The *Drosophila* *su(Hw)* gene, which controls the phenotypic effect of the gypsy transposable element, encodes a putative DNA-binding protein

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Homozygous mutations at the *suppressor of Hairy-wing* [*su(Hw)*] locus reverse the phenotype of gypsy-induced alleles in a number of genes located throughout the *Drosophila* genome. To understand the molecular basis of this phenomenon, the *su(Hw)* locus was isolated by chromosomal walking from a cloned homeo-box-containing sequence. The exact location of the gene was determined by Southern analysis of the DNA alterations associated with several *su(Hw)* alleles. A 9.5-kb *KpnI*–*SalI* fragment, where all the DNA changes associated with *su(Hw)* mutations were mapped, was able to rescue the *su(Hw)* mutant phenotype after P-element-mediated germ-line transformation. This DNA fragment encodes a 3.3-kb RNA that is expressed in all stages of *Drosophila* development; the size or abundance of this RNA is affected in several *su(Hw)* alleles tested. This transcript encodes a protein that contains a highly acidic region and 12 repeats of the 'Zn finger' domain characteristic of some DNA-binding and transcription-activating proteins, supporting the hypothesis that the *su(Hw)* locus might encode a transcription factor that plays a role in the expression of the gypsy element.

[**Key Words:** Transposable element; suppression; transcriptional control; regulatory factor]

Received June 2, 1988; revised version accepted August 1, 1988.

Some transposable elements affect the genes they mutate by bringing their expression under the control of a second unlinked locus that is termed a modifier. The modifier gene is referred to as an enhancer or a suppressor locus, depending on the type of effect exerted by mutations in this gene on the phenotype of the transposable element-induced mutation. This type of phenomenon has been documented extensively in yeast and *Drosophila* (for reviews, see Roeder and Fink 1983; Rubin 1983; Kubli 1986; Parkhurst and Corces 1986b; Rutledge et al. 1988).

When the Ty transposable element of yeast inserts in the 5'-noncoding region of the *HIS4* and *LYS2* genes, it causes a mutant phenotype that can be reversed by second-site mutations at a variety of *SPT* loci (Winston et al. 1984a,b). The Ty-induced mutant phenotype might be due to the presence of transcription signals on the Ty element that interfere with the transcription of adjacent genes. The various *spt* mutations described probably

differ in the mechanism by which they reverse the mutant phenotype. For example, *spt3*, *spt7*, and *spt8* suppress mutations caused by the insertion of complete Ty elements or the long terminal repeats (called δ sequences) by abolishing transcription from the normal Ty promoter (Winston et al. 1984b, 1987). In contrast, *spt4*, *spt5*, and *spt6* mutations are strong suppressors of solo δ insertion mutations but have little effect on Ty-induced mutations and do not affect the transcription of this element (Clark-Adams and Winston 1987). A third class of suppressor genes, represented by *SPT13* and *SPT14*, suppress the phenotype of complete Ty insertion mutations, but not of solo δ insertion mutations, as a result of increased levels of transcription of the adjacent gene (Fassler and Winston 1988).

In *Drosophila*, several suppressor and enhancer mutations have been described that exert an effect on spontaneous mutant alleles in a transposable element-specific fashion, although the molecular mechanisms of these phenomena are poorly understood. Mutations at the *suppressor of Hairy-wing* [*su(Hw)*, 3-54.8] locus reverse the phenotype of at least 15 gypsy-induced mutations at 11 different loci (Modolell et al. 1983; Parkhurst and Corces 1986b); *suppressor of white-apricot* [*su(w^a)*, 1-0.1]

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partially restores the wild-type eye color of the copia-induced allele *white-apricot* (w^a) (Levis et al. 1984; Zachar et al. 1985; Mount et al. 1988); mutations at *suppressor of sable* [*su(s)*, 1-0] reverse the phenotype of spontaneous alleles at several loci caused by the insertion of the 412 retrotransposon (Chang et al. 1986; Searles and Voelker 1986). Additional interactions between some of the mutant alleles and their modifier loci can also take place (Rutledge et al. 1988). Mutations at the *suppressor of forked* [*su(f)*, 1-65.9] locus can revert the phenotype of the same *forked* (*f*, 1-56.7) (Green 1955) and *lozenge* (*lz*, 1-27.7) (Snyder and Smith 1976) alleles suppressed by *su(Hw)*, but enhance the phenotype of w^a (Green 1959) and *lz*³⁷ (Schalet 1970). The molecular bases of the transposable element-mediated interactions between the modifier loci and the mutant genes they affect are being studied using models such as the copia-induced w^a and gypsy-induced *yellow* (y^2) alleles. The copia element is inserted in an intron of the *white* gene in w^a (Levis et al. 1984), and the mutagenic effect of this transposon may be due to its interference with proper splicing of the *white* RNA. This explanation is supported by recent findings indicating that *su(w^a)* encodes a protein involved in regulation of splicing (Chou et al. 1987; Zachar et al. 1987), although additional effects of the copia element on the developmental expression of *white* RNA cannot be discarded (Zachar et al. 1985; Mount et al. 1988). The effect of the gypsy element on the expression of the *yellow* locus in the y^2 allele seems to be caused via a different mechanism, as this mutation is due to the insertion of gypsy in the 5' region of the gene, at ~700 bp from the start of transcription (Chia et al. 1986; Geyer et al. 1986; Parkhurst and Corces 1986a). The adult tissue-specific phenotype in these mutant flies might be due to the gypsy-mediated interaction of the *su(Hw)*-encoded protein with transcriptional enhancer elements that are located upstream of the gypsy insertion point and control the expression of the *yellow* gene in the particular tissues that show a mutant phenotype (Geyer and Corces 1987; Geyer et al. 1988).

To further our understanding of the molecular mechanisms by which mutations at the *su(Hw)* locus reverse the phenotype of gypsy-induced alleles, we have cloned and characterized this gene. Here we present evidence suggesting that *su(Hw)* encodes a DNA-binding protein, supporting the hypothesis that this protein might interact with the gypsy element to control its transcription and, as a consequence, its mutagenic effect.

Results

Molecular mapping of chromosomal deficiencies associated with su(Hw)

The *su(Hw)* locus maps on the third chromosome at 54.8 cM, in close proximity to the *red* (*red*, 3-53.6) locus (Lindsley and Grell 1968). The phenotype of these two mutations, in addition to *trithorax* (*trx*, 3-54.2), is uncovered by two small cytologically visible deficiencies designated *Df(3R)red^{P52}* and *Df(3R)red^{P93}* (Lewis 1981).

