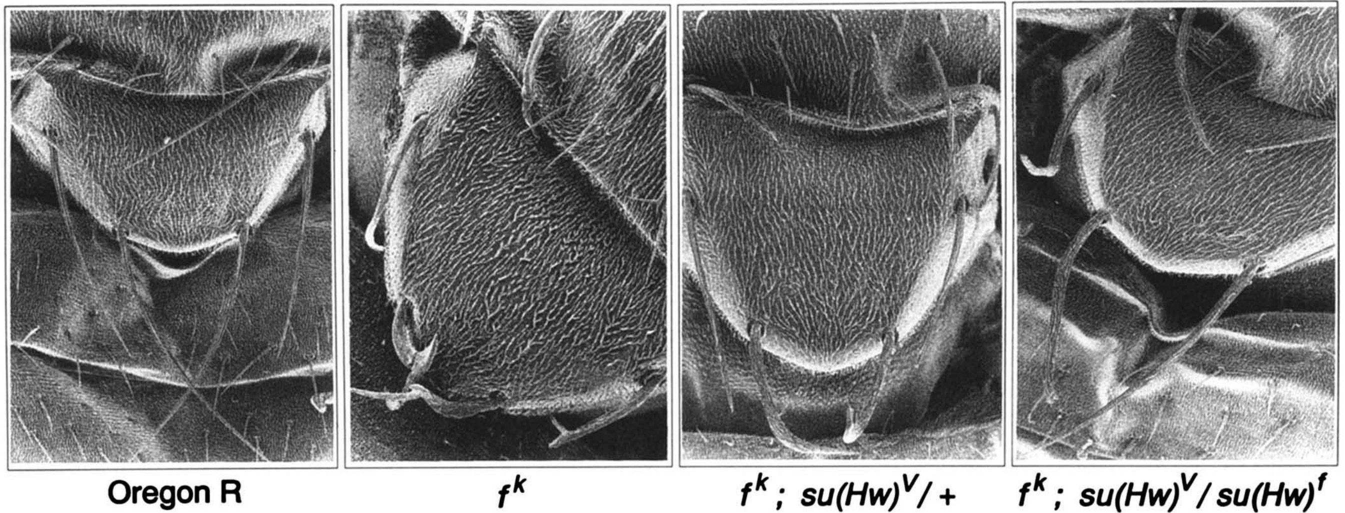


FIGURE 1.—Dominant action of *su(Hw)* on bristles of f^k mutants. Scanning electron micrographs of scutellar macrochaetae demonstrate a direct correlation between bristle forking and *su(Hw)* dosage. The first two panels show a wild-type scutellum and that of a f^k mutant. Decreasing *su(Hw)* dosage to half, correspondingly decreases the extent of bristle forking in f^k, $su(Hw)^V/su(Hw)^+$ flies. Flies of the genotype f^k, $su(Hw)^V/su(Hw)^f$ show a further reduction of bristle forking.

