The *suppressor of Hairy-wing* Protein Regulates the Tissue-Specific Expression of the Drosophila *gypsy* Retrotransposon

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ABSTRACT

The *gypsy* retrotransposon of *Drosophila melanogaster* causes mutations that show temporal and tissue-specific phenotypes. These mutant phenotypes can be reversed by mutations in *su(Hw)*, a gene that also regulates the transcription of the *gypsy* element. *Gypsy* encodes a full-length 7.0-kb RNA that is expressed in the salivary gland precursors and fat body of the embryo, imaginal discs and fat body of larvae, and fat body and ovaries of adult females. The *su(Hw)*-binding region inserted upstream of the promoter of a *lacZ* reporter gene can induce β-galactosidase expression in a subset of the embryonic and larval tissues where *gypsy* is normally transcribed. This expression is dependent on the presence of a functional *su(Hw)* product, suggesting that this protein is a positive activator of *gypsy* transcription. Flies transformed with a construct in which the 5′ LTR and leader sequences of *gypsy* are fused to *lacZ* show β-galactosidase expression in all tissues where *gypsy* is normally expressed, indicating that sequences other than the *su(Hw)*-binding site are required for proper spatial and temporal expression of *gypsy*. Mutations in the zinc fingers of *su(Hw)* affect its ability to bind DNA and to induce transcription of the *lacZ* reporter gene. Two other structural domains of *su(Hw)* also play an important role in transcriptional regulation of *gypsy*. Deletion of the amino-terminal acidic domain results in the loss of *lacZ* expression in larval fat body and adult ovaries, whereas mutations in the leucine zipper region result in an increase of *lacZ* expression in larval fat body and a decrease in adult ovaries. These effects might be the result of interactions of *su(Hw)* with activator and repressor proteins through the acidic and leucine zipper domains to produce the final pattern of tissue-specific expression of *gypsy*.

THE *gypsy* transposable element of *Drosophila melanogaster* is a 7.5-kb long terminal repeat (LTR)–containing retrotransposon. *Gypsy* contains two 482-bp LTRs and three open reading frames that encode putative products similar to the *gag, pol* and *env* proteins found in vertebrate retroviruses (MARLOR *et al.* 1986; BOEKE and CORCES 1989). Insertion of *gypsy* into genes such as *cut, forked, yellow* and *scute* results in mutant phenotypes that can be reversed by second-site mutations in the *suppressor of Hairy-wing* [*su(Hw)*] gene (MODOLELL *et al.* 1983; RUTLEDGE *et al.* 1988).

The molecular basis for the mutagenic effect of *gypsy* has been well studied. Analyses of *gypsy*-induced mutations in the *yellow, cut* and *Ubx* genes indicate that insertion of *gypsy* between *cis*-regulatory sequences and the promoter is responsible for the manifestation of the mutant phenotype (GEYER *et al.* 1986, 1988; PEIFER and BENDER 1986; JACK *et al.* 1991). The *su(Hw)* protein has 12 zinc fingers and binds to a region in the 5′ transcribed, untranslated portion of *gypsy* containing 12 copies of a sequence homologous to the octamer motif found in mammalian transcriptional enhancers (SPANA *et al.* 1988; MAZO *et al.* 1989). This sequence has been shown to be necessary and sufficient for *gypsy* mutagenesis in the case of *yellow* and *hsp70*, a gene that is not normally a target of *gypsy* insertions (SPANA and CORCES 1990; HOLDRIDGE and DORSETT 1991; GEYER and CORCES 1992; SMITH and CORCES 1992). From these data, it has been suggested that the *su(Hw)* protein mediates the mutagenic effect of *gypsy* by repressing the ability of enhancers to regulate the expression of the affected gene. A functional analysis of the *su(Hw)* protein has shown that the leucine zipper motif and, to a lesser extent, the acidic domains are essential for the repressive effect on enhancer function. This suggests that *su(Hw)* interacts with other proteins through these regions to repress gene expression (HARRISON *et al.* 1993).