The localization of *su(Hw)* within the DNA deleted in these two deficiencies allows the mapping of this gene to the 88BC region (Fig. 1A). As a first step to initiate a chromosomal walk to the *su(Hw)* locus, we determined the chromosomal location of a homeo-box-containing clone, known to map to the 88BC region (M. Levine, pers. comm.), with respect to the breakpoints of the two deficiencies mentioned above. A λ clone containing this sequence (λ S63) was hybridized to *Drosophila* polytene chromosomes obtained from larvae of the genotypes *Df(3R)red^{P52}/Df(3R)293^{γ5}* and *Df(3R)red^{P93}/Df(3R)293^{γ5}*. The presence of *Df(3R)293^{γ5}*, which deletes the 87E-88A region (Fig. 1A), results in asynapsis of the chromosomes in this area and facilitates the identification of the bands (R. Kelley and A. Spradling, in prep.). The clone λ S63 hybridized only to the chromosome containing *Df(3R)293^{γ5}* in the *Df(3R)red^{P93}/Df(3R)293^{γ5}* stock (Fig. 1B) and to both chromosomes in the *Df(3R)red^{P52}/Df(3R)293^{γ5}* stock (data not shown). These results suggest that sequences contained in this λ clone are deleted in *Df(3R)red^{P93}* but not in *Df(3R)red^{P52}* or *Df(3R)293^{γ5}*.

λ S63 was then used to probe a wild-type Canton S library, and λ clones extending in both directions, designated λ R9 and λ L11, were isolated (Fig. 2A). The end restriction fragments from these clones were used as hybridization probes to further the chromosomal walk, and clones λ 3R3 and λ 3L2 were obtained. The different λ clones were used as probes to carry out in situ hybridizations to polytene chromosomes from the *Df(3R)red^{P93}/Df(3R)293^{γ5}* stock. Clones λ R9 and λ 3R3 hybridized only to the *Df(3R)293^{γ5}* chromosome (data not shown), indicating that DNA sequences contained in these clones were deleted in *Df(3R)red^{P93}*, whereas clone λ L11 hybridized to both chromosomes (Fig. 1C). Therefore, sequences located in this clone are found outside of the deleted region. These results indicate that clone λ L11 overlaps with the distal breakpoint of *Df(3R)red^{P93}*, whereas clones λ R9 and λ 3R3 are located closer to the *red* locus and the centromere; therefore, they specify the direction of the location of the *su(Hw)* locus for the chromosomal walk to proceed. The exact position of the distal breakpoint in *Df(3R)red^{P93}* was located to a 1.8-kb *Eco*RI fragment using Southern blots (Fig. 2A and data not shown). The same type of strategy, using in situ hybridization of various λ clones to polytene chromosomes of the stock *Df(3R)red^{P52}/Df(3R)293^{γ5}*, was used to map approximately DNA sequences deleted in *Df(3R)red^{P52}*; the distal breakpoint in this deficiency was localized to clone λ 3R3 and to one of a series of small (~0.5 kb) *Eco*RI fragments located in this clone (see Fig. 2A) by Southern analysis.

Cloning of the su(Hw) locus by chromosomal walking

To determine the exact location of the *su(Hw)* locus, we made use of several spontaneous and γ -ray-induced alleles of this mutation. Spontaneous alleles usually are associated with the insertion of transposable elements, whereas γ -rays often result in the deletion of DNA sequences. Both types of alterations can be easily detected by Southern analysis. DNA was prepared from adult

Figure 1. Cytological analysis of chromosomal deletions. (A) Preparation of polytene chromosomes from the stock *Df(3R)red^{P93}/Df(3R)293^{γ5}*. (B) In situ hybridization of ³H-labeled λS63 DNA to polytene chromosomes from *Df(3R)red^{P93}/Df(3R)293^{γ5}* larvae. (C) In situ hybridization of ³H-labeled λL11 DNA to polytene chromosomes from the *Df(3R)red^{P93}/Df(3R)293^{γ5}* stock.

flies of different *su(Hw)* mutant stocks. The *su(Hw)^{f3}* stock is fertile and homozygous viable, whereas *su(Hw)^B* and *su(Hw)^C* are sterile but viable as homozygotes. In addition, DNA was also made from transheterozygous adults of the stock *su(Hw)²/su(Hw)^f*, due to the low viability of homozygous *su(Hw)²* flies. The alleles *su(Hw)^B* and *su(Hw)^C* were induced by γ -ray irradiation, whereas the rest of the mutations analyzed arose spontaneously. Genomic DNA from these various stocks was digested with different restriction enzymes and subjected to Southern analysis; individual *Eco*RI fragments obtained from the different λ clones of the chromosomal walk (Fig. 2A) were used as hybridization probes. No consistent changes in the restriction patterns obtained with *Eco*RI, *Bam*HI, and *Xho*I were detected among Canton S, different *su(Hw)* alleles, and their parental stocks when probes from λ 3L2, λ L11, λ S63, and λ R9 were used in the Southern analysis (data not shown). In marked contrast, DNA alterations were found associated with all four alleles examined when a 4.0-kb *Eco*RI fragment from λ 3R3 (probe a, Fig. 2B) was used as a hybridization probe. Sequences contained in this clone are deleted in both *Df(3R)red^{P52}* and *Df(3R)red^{P93}*. These alterations were not present in the parental stocks. Some selected examples of the Southern analyses carried out are shown in Figure 3. Figure 3A shows that the *su(Hw)^{f3}* allele contains an insertion of 0.7 kb into the 4.0-kb *Hind*III

fragment (probe b, Fig. 2B) when compared with the parental stock, *Ubx⁷⁸*. Figure 3B shows that the *su(Hw)²* allele is caused by an insertion of 1.3 kb into the 1.2-kb *Xba*I–*Apa*LI fragment (probe c, Fig. 2B). These results have now been confirmed by cloning and sequence analysis of DNA from the *su(Hw)^{f3}* and *su(Hw)²* alleles (D.A. Harrison and V.G. Corces, in prep.). DNA from the *su(Hw)^f* allele shows no alterations in the restriction pattern, suggesting that the phenotype is probably caused by a point mutation (see below for additional evidence). The γ -ray-induced alleles *su(Hw)^B* and *su(Hw)^C* are associated with small deletions, as the sizes of the *Hind*III restriction fragment (Fig. 3C) are smaller than that of wild type. The approximate location of the molecular defects associated with these four alleles is indicated in Figure 2B by horizontal lines spanning the affected DNA fragments. These results delimit the region where the *su(Hw)* gene is expected to reside.

Changes in RNA transcripts associated with su(Hw) alleles

To localize further the exact position of the *su(Hw)* locus, we determined the pattern of developmental transcription in the region where the different DNA abnormalities related to *su(Hw)* had been mapped. RNA was prepared from larvae, pupae, and adults of wild-type and

Figure 2. DNA structure of the *su(Hw)* locus and adjacent regions. (A) Restriction map of the 88BC region covered by the chromosomal walk. Vertical lines denote *EcoRI* sites, and the black square indicates the location of the homeo box homology contained in the clone λ S63 used to initiate the walk. The different clones isolated during this chromosomal walk are indicated at the bottom of A by horizontal lines. The top of the A shows the distal breakpoints of the deficiencies *Df(3)red*^{P93} and *Df(3)red*^{P52}. The region underlined by a crosshatched bar on the left side of the map is shown in detail in B. (B) Restriction map of the *su(Hw)* locus. Only some *PstI* and *ApaI* sites are indicated. Horizontal lines located immediately under the map show the location of various hybridization probes used in these studies. Also indicated is the location of the different transcripts detected in the region. The exon-intron structure of the *su(Hw)* gene is shown in detail, with the location of the different Zn finger domains indicated by vertical lines. The restriction fragments where the *su(Hw)*^{f3}, *su(Hw)*², *su(Hw)*^B, and *su(Hw)*^C alleles have been mapped are shown by horizontal lines at the bottom of B.

mutant stocks and subjected to Northern analysis using the entire λ 3R3 clone (Fig. 2A) as a probe. This DNA hybridized to four different transcripts, 3.3, 2.2, 1.4, and 1.1 kb, on Northern blots containing poly(A)⁺ RNA from wild-type flies (Fig. 4). The approximate location of these four transcripts was mapped by using different *EcoRI* restriction fragments from the region as hybridization probes (data not shown). These results are summarized in Figure 2B by solid lines that denote the size of the RNAs, flanked by broken lines that indicate the limits of the region containing the different transcripts. The structure of the 3.3-kb RNA is indicated in more detail based on results discussed below. Also shown in Figure 2B is the location of two additional transcripts, 1.6 and 1.0 kb in length, that were not contained in the λ 3R3 clone used in the experiment described in Figure 4.