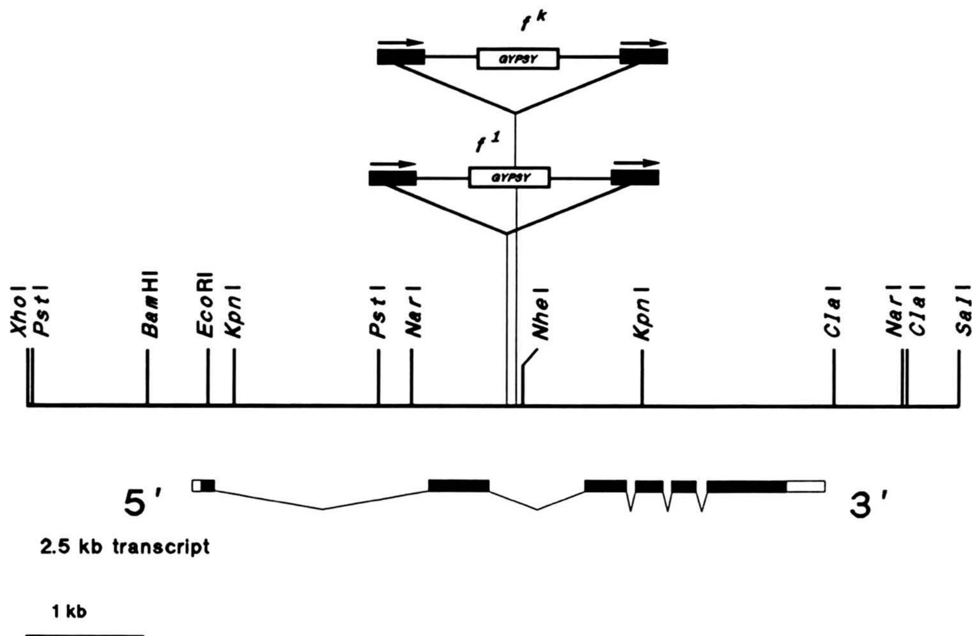


FIGURE 2.—Map of the f^1 and f^k mutations and major transcript of *forked*. The *gypsy* retrotransposon responsible for these mutations is located in the second intron of the gene, in the same orientation as *forked*. Open boxes signify transcribed, untranslated regions of the message while closed boxes represent transcribed translated portions.

$su(Hw)^V$ null mutation, suppresses the bristle phenotype. These results could be interpreted as a consequence of the different insertion point of the *gypsy* element in both alleles. Nevertheless, the fact that both insertions are only 150 bp apart in a region of the intron that should not be important for splicing suggests that the differential response to *su(Hw)* dosage might be a consequence of structural changes within the *gypsy* element. To test this possibility, we carried out a careful comparison of the two copies of *gypsy* responsible for the mutant phenotype of each allele. The f^1 mutation contains a full-length 7469-bp *gypsy* retrotransposon (MARLOR, PARKHURST and CORCES 1986). Restriction analysis of the *gypsy* element present in f^k shows no alteration from the copy of this element responsible for the f^1 allele. Sequencing of the f^k *gypsy* reveals a 29-bp deletion in the 5′ transcribed untranslated region that interacts with the *su(Hw)* product. This deletion eliminates part of the fourth and fifth of the 12 octamer repeats as well as the A/T-rich region spanning them (Figure 3). These results are consistent with the hypothesis that the increased sensitivity of f^k to changes in *su(Hw)* levels might be a consequence of a lower affinity of the *su(Hw)* protein for the *gypsy* element present in f^k.

Interaction of *cut* alleles with mutations in *su(Hw)*: To further test the possible correlation between phenotypic sensitivity to *su(Hw)* levels and the integrity of the *gypsy* region containing *su(Hw)* binding sites, we analyzed the *gypsy*-induced alleles ct^k and ct^{MRpN30}. Mutations in *su(Hw)* act as recessives to reverse the phenotype of the *gypsy*-induced ct^6 allele, but act as