The same four transcripts detected in wild-type RNA preparations were also observed when RNA from the stock *su(Hw)*⁷/*su(Hw)*^f was analyzed (data not shown). Because *su(Hw)*⁷ is caused by a large cytologically vis-

ible deletion, the RNA detected in this sample must originate from the *su(Hw)*^f chromosome, supporting the hypothesis that *su(Hw)*^f is caused by a point mutation or a very small deletion that results in normal levels of transcription of a less functional product. The same pattern of RNAs was observed in *su(Hw)*^{69k}/*su(Hw)*^f flies, in agreement with the fact that no DNA aberrations could be associated with either of these mutations by Southern analysis (data not shown). In contrast, RNA from the *su(Hw)*^{f3} allele contains normal levels of the 2.2-, 1.4-, and 1.1-kb transcripts, but very low levels of the 3.3-kb RNA, suggesting that this transcript is encoded by the *su(Hw)* locus (Fig. 4). This conclusion is supported by the fact that RNA from the *su(Hw)*²/*su(Hw)*^f stock contains an additional 1.6-kb transcript that could arise as a consequence of premature termination of transcription of the 3.3-kb RNA, whereas the 1.4- and 1.1-kb RNAs are present at normal levels (Fig. 4). The 1.6-kb RNA hybridizes to a *HindIII*-*PstI* fragment (probe d, Fig. 2B) that contains sequences specific for the

Figure 3. Southern analysis of *su(Hw)* mutant alleles. Genomic DNA from wild-type and mutant stocks was digested with various restriction enzymes, electrophoresed on a 1% agarose gel, transferred to nitrocellulose paper, and hybridized with ³²P-labeled DNA containing the *su(Hw)* locus. (A) DNA from the *su(Hw)*^{f3} allele and its parental stock *Ubx*^{rfg} were digested with *Hind*III or *Pst*I and subjected to Southern analysis using the 4.0-kb *Eco*RI fragment containing the 5' region of the *su(Hw)* gene as a hybridization probe (probe a, Fig. 2B). (B) DNA was isolated from the stock *su(Hw)*²/*su(Hw)*^f, the parental chromosome in which the *su(Hw)*^f allele was induced (*TM6*), and a wild-type stock for the *su(Hw)* locus (*TM6*/*Mkrs*). The DNA was digested with a mixture of *Xba*I and *Apa*LI and subjected to Southern analysis using the 1.2-kb *Xba*I–*Apa*LI fragment as a probe (probe c, Fig. 2B). (C) DNA from the *su(Hw)*^B and *su(Hw)*^C mutant alleles and their parental stock *bx*^{34e} were digested with *Hind*III, and the Southern blot was hybridized with the 4.0-kb *Hind*III fragment (probe b, Fig. 2B).

Figure 4. RNA analysis of wild-type and *su(Hw)* mutant alleles. Poly (A)⁺ RNA was isolated from larvae (L), pupae (P), and adults (A) of wild-type, *su(Hw)*^{f3}, and *su(Hw)*²/*su(Hw)*^f flies. Five micrograms were electrophoresed on a 1% agarose–formaldehyde gel, transferred to a nylon membrane, and hybridized with ³²P-labeled DNA from the clone λ 3R3 (see Fig. 2A). (Bottom) The same blots hybridized with the *Drosophila ras2* gene, which is expressed at approximately constant levels throughout development, as a control for the amount of RNA loaded in each lane.

su(Hw) locus (data not shown). The 3.3-kb transcript present in RNA samples from *su(Hw)²/su(Hw)^f* flies presumably originates from the *su(Hw)^f* allele (see above).

The su(Hw) mutant phenotype can be rescued by P-element-mediated transformation

Confirmation of the precise location of the *su(Hw)* locus in a specific region was obtained by the ability of DNA fragments from this region to rescue the mutant phenotype of *su(Hw)* flies. The 18-kb *KpnI*–*XbaI* and the 9.5-kb *KpnI*–*SalI* fragments depicted in Figure 2B were cloned into the CaSpeR vector, which contains the *Drosophila white* gene as a marker for transformation (Pirrotta et al. 1985). Transformants were obtained using the stock, *Df(1)w, y²sc¹w^{67c23(2)} ct⁶f¹; su(Hw)^{69k} e/TM6, su(Hw)^f* and identified on the basis of eye color. The presence of the transforming DNA was confirmed by Southern analysis (data not shown). One wild-type eye color transformant line was established containing each of the plasmids mentioned above. Both of these transformants were genotypically *y² sc¹ ct⁶ f¹; su(Hw)^{69k}/su(Hw)^f* but had yellow, scute, cut, and forked phenotypes. This conclusion indicates that the *su(Hw)* phenotype was rescued by the injected plasmids, because the *su(Hw)* mutation in the transformed flies was not able to reverse the gypsy-induced phenotype of the *y,sc,ct*, and *f* mutations. In addition, the transforming DNA was also able to rescue the female sterility associated with *su(Hw)^{69k}*. On the basis of this evidence, the *su(Hw)* locus can be localized to the 9.5-kb *KpnI*–*SalI* fragment (Fig. 2B).

Temporal and tissue-specific expression of the su(Hw) locus

Several *su(Hw)* alleles cause female sterility, suggesting that the product encoded by this locus might be necessary in the ovary during the development of the egg and should therefore be transcribed there. Poly(A)⁺ RNA was prepared from the ovaries of mature adult females obtained from Canton S and *su(Hw)^{f3}* stocks and subjected to Northern analysis using λ 3R3 as hybridization probe. Figure 5A shows that ovaries from wild-type flies only express the 3.3- and 2.2-kb RNAs and that the accumulation of the former transcript is severely affected in *su(Hw)^{f3}*. A novel 4.0-kb RNA can also be detected in these mutant flies, presumably due to transcription through the DNA insert responsible for the mutation. A large transcript of similar size was also observed in RNA preparations from ovaries of *su(Hw)^{69k}/su(Hw)^f* females, although this aberrant RNA could not be seen in preparations from whole adult animals of the same stock (data not shown). Two additional 1.4- and 1.1-kb RNAs encoded within the λ 3R3 clone and present in both wild-type and mutant flies could not be detected in the ovary. These results support the conclusion that the 3.3-kb RNA is the only *su(Hw)*-encoded transcript.