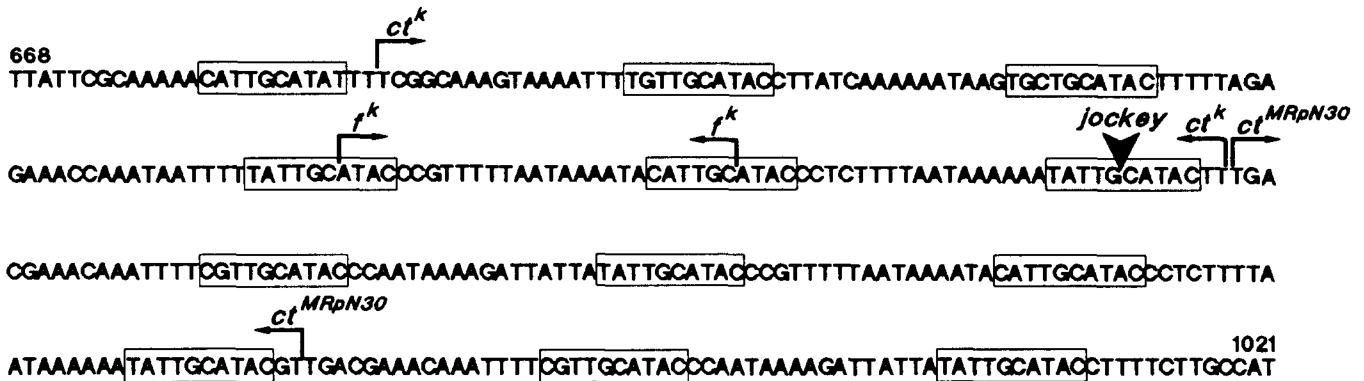


FIGURE 3.—Sequence of the *gypsy* repeat region. Alteration of this sequence in the alleles studied results in an increased phenotypic sensitivity to fluctuations in levels of *su(Hw)* gene. Boxes enclose each of the octamer-like repeats. The regions of *gypsy* deleted in each of the mutants f^k, ct^k and ct^{MRpN30} are enclosed in brackets. The insertion site of jockey in ct^{MRpN30} is also indicated.

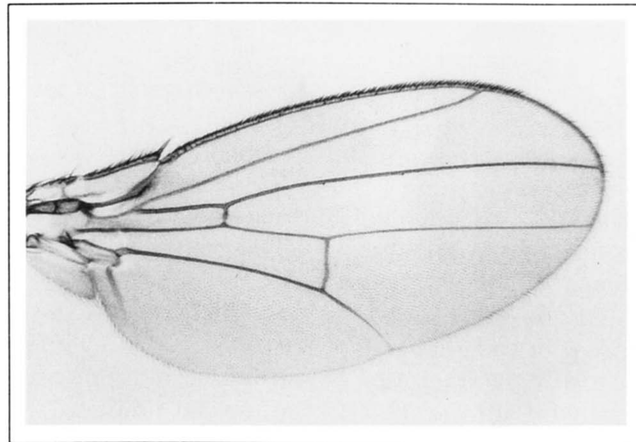
dominants on ct^k and ct^{MRpN30} (RUTLEDGE *et al.* 1988; T. GERASIMOVA, unpublished results). Both $su(Hw)^V$ and $su(Hw)^f$ alleles almost completely revert the wing phenotype of ct^k flies as heterozygotes (Figure 4). The $su(Hw)^V$ heterozygote suppresses the phenotype of ct^{MRpN30} more strongly than the $su(Hw)^f$ heterozygote, in agreement with the fact that $su(Hw)^V$ is a deletion of the locus and therefore a null allele (HARRISON, MORTIN and CORCES 1992), whereas $su(Hw)^f$ is a point mutation that is still partially active (D. HARRISON and V. CORCES, unpublished results). Crossing the $su(Hw)^V$ null mutation into either the ct^k or the ct^{MRpN30} mutant stocks effectively reduces the amount of *su(Hw)* by half and the resulting suppression demonstrates a phenotypic sensitivity to this reduction. Heterozygous combinations of both *su(Hw)* alleles further reverse the phenotype to wild type. In contrast, the phenotype of ct^{MR2}, the parental stock of the ct^{MRpN30} partial revertant, is reverted to wild type by homozygous mutations in *su(Hw)*, but it is not affected in a dominant fashion by mutations in this gene (data not shown).

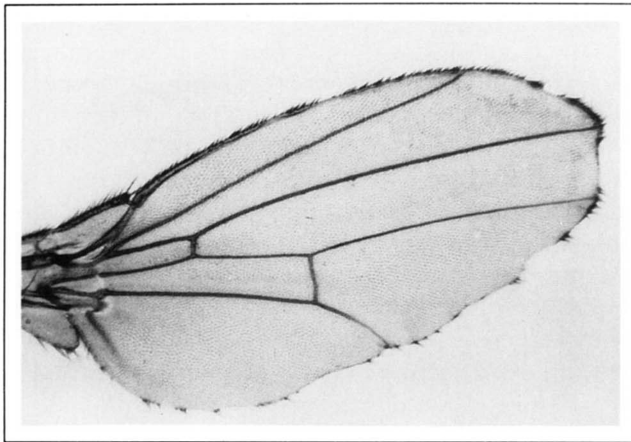
The structure of the *cut* locus in the ct^k and ct^{MRpN30} alleles: The *cut* locus from *D. melanogaster* has been cloned and the insertion sites for various transposable elements responsible for the generation of *ct* spontaneous alleles has been described (JACK 1985; TCHURIKOV *et al.* 1989; LIU, MCLEOD and JACK 1991; JACK *et al.* 1991). All gypsy-induced alleles of *ct* are caused by the insertion of this element in the 5′ regulatory region (JACK 1985; TCHURIKOV *et al.* 1989; LIU, MCLEOD and JACK 1991; JACK *et al.* 1991). The ct^k allele is caused by the insertion of *gypsy* 6 kb upstream of the 5′ most identified exon in a transcriptional orientation opposite to *cut* (LIU, MCLEOD and JACK 1991). A genomic library was prepared from a ct^k mutant stock. This library was probed with a 3.8-kb *Eco*RI genomic fragment located in the +70 to +80 interval of the chromosomal walk in the *cut* region described by JACK (1985). Positive clones were subsequently screened with the 6.5-kb *gypsy Xho*I fragment (MARLOR, PARKHURST and CORCES 1986) and then analyzed via restriction mapping and sequencing. Restriction mapping of the *gypsy* retrotransposon responsible for the mutant phenotype in ct^k reveals the presence of a deletion in the *su(Hw)* binding region of this element. Sequence analysis of this region indicates a 129 bp deletion that extends from the first to the sixth octamer repeat and spans a total of five repeats (Figure 3).

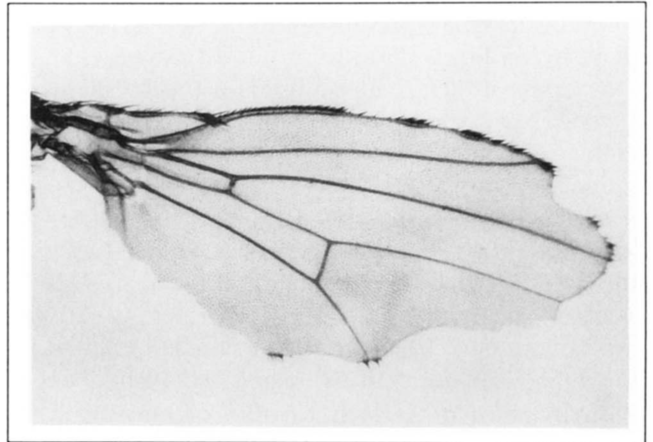
ct^{MRpN30} is a partial revertant of the ct^{MR2} mutation (TCHURIKOV *et al.* 1989). The *gypsy* element responsible for the ct^{MR2} mutation is inserted approximately 79 kb upstream of the most 5′ exon in the same orientation as *cut*. The *gypsy* element in ct^{MR2} has been shown by southern analysis to be in the same location and orientation as the ct^6 *gypsy* insertion (MIZROKHI *et al.* 1985; TCHURIKOV *et al.* 1989). The *gypsy* retrotransposon in ct^{MR2} has a full length *su(Hw)* binding region of 340 bp and is identical to that characterized from f^1 (MIZROKHI *et al.* 1985). Homozygous mutations of the *su(Hw)* locus are required for suppression of this mutant, while heterozygous combinations are sufficient to reverse the phenotype of its derivative ct^{MRpN30}. To ascertain how ct^{MRpN30} has become sensitized to fluctuations in *su(Hw)* levels, clones from the *cut* locus were isolated using a subcloned fragment of the lB4 phage as a probe (TCHURIKOV *et al.* 1989). This subcloned fragment was used to screen a lambda library made with genomic DNA from the ct^{MRpN30} mutant. Restriction mapping of the *gypsy* retrotransposon present in ct^{MRpN30} shows an alteration in the *su(Hw)* binding region. Sequence analysis reveals that two features compromise the integrity of this region: a 109 bp deletion spans four octamer repeats, and a 2.9-kb *jockey* element divides the remaining eight repeats (Figure 3). These alterations account for partial reversion of the ct^{MRpN30} phenotype as well as an increased sensitivity to *su(Hw)* dosage (Figure 4).