To study the temporal expression of the *su(Hw)*-encoded-transcript, we prepared poly(A)⁺ RNA from different stages of *Drosophila* development, collecting

samples at 24-hr intervals. These RNAs were subjected to Northern analysis using a *su(Hw)* cDNA clone as hybridization probe. The result of this experiment is shown in Figure 5B and indicates that the *su(Hw)* locus is expressed in all stages of *Drosophila* development, with slightly higher levels of accumulation in larvae and adults. Figure 5C shows the same filter probed with the *Drosophila ras2* gene, which is expressed at approximately the same levels throughout the development of the fly (Mozer et al. 1985) and is used here as a measure of the amount of RNA loaded in the different lanes.

The su(Hw)-encoded protein contains 12 copies of the 'Zn finger' motif

The direction of transcription of the *su(Hw)* RNA was determined by hybridizing single-stranded RNA probes specific for each DNA strand (Melton et al. 1984) to Northern blots containing poly(A)⁺ RNA from adult flies. Only one strand-specific probe hybridized to the RNA (data not shown), indicating that the direction of transcription is toward the centromere (Fig. 2B).

To determine the organization of the *su(Hw)* locus, we isolated two overlapping cDNA clones from an embryonic wild-type cDNA library (Poole et al. 1985). One of these clones, designated cDNA-1, corresponded to a complete copy of the transcribed region, whereas the second (cDNA-2) was missing ~500 bp from the 5' region. Both genomic and cDNA clones were sequenced. Comparison of these two sequences shows that *su(Hw)* contains seven exons separated by six introns (Fig. 2B). All but the first of these introns are quite small, in the range of 50–60 bp. The complete sequence of the protein-coding region of the *su(Hw)* locus obtained from Canton S is represented in Figure 6, starting at the mRNA cap site. The predicted molecular weight is 109,000 daltons. A careful analysis of this sequence shows the presence of 12 repeats of the Zn finger motif, which is characteristic of some DNA-binding proteins and transcription factors (Klug and Rhodes 1987). These various finger domains are located in the central portion of the protein and arranged in several exons, and some of them are interrupted by introns. Figure 7 shows a comparison of the amino acid sequence of the different Zn fingers of the *su(Hw)* protein and a consensus sequence derived from various *Drosophila*, *Xenopus*, and mammalian finger-containing protein (Berg 1988). The consensus structure of the Zn finger motif is Phe/Tyr Xaa Cys Xaa Xaa Cys Xaa Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa His Xaa Xaa Xaa Xaa His. This structure is quite well conserved among the different Zn fingers found to date, although invariant residues present in the consensus sequence, such as Leu and Phe, are not found in some particular finger domains described in other proteins (e.g., Tautz et al. 1987); and the number of amino acids present between the two His residues is variable, ranging between 3 and 5 in different Zn fingers. The amino acids conserved in the *su(Hw)* protein with respect to the consensus are underlined in Figure 7, and it is apparent that the critical invariant residues are also

Figure 5. Developmental and tissue-specific transcription of the *su(Hw)* gene. (A) Five micrograms of poly(A)⁺ RNA, isolated from ovaries of mature females from Canton S and *su(Hw)*^{f3} stocks, was electrophoresed on a 1% agarose-formaldehyde gel, transferred to a nylon membrane, and hybridized with ³²P-labeled λ 3R3 (see Fig. 2A). (B) Five micrograms of poly(A)⁺ RNA from different stages of *Drosophila* development was subjected to Northern analysis using a *su(Hw)* cDNA clone as hybridization probe. (C) The same filter shown in B was hybridized with a clone containing the *Drosophila ras2* gene.

maintained in most of the *su(Hw)* Zn fingers. Two exceptions are finger 2, which contains a Tyr instead of Leu, and finger 3, which contains a Leu instead of Phe. The same type of changes have been observed in the *hunchback* protein (Tautz et al. 1987). The terminal His has been replaced by a Cys in fingers 1 and 4. This type of change does not affect the functionality of the finger, as both Cys and His are able to complex Zn and maintain the structure of the protein domain (Johnston 1987).

In addition to the Zn finger domains, a highly acidic region (48% Asp and Glu) is present in the *su(Hw)* protein between amino acids 154 and 202, immediately preceding the first Zn finger motif. These amino acids have been underlined in Figure 6. Similar acidic regions have been described in yeast transcriptional activator proteins such as GCN4 and GAL4 (Hope and Struhl 1986; Ma and Ptashne 1987).

Discussion

The *su(Hw)* locus was isolated by chromosomal walking

from a previously cloned sequence. The exact location of the *su(Hw)* gene was mapped to a 9.5-kb *KpnI*–*Sall* restriction fragment based on the ability of this DNA to rescue both the female sterility of the *su(Hw)*^{69k} allele and its capacity to reverse the phenotype of gypsy-induced mutations. The DNA sequences contained in the 9.5-kb fragment encode three different RNAs, 3.3, 1.4, and 1.1 kb long. The former transcript was determined to correspond to the *su(Hw)* locus on the basis of several criteria. This RNA is expressed in the ovaries of adult females as expected for a *su(Hw)*-encoded RNA because mutations at this locus cause female sterility, whereas the other two transcripts are not. In addition, DNA alterations associated with four different *su(Hw)* alleles mapped within the RNA-coding region of the 3.3-kb transcript. Finally, *su(Hw)* mutations affect the levels or size of the 3.3-kb RNA but not the 1.4- or 1.1-kb transcripts.

The *su(Hw)* locus is expressed in all stages of *Drosophila* development and encodes a putative 109,000-dalton protein that contains 12 repeats of the Zn finger