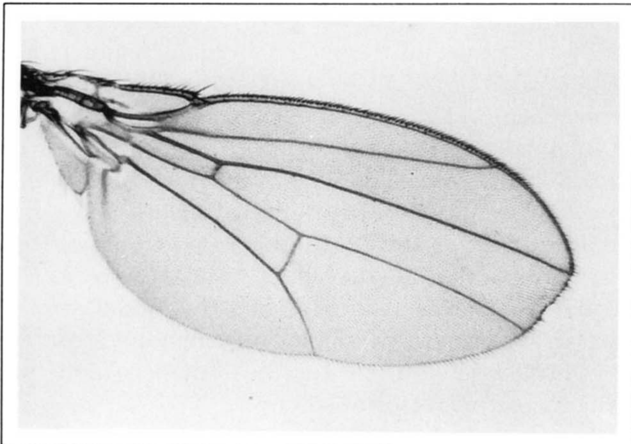
DISCUSSION

Extensive binding studies have shown that the *su(Hw)* protein interacts with specific domains in the









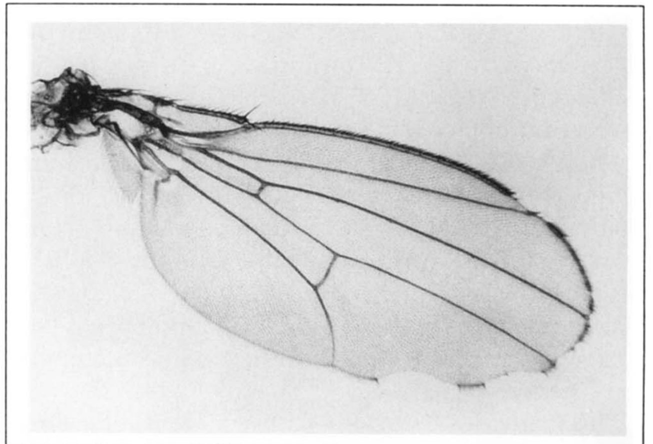


FIGURE 4.—Dosage effect of *su(Hw)* gene on the wing phenotype of ct^{MRpN30} and ct^k alleles. $su(Hw)^V$, a null mutation, acts as a dominant to suppress the wing phenotype of ct^{MRpN30} and ct^k. The wing phenotype is partially restored to wild type in flies that are heterozygous for $su(Hw)^V$. The more severe ct^{MRpN30} wing is also suppressed in the presence of one copy of *su(Hw)*.