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ATACACAAACGCTTGTCTGTTGGAATTTAAAGCATAAAATTAG	gtaaatg...	536 bp...	aattgcag	GCTGCTATCACCAAC	ATG	AGT	GCC	TCC	AAG	GAG	GGC	AAG	635																		
GAA	AAG	AAG	GGC	AAG	CTG	CTG	GGT	GTG	GAA	AAC	ATT	TCG	CCG	CCT	AAG	GAC	AAA	CGT	CCC	GCC	ACC	AAG	ATG	AAG	CTC	TTG	AAT	GAC	GTG	725	
Glu	Lys	Lys	Gly	Lys	Leu	Leu	Gly	Val	Glu	Asn	Ile	Ser	Pro	Pro	Lys	Asp	Lys	Arg	Pro	Ala	Thr	Lys	MET	Lys	Leu	Leu	Asn	Asp	Val	815	
GGG	GCT	GGT	GAG	GAT	TCG	GAG	GCA	AGC	ACT	ACT	ACT	ACC	ACA	TCA	AGA	ACT	CCC	TCC	AAC	AAG	CAG	GAG	AAG	CGT	GAC	AGT	GTC	GCT	GAA	815	
Gly	Ala	Gly	Glu	Asp	Ser	Glu	Ala	Ser	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Thr	Ser	Asn	Lys	Gln	Glu	Lys	Arg	Arg	Arg	GTC	GCT	GAA	815	
TCG	CGC	ATC	AAA	ATA	CTC	AAT	GAA	GAA	ATA	CTG	GGC	ACT	CCG	AAA	ACT	GAG	AAA	CGG	GGC	GCC	ACA	AAG	AGC	ACA	GCT	CCG	GCT	GCT	TCC	905	
Ser	Arg	Ile	Lys	Ile	Leu	Asn	Glu	Glu	Ile	Leu	Gly	Thr	Pro	Lys	Thr	Glu	Lys	Arg	Gly	Ala	Thr	Lys	Ser	Thr	Ala	GCT	CCG	GCT	GCT	TCC	905
ACT	GTG	AAG	ATT	CTG	AAC	GAG	AAA	AAG	ACA	CCC	AGT	GCT	ACC	GTG	ACA	GCC	GTG	GAA	ACT	ACC	AAG	ATC	AAG	ACG	TCT	CCC	AGT	AAA	AGA	995	
Thr	Val	Lys	Ile	Leu	Asn	Glu	Lys	Thr	Pro	Ser	Ala	Thr	Val	Val	Val	Val	Val	Val	Val	Val	Val	Val	Val	Val	Val	Val	Val	Val	Val	995	
AAG	AAA	ATG	GAG	CAC	TAT	GTA	CTT	CAG	GCT	GTG	AAA	TCA	GAA	AAT	ACC	AAG	GCG	GAC	ACC	ACG	GTT	ACC	GTG	GTC	ACC	GAA	GAA	GAT	GAC	1085	
Lys	Lys	MET	Glu	His	Tyr	Val	Leu	Gln	Ala	Val	Lys	Ser	Glu	Asn	Thr	Lys	Ala	Asp	Thr	Val	Val	Val	Val	Val	Val	Val	Val	Val	Val	1085	
ACC	ATT	GAT	TTC	ATT	CTA	GCG	GAT	GAT	GAG	GAA	GTA	GTT	CCG	GGC	AGA	ATA	GAA	AAT	AAC	AAC	GGC	CAG	GAG	ATC	GTT	GTC	ACC	GAG	GAC	1175	
Thr	Ile	Asp	Phe	Ile	Leu	Ala	Asp	Asp	Glu	Glu	Val	Val	Pro	Gly	Arg	Ile	Glu	Asn	Asn	Asn	Gly	Gln	Glu	Ile	Val	Val	Val	Val	Val	1175	
GAT	GAG	GAT	CTA	GGG	GAG	GAT	GGT	GAT	GAG	GAC	GGT	GAA	GAC	TCC	TCA	GGC	AAG	GGC	AAC	TCT	AGC	CAG	ACA	AAG	ATC	AAG	GAG	ATC	GTG	1265	
Asp	Glu	Asp	Leu	Gly	Glu	Asp	Gly	Asp	Gly	Asp	Gly	Asp	Gly	Asp	Ser	Ser	Gly	Lys	Gly	Asn	Ser	Ser	Gln	Thr	Lys	Ile	Lys	Glu	Val	1265	
GAG	EAT	GTG	TGT	GGC	AAA	TGC	TAC	AAG	ACC	TTC	CGT	CGG	GTT	CAG	AGT	CTG	AAA	AAG	CAT	CTA	GAG	TTC	TGT	CCG	TAT	GAC	AGT	GGG	TAT	1355	
Glu	His	Val	Cys	Gly	Lys	Cys	Tyr	Lys	Thr	Phe	Arg	Arg	Val	Gln	Ser	Leu	Lys	Lys	Lys	Leu	Glu	Phe	Cys	Arg	Tyr	Asp	Arg	Gly	Tyr	1355	
CAT	TTG	CGC	AAA	GGC	GAC	ATG	CTT	AAG	AAC	CTG	GAG	AAG	ATC	GAG	AAG	GAT	GGC	GTG	GTG	ATG	GAG	AAG	AAG	GAC	ATA	TGC	TTC	TGC	TGC	1445	
His	Leu	Arg	Lys	Ala	Asp	MET	Leu	Lys	Asn	Leu	Glu	Lys	Ile	Glu	Lys	Asp	Ala	Val	Val	MET	Glu	Lys	Lys	Asp	Ile	Cys	Phe	Cys	Cys	1445	
AGC	GAA	AGT	TAC	GAC	ACC	TTC	CAT	gtaagtca...	50 bp...	cttttcag	TTG	GGT	CAC	ATT	AAC	TGT	CCC	CAC	TGC	CCC	AAG	TGG	TTT	AAG	ACA	Ser	Glu	Ser	Tyr	1580	
Ser	Glu	Ser	Tyr	Asp	Thr	Phe	His																							1580	
GAG	ACC	AGT	TAC	GAA	CGC	CAC	ATT	TTC	ATC	AGT	CAC	TCC	GAG	TTC	AGC	GAT	TTT	CGT	TCC	TCC	ATT	TGC	AAT	GGC	AAC	TTC	GGC	AGC	CAG	1670	
Gln	Thr	Ser	Thr	Glu	Arg	His	Ile	Phe	Ile	Thr	His	Ser	Glu	Phe	Ser	Asp	Phe	Pro	Cys	Ser	Ile	Cys	Asn	Ala	Asn	Leu	Arg	Ser	Glu	1670	
GGC	TTG	TTG	GGC	CTG	GAC	GAG	GAA	CAG	CAC	AAG	TCA	CGT	GCG	AAG	TCG	TAT	GCA	TGC	AAG	ATC	TGC	GGC	AAG	CAC	TTC	ACC	GGC	TGC	TAC	1760	
Ala	Leu	Leu	Ala	Leu	His	Glu	Glu	Gln	His	Lys	Ser	Arg	Gly	Lys	Ser	Tyr	Ala	Cys	Lys	Ile	Cys	Gly	Lys	Asp	Phe	Thr	Arg	Ser	Tyr	1760	
CAC	TTG	AAG	CGC	CAG	CAG	AAG	TAG	TCA	TGG	TGG	TCA	TCA	AAC	GAG	ACC	GAT	ACC	ATG	AGC	TGC	AAG	GTG	TGC	GAT	CCG	GTC	TTC	TAT	CGG	1850	
His	Leu	Lys	Arg	His	Gln	Lys	Tyr	Ser	Ser	Cys	Ser	Ser	Asn	Glu	Thr	Asp	Thr	MET	Ser	Cys	Lys	Val	Cys	Asp	Arg	Val	Phe	Thr	Arg	1850	
CTT	GAC	AAC	CTG	CGA	TGG	CAT	TTG	AAG	CAG	CAT	TTG	GGC	ACA	CAG	GTG	GTT	AAA	AAG	CCG	GAG	TAC	ATC	TGC	CAC	ACC	TGC	AAG	AAT	TGT	1940	
Leu	Asp	Asn	Leu	Arg	Ser	His	Lys	Gln	His	Leu	Gly	Thr	Gln	Val	Val	Lys	Lys	Pro	Glu	Tyr	MET	Cys	His	Thr	Lys	Lys	Asn	Cys		1940	
TTC	TAC	AGC	CTG	TCC	ACG	CTG	AA	gtaagttc...	41 bp...	ttttcgag	C	ATT	CAC	ATA	CGC	ACT	CAT	ACG	GGC	GAG	AAA	CCA	TAC	AGT	TGC	ACT	GTG	gtt		2066	
Phe	Tyr	Ser	Leu	Ser	Thr	Leu	As																							2066	
CTT	TGC	GAC	AAG	AAG	TTT	TCT	GCA	TTG	GTG	GGC	ETT	AAA	AAG	CAT	GGA	GGC	TAT	CAC	ACT	GGC	GAG	AAA	CCA	TAC	AGT	TGC	ACT	GTG	gtt	2156	
Leu	Cys	Asp	Lys	Lys	Phe	Ser	Ala	Leu	Val	Ala	Leu	Lys	Lys	His	Arg	Tyr	His	Thr	Gly	Glu	Lys	Pro	Tyr	Ser	Gly	Thr	Val			2156	
agtct...	53 bp...	acctttag	TGC	AAC	GAA	GCA	TTT	GGC	GTA	AAG	GAA	GTG	GTC	AAT	GGT	CAC	ATC	AAA	GGC	GAC	ACT	GGA	CAG	CGT	CCG	GAC					2294
			Cys	Asn	Gln	Ala	Phe	Ala	Val	Lys	Glu	Val	Leu	Asn	Arg	His	MET	Lys	Arg	His	Thr	Gly	Glu	Arg	Pro	His					2294
AAG	TGT	GAT	GAA	TGT	GGC	AAG	AGT	TTT	ATC	CGG	GGC	ACC	CAG	CTG	CGC	ACC	CAT	TCC	AAA	ACC	CAT	ATT	CGC	CCC	TTT	CCG	TGT	GAG	CAA	2384	
Lys	Cys	Asp	Glu	Cys	Gly	Lys	Ser	Phe	Ile	Arg	Ala	Thr	Gln	Leu	Arg	Thr	His	Ser	Lys	Thr	His	Ile	Arg	Pro	Phe	Pro	Cys	Glu	Gln	2384	
TGC	GAC	GAA	AAA	TTC	AAA	ACA	GAG	AAA	CA	gtaagata...	52 bp...	ttttcgag	A	CTT	GAG	CGC	CAT	GTG	AAG	ACC	CAT	TCT	CCG	ACA	AAA	CGC					2521
Cys	Asp	Glu	Lys	Phe	Lys	Thr	Glu	Lys	GI																					2521	
CCC	GTG	TTT	TCC	TGC	GGC	GAG	TGC	AAG	CGT	AAT	TTC	CGC	ACT	CCA	GCA	TTC	GTC	AAG	GAC	CAT	ATG	GAC	GAG	GGG	AAC	AGC	C	gtaag		2611	
Pro	Val	Phe	Ser	Cys	Ala	Glu	Cys	Lys	Arg	Asn	Phe	Arg	Thr	Pro	Ala	Leu	Leu	Lys	Glu	His	MET	Asp	Glu	Gly	Lys	His	Ser	P		2611	
ttg...	45 bp...	ctttgtag	CG	AAA	CAA	CAA	CGG	TCA	TCA	ATG	CGA	TGC	GCC	GTC	AAA	ATT	ATG	GAA	CGC	AGC	GAT	TGC	GGC	ATT	TGC	CAT				2738	
			ro	Lys	Gln	Gln	Arg	Ser	Ser	Met	Arg	Ser	Ala	Leu	Lys	Ile	MET	Glu	Arg	Thr	Asp	Cys	Ala	Ile	Cys	Asp				2738	
AAG	AAC	TTT	GAC	AGC	AGC	GAT	ACC	CTG	CGA	GGC	CAC	ATA	CGC	ACC	GTT	CAC	GAA	TGC	GAT	CCG	GAT	GAC	ATT	TTT	GGC	GTT	GAG	CCG	CAT	2828	
Lys	Asn	Phe	Asp	Ser	Ser	Asp	Thr	Leu	Arg	Arg	His	Ile	Arg	Thr	Val	His	Glu	Cys	Asp	Pro	Asp	Asp	Ile	Phe	Val	Glu	Pro	His		2828	
CCA	TCA	AAG	CGT	GCA	AAG	AAG	CAC	ATC	GAA	TGC	GAG	GAT	GTT	CTA	CCA	GTC	GGG	TTA	AAC	TCA	CGG	GGC	TGC	TTA	ATT	TCC	AGC	CAA	2918		
Pro	Ser	Lys	Arg	Ala	Lys	Lys	Asp	Ile	Glu	Ser	Glu	Glu	Val	Val	Pro	Val	Ala	Leu	Asn	Thr	Ser	Ala	Gly	Ser	Leu	Ile	Ser	Ser	Gln	2918	
ACG	GAC	GGC	AAT	GAC	GTT	GTG	GTG	CGT	GAA	TTC	CTC	GTG	GAC	GAA	GGC	GAT	GGC	GGC	GGC	CAG	ACC	ATT	ACA	CTA	GAA	AAC	GAA	ACA	TAC	3008	
Thr	Asp	Gly	Asn	Asp	Val	Val	Arg	Glu	Phe	Leu	Val	Arg	Glu	Gly	Asp	Gly	Ala	Ala	Ala	Ala	Thr	Ile	Thr	Leu	Glu	Asn	Glu	Thr	Tyr	3008	
ACA	ATT	CTG	CCC	CTG	GAC	GGG	GCC	ATC	GAG	GGA	GAG	CAG	CTG	ACG	GAT	GAG	GCT	GGC	GTT	AAG	CCC	GAG	CGC	AAG	AAG	GAG	GAG	CGC	CAG	3098	
Thr	Ile	Leu	Pro	Leu	Asp	Gly	Ala	Ile	Glu	Gly	Glu	Gln	Leu	Thr	Asp	Glu	Ala	Gly	Val	Lys	Pro	Glu	Ala	Lys	Lys	Glu	Glu	Ala	Gln	3098	
GTC	TGC	CGG	CTG	GTA	AAG	AAA	GAG	CAG	CGT	AAA	TCC	CTG	GCC	GCC	AGT	CTG	GCC	GCT	GCC	ATC	GCA	GAT	AAT	TTG	GAA	GAA	TCA	TGT	AGC	3188	
Val	Ser	Pro	Val	Val	Lys	Ala	Glu	Gln	Arg	Lys	Ser	Leu	Ala	Ala	Ser	Leu	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ser	3188	
GAG	GAT	GAC	TTC	AGT	GGC	GAA	ATT	CTA	ACT	GAG	GAG	GAC	ATA	AAG	CTC	AAA	GAG	AAT	GTG	GGT	AAA	CTT	ATT	GAT	ATG	CTA	GTG	GAT	CCA	3278	
Glu	Asp	Asp	Phe	Ser	Gly	Glu	Ile	Leu	Thr	Glu	Glu	Asp	Ile	Lys	Leu	Lys	Cys	Ala	Val	Gly	Lys	Leu	Ile	Asp	MET	Leu	Val	Asp	Pro	3278	
CCG	ATC	CTA	AAG	AAA	TAC	GGC	TGG	CCC	AAT	GCC	CCC	GAG	GAG	ACG	GTG	CTC	TGC	GAA	GTG	ATC	GAG	AAC	TGT	GGC	CAC	GAC	CTG	ACT	AAA	3368	
Pro	Ile	Leu	Lys	Lys	Tyr	Gly	Trp	Pro	Asn	Ala	Pro	Glu	Glu	Thr	Val	Leu	Cys	Lys	Val	Ile	Glu	Asn	Cys	Gly	His	Asp	Leu	Thr	Lys	3368	
GGT	GGC	GAA	AAC	TAC	GCT	GAG	CTT	GAC	TAC	GGT	AGC	CGC	ATG	CGA	GAG	TAC	TGC	AAA	CTG	CTC	TTT	ACC	GTG	GTC	ATT	CAC	AAC	GAT	TCC	3458	
Gly	Gly	Glu	Asn	Tyr	Ala	Glu	Leu	Asp	Tyr	Gly	Ser	Arg	MET	Arg	Glu	Tyr	Cys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	Lys	3458	
ATC	AAG	TGC	CTG	CTC	AAT	AAT	TTC	CCC	ATC	GAC	GAT	GTG	ATC	GAA	TAT	GTA	CTG	GGA	GAT	GAG	GAT	CAG	GAC	GAA	GGT	GGA	TTG	GAC	AAA	3548	
Ile	Lys	Ser	Leu	Leu	Asn	Asn	Phe	Pro	Ile	Asp	Asp	Val	Ile	Glu	Tyr	Val	Leu	Gly	Asp	Glu	Asp	Gln	Asp	Glu	Gly	Gly	Leu	Asp	Lys	3548	
GAC	AAT	GAA	GAC	AAT	GAG	TCA	GAT	TCA	GGT	GAT	GAA	GAA	GGG	GTG	GTC	ACA	GGC	GAA													