repeat region of *gypsy*. Each domain is composed of one of the octamer-like repeats and adjacent bases (SPANA and CORCES 1990). This implies the presence of up to 12 *su(Hw)* binding units per copy of *gypsy* DNA. Isolated individual repeat units maintain their global level of affinity to *su(Hw)* protein. The repeat unit therefore contains the information to confer the observed affinity to *su(Hw)* protein. The significance of the *gypsy* octamer repeat region in eliciting a mutant effect can be explained based on the following model. Homology of the repeat unit to vertebrate transcriptional enhancer elements, the specific binding of *su(Hw)* product to this motif, and the similarity of *su(Hw)* protein to transcriptional activators suggest that the region may control levels of *gypsy* transcription and thereby regulate its mutant effect. This model is supported by observations that in cultured cells, the repeat region is necessary for positive regulation of *gypsy* expression (MAZO *et al.* 1989). Also, mutations in *su(Hw)* have been found to reduce *gypsy* transcription levels (PARKHURST and CORCES 1986a). The model parallels that proposed for the *Ty1* element of yeast. This element contains four copies of an octameric sequence homologous to the *gypsy* repeat sequences (ERREDE *et al.* 1985). These sequences are necessary for the expression of *Ty* elements (ROEDER, ROSE and FINK 1985). They are also required for the mutant effect that *Ty1* has upon the affected gene (ROEDER, ROSE and FINK 1985; ERREDE, COMPANY and HUTCHINSON 1987). Studies of the *yellow* locus in *D. melanogaster* demonstrate that the 340 bp *su(Hw)* binding region of *gypsy* is capable of eliciting a y^2 phenotype when inserted in the same location as the *gypsy* in y^2 (P. GEYER and V. CORCES, submitted for publication). Transcriptional competence of the *gypsy* element in y^2 is therefore not a factor in conferring mutagenicity to this retrotransposon. In this model, binding of *su(Hw)* product to the octamer repeats disrupts the ability of y^2 enhancers to interact with the promoter. It is not clear, however, whether the interference results from steric hindrance or the direct interaction between *su(Hw)* product and *trans*-acting factors that bind to *yellow* enhancer regions. Though this model is predicted from the studies of the *yellow* locus, it also provides a plausible mechanism to explain the mutant effect of *gypsy* in other suppressible mutations (JACK *et al.* 1991).

This model is supported by observations indicating that alterations in the *su(Hw)* binding region affect the severity of the gypsy-induced mutant phenotype. The specific conformational accessibility of the *su(Hw)* binding site is determined by the bending of the AT-rich sequences that flank the octamer-like repeat (SPANA and CORCES 1990). Deletions that remove octamer-like sequences and/or A/T-rich stretches separating them are likely to affect the three-dimensional structural availability of remaining binding sites and the overall global conformation of the repeat region. Compromising the integrity of this region results in a range from partial to complete phenotypic reversion of certain *gypsy*-induced alleles. Partial revertants of y^2, Hw^1 (GEYER, GREEN and CORCES 1988) and ct^{MR2} (MIZROKHI, GEORGIEVA and ILYIN 1988; FLAVELL *et al.* 1990) are caused by insertion of transposable elements into the *gypsy su(Hw)* binding region. Deletions disrupting this region result in partial revertants of bxd^1 (PFEIFFER and BENDER 1988) and y^2 (SMITH and CORCES 1992). The insertion of *jockey* combined with the deletion of more than half the *gypsy* octamer repeats decreases the phenotype of ct^{MR2} revertants (FLAVELL *et al.* 1990). While the correlation between structural integrity of the repeat region and intensity of the *gypsy*-induced phenotype is well documented, the data presented here are the first to demonstrate that alterations of the *su(Hw)* binding region in certain alleles changes *su(Hw)* action from recessive to dominant.

The mutations we examined in this manuscript show, in addition to an increased sensitivity to *su(Hw)* gene dosage, a decrease in the intensity of the mutant phenotype induced by gypsy insertion. This observation suggests that both effects are linked to the interaction of *su(Hw)* protein with gypsy sequences. Reduction of the *gypsy* mutant effect is indiscriminate of the specific octamer repeats lost. The deletion of *gypsy* sequences in the f^k allele overlaps the fourth and fifth octamer repeats and the A/T-rich region spanning them. The ct^k *gypsy* deletion ranges from the first to the sixth repeat and removes five repeats in all. The ct^{MRpN30} partial revertant has a 109-bp deletion that removes the seventh through the tenth repeats, and contains a 2.9-kb *jockey* element that disrupts the remaining eight repeats (Figure 3). Inherent to the repetitive nature of the *su(Hw)* binding region, these deletions probably arose as a result of homologous recombination at the intramolecular level or as a result of unequal crossing over between *gypsy* elements on paired chromosomes. These observations indicate that alterations in the *su(Hw)* binding region of a gypsy element affecting the intensity of its phenotypic effect also sensitize the mutant gene to variations in *su(Hw)* gene dosage. This result supports the model proposing that the presence of *su(Hw)* protein bound to gypsy sequences is responsible for the mutant phenotypes induced by this retrotransposon.

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