#1	His	Val	<u>Cys</u>	Gly	Lys	<u>Cys</u>	Tyr	Lys	Thr	<u>Phe</u>	Arg	Arg	Val	Gln	Ser	<u>Leu</u>	Lys	Lys	<u>His</u>	Leu	Glu		Phe	Cys	
#2	Ile	Asn	<u>Cys</u>	Pro	Asp	<u>Cys</u>	Pro	Lys	Ser	<u>Phe</u>	Lys	Thr	Gln	Thr	Ser	Tyr	Glu	Arg	<u>His</u>	Ile	Phe	Ile	Thr	<u>His</u>	
#3	<u>Phe</u>	Pro	<u>Cys</u>	Ser	Ile	<u>Cys</u>	Asn	Ala	Asn	<u>Leu</u>	Arg	Ser	Glu	Ala	Leu	<u>Leu</u>	Ala	Leu	<u>His</u>	Glu	Glu		Gln	<u>His</u>	
#4	<u>Tyr</u>	Ala	<u>Cys</u>	Lys	Ile	<u>Cys</u>	Gly	Lys	Asp	<u>Phe</u>	Thr	Arg	Ser	Tyr	His	<u>Leu</u>	Lys	Arg	<u>His</u>	Gln	Lys	Tyr	Ser	Ser	Cys
#5	Met	Ser	<u>Cys</u>	Lys	Val	<u>Cys</u>	Asp	Arg	Val	<u>Phe</u>	Tyr	Arg	Leu	Asp	Asn	<u>Leu</u>	Arg	Ser	<u>His</u>	Leu	Lys		Gln	<u>His</u>	
#6	<u>Tyr</u>	Met	<u>Cys</u>	His	Thr	<u>Cys</u>	Lys	Asn	Cys	<u>Phe</u>	Tyr	Ser	Leu	Pro	Thr	<u>Leu</u>	Asn	Ile	<u>His</u>	Ile	Arg		Thr	<u>His</u>	
#7	<u>Phe</u>	Asp	<u>Cys</u>	Asp	Leu	<u>Cys</u>	Asp	Lys	Lys	<u>Phe</u>	Ser	Ala	Leu	Val	Ala	<u>Leu</u>	Lys	Lys	<u>His</u>	Arg	Arg		Tyr	<u>His</u>	
#8	<u>Tyr</u>	Ser	<u>Cys</u>	Thr	Val	<u>Cys</u>	Asn	Gln	Ala	<u>Phe</u>	Ala	Val	Lys	Glu	Val	<u>Leu</u>	Asn	Arg	<u>His</u>	Met	Lys		Arg	<u>His</u>	
#9	His	Lys	<u>Cys</u>	Asp	Glu	<u>Cys</u>	Gly	Lys	Ser	<u>Phe</u>	Ile	Arg	Ala	Thr	Gln	<u>Leu</u>	Arg	Thr	<u>His</u>	Ser	Lys		Thr	<u>His</u>	
#10	<u>Phe</u>	Pro	<u>Cys</u>	Glu	Gln	<u>Cys</u>	Asp	Glu	Lys	<u>Phe</u>	Lys	Thr	Glu	Lys	Gln	<u>Leu</u>	Glu	Arg	<u>His</u>	Val	Lys		Thr	<u>His</u>	
#11	<u>Phe</u>	Ser	<u>Cys</u>	Ala	Glu	<u>Cys</u>	Lys	Arg	Asn	<u>Phe</u>	Arg	Thr	Pro	Ala	Leu	<u>Leu</u>	Lys	Glu	<u>His</u>	Met	Asp	Glu	Gly	Lys	<u>His</u>
#12	Thr	Asp	<u>Cys</u>	Ala	Ile	<u>Cys</u>	Asp	Lys	Asn	<u>Phe</u>	Asp	Ser	Ser	Asp	Thr	<u>Leu</u>	Arg	Arg	<u>His</u>	Ile	Arg	Thr	Val	<u>His</u>	

Consensus Phe Xaa Cys Xaa Xaa Cys Xaa Xaa Xaa Phe Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa His Xaa Xaa Xaa Xaa His
Tyr

Figure 7. Amino acid structure of the Zn finger domains of the *su(Hw)*-encoded protein. The protein sequences of the different Zn finger regions contained in the *su(Hw)* protein are numbered 1–12, starting at the amino-terminal end of the protein. Also shown is a consensus sequence derived from similar regions found in other organisms. Residues conserved with respect to the consensus sequence have been underlined.

domain. This type of structure has been found in some nucleic-acid-binding proteins. For example, TFI_{II}A, a polymerase III transcription factor that interacts with both DNA and RNA contains nine copies of the Zn finger domain (Miller et al. 1985). Other proteins in which this type of structure has been found include the *Drosophila Krüppel* (Rosenberg et al. 1986), *hunchback* (Tautz et al. 1987), and *serendipity* (Vincent et al. 1985) gene products, the yeast ADR1 (Blumberg et al. 1987) and GAL4 (Johnston 1987) proteins, and the human estrogen and glucocorticoid receptors (Giguere et al. 1986; Green and Chambon 1987). The structure of the different fingers present in the *su(Hw)* protein is well conserved with respect to the proposed consensus sequence for this type of functional domain. The few deviations from the consensus have also been found in other fingers shown to be functional, suggesting that all 12 fingers present in the *su(Hw)* protein should be able to bind DNA.

In addition, the *su(Hw)*-encoded product contains a very acidic region (48% Asp and Glu) located upstream of the first finger domain. Similar acidic tracts have been found in the GCN4 and GAL4 proteins of yeast (Hope and Struhl 1986; Ma and Ptashne 1987), which are transcriptional activators of genes encoding proteins involved in amino acid biosynthesis and galactose catabolism, respectively. The acidic domain of these two proteins is not involved in DNA binding but rather in transcriptional activation via protein–protein interactions, presumably with a TATA-binding protein or RNA polymerase II (Hope et al. 1988).

The presence of this modular structure, acidic domain and Zn finger motifs, in the *su(Hw)* product suggests that this protein might be a transcription factor involved in the control of the expression of normal cellular genes. In addition, the existence of these two structural characteristics correlates with previously proposed roles for the *su(Hw)* protein to explain the mechanism of gypsy mu-

tagenesis and the reversion of the gypsy-induced phenotypes by homozygous mutations at the *su(Hw)* locus. We have shown that levels of gypsy RNA are 25-fold lower in *su(Hw)*[−] mutants as compared to wild type and have suggested that the mutagenicity of the gypsy element might be related to the *su(Hw)*-dependent transcriptional activation of this element during the pupal stages of development (Parkhurst and Corces 1985, 1986a). The case we have studied in most detail is that of the *y*² allele, in which the insertion of gypsy at −700 bp from the start of transcription of the *yellow* RNA causes an adult-specific phenotype that results in wild-type colored bristles but mutant wing blades and body cuticle (Biessmann 1985; Chia et al. 1986; Geyer et al. 1986; Parkhurst and Corces 1986a). To explain the temporal and spatial restrictions of this phenotype and its reversibility by *su(Hw)* mutations, we have proposed two possible complementary and overlapping explanations. The tissue-specific *y*² phenotype might be a consequence of changes in the chromatin structure of the *yellow* locus due to the transcriptional activation of the gypsy element during pupal development in the affected tissues, when *yellow* expression is necessary for proper coloration of adult cuticular structures (Parkhurst and Corces 1986b). Alternatively, transcription of gypsy might not be necessary per se, and it is merely the binding of the *su(Hw)*-encoded protein to particular sequences located in the central portion of the gypsy element that affects the proper interaction of tissue-specific transcriptional enhancers with the *yellow* promoter and results in mutant phenotype in these particular tissues (Geyer and Corces 1987; Geyer et al. 1988). Both hypotheses require the interaction of the *su(Hw)* protein with DNA sequences located in the gypsy element, a condition supported by the presence of Zn finger domains in the predicted amino acid sequence of the *su(Hw)* product.

The cloning of the *su(Hw)* locus will afford the isola-

tion of the encoded protein and the study of its interaction in vitro with gypsy DNA. From this type of analysis we hope to gain further understanding of the mechanisms by which transposable elements induce mutant phenotypes and how these can be enhanced or reversed by second site mutations at modifier loci.

Materials and methods

Isolation and maintenance of *Drosophila* strains

Fly stocks were maintained at 22.5°C and 65% relative humidity. The *su(Hw)^B* and *su(Hw)^C* alleles were obtained by crossing *bx^{34e}* males, which had been irradiated with γ -rays (3000 rads), with females of the genotype *TM3, su(Hw)² Sb bx^{34e}/TM6, su(Hw)^f Ubx^{P15} e^s*, and the progeny were scored for *bx⁺* phenotype. Some *su(Hw)* alleles used in this study, such as *su(Hw)^{69k}* and *su(Hw)²*, are female sterile and are maintained as balanced stocks over the chromosome *TM6, su(Hw)^f*. These two particular alleles show very low viability as homozygotes, probably due to the accumulation of lethal mutations in these chromosomes that have no effects in the balanced stocks. This is evidenced by the fact that these alleles exhibit high viability when compounded with a deficiency of the *su(Hw)* region.

Isolation and enzymology of nucleic acids

Isolation of plasmid DNA, construction and screening of λ libraries, and DNA labeling and enzymology were carried out by standard procedures (Maniatis et al. 1982). Genomic DNA from *Drosophila* adults was prepared as described previously (Parkhurst and Corces 1985). Total RNA was isolated from synchronously developing *Drosophila* cultures by homogenization in 4 M guanidine isothiocyanate, 0.2% *N*-lauroyl sarcosine, 150 mM mercaptoethanol, 12.5 mM EDTA, and 50 mM Tris-hydrochloride (pH 7.5), followed by phenol extraction and ethanol precipitation (Parkhurst and Corces 1985). Poly(A)⁺ RNA was selected by chromatography on oligo(dT)-cellulose (Aviv and Leder 1972). Ovaries for RNA preparation were dissected from mature adult females in 130 mM NaCl, 5 mM KCl, 2 mM CaCl₂, and 10 mM HEPES (pH 6.9). The tissue was then homogenized in 10 mM EDTA, 100 mM NaCl, 0.5% SDS, 250 μ g/ml proteinase K, and 50 mM Tris-hydrochloride (pH 7.5), phenol extracted three times, and ethanol-precipitated (Parks et al. 1986). Southern and Northern analysis was done as previously described (Parkhurst and Corces 1985). DNA sequence analysis was performed according to Maxam and Gilbert 1980.

P-element-mediated transformation was carried out as described by Rubin and Spradling (1982), using the *white* gene as a selectable marker in the CaSpeR vector (Pirrotta et al. 1985). In situ hybridizations to polytene chromosomes were done by the method of Pardue and Gall (1975).

Acknowledgments

We would like to thank Dr. M. Green for many fruitful discussions during the preparation of this manuscript and Dr. A. Spradling for support and advice. The mutagenesis experiments leading to the isolation of *su(Hw)^B* and *su(Hw)^C* were carried out by Robert Coyne during his stay in the Department of Biochemistry and Molecular Biology at Harvard University. We would like to thank Dr. M. Meselson for his constant encouragement and support throughout those studies. We also thank Drs. M. Levine for the gift of λ S63, V. Pirrotta for the CaSpeR vector, M. Peifer, W. Bender, and E. Grell for several *su(Hw)*

alleles and their parental stocks. A. Greenleaf and colleagues for unpublished mapping data, S. Parks for protocols to prepare ovarian RNA, and T. Kornberg and L. Kauvar for cDNA libraries. S.M.P. would like to thank Drs. Suki Parks, Ward Odenwald, Brian Mozer, and colleagues in the Hopkins/Carnegie fly groups for valuable suggestions, sharing unpublished data, and moral support during the course of these studies. The work reported here was supported by U.S. Public Health Service grant GM-35463, and American Cancer Society grant NP-546.

Note

Sequence data described in this paper have been submitted to the EMBL/GenBank Data Libraries under accession number Y00228.

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The *Drosophila* su(Hw) gene, which controls the phenotypic effect of the gypsy transposable element, encodes a putative DNA-binding protein.

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Genes Dev. 1988, **2**:

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