It is not known how the repressive role of the *su(Hw)* protein in *gypsy* mutagenesis is related to its normal cellular function in Drosophila. Additional results indicate that *su(Hw)* may not act solely as a repressor. A role of *su(Hw)* protein as a general cellular transcription factor capable of activating gene expression is implied by the fact that several alleles of *su(Hw)* are female-sterile, suggesting that the function of this protein is necessary for the expression of genes required during oogenesis (LINDSLEY and ZIMM 1992; HARRISON *et al.* 1993). The *su(Hw)* protein has been proposed to regu-

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late *gypsy* transcription, because *gypsy* RNA levels decrease 25-fold in a *su(Hw)* homozygous mutant background (PARKHURST and CORCES 1986). This effect could take place at the level of transcription initiation or, because the *su(Hw)* binding region is located in the transcribed untranslated region of *gypsy*, on RNA stability. The *su(Hw)*-binding region has been shown to potentiate polyadenylation at upstream termination sites (DORSETT *et al.* 1989; DORSETT 1990). The normal role of *su(Hw)* might be related to its function in the control of *gypsy* expression; therefore, information about the regulation of expression of the *gypsy* retrotransposon and the role of *su(Hw)* protein in this process may provide clues as to the function of this protein in the transcription of cellular genes. Thus, experiments described in this paper were aimed at further elucidating the role of the *su(Hw)* protein in *gypsy* expression and identifying the protein domains required in this process.

We present results indicating that *su(Hw)* acts as a transcriptional activator of *gypsy* expression during development. *Gypsy* is expressed in the gonads, fat body and salivary gland precursors of the embryo. Its expression continues at high levels in the fat body and at very low levels in the salivary glands during larval development. In adult females, *gypsy* is expressed in the fat body as well as the nurse and follicle cells during oogenesis. A β-galactosidase reporter gene containing the *su(Hw)*-binding region upstream of the *hsp70* promoter can reproduce part of the embryonic and larval expression patterns of an intact *gypsy* element, suggesting that *su(Hw)* activates the tissue-specific expression of *gypsy* at the level of transcription initiation. Additional *gypsy* sequences from the LTR and 5′-transcribed untranslated region give rise to the normal expression pattern. Analyses of specific mutant *su(Hw)* alleles on the expression of this reporter gene indicate that both the amino-terminal acidic and the leucine zipper domains of *su(Hw)* are essential for the proper regulation of *gypsy* expression in larval tissues and in adult ovaries. These data suggest that the *su(Hw)* protein interacts with other proteins through its acidic and leucine zipper domains to produce the tissue-specific expression of *gypsy*.

MATERIALS AND METHODS

Drosophila strains: Fly stocks were maintained at 22.5°. The y^{59b} *z w f* flies were obtained from Dr. JOHNG LIM. Seven alleles of *su(Hw)* were used (Figure 7). The $su(Hw)^{E8}$ mutation was generated in an EMS screen as described by KENNISON and TAMKUN (1988). The strains $su(Hw)^{V}$ and $su(Hw)^{J}$ were induced in a different EMS screen (PARKHURST *et al.* 1988; HARRISON *et al.* 1993). The $su(Hw)^{V}P[CaS\ X/K\ 5.3]$ strain is a fly line in which the lethality associated with the deletion of the *DmRPII15* gene has been rescued by transformation with a 5.3-kb genomic DNA fragment (HARRISON *et al.* 1992). The $su(Hw)^{\Delta 100}$, $su(Hw)^{NoAD}$ and $su(Hw)^{\Delta 283}$ mutations were generated *in vitro* and are described by HARRISON *et al.* (1993). With the exception of $su(Hw)^{J}$, all of the *su(Hw)* mutations were maintained over the *TM6B Tb Hu e* chromosome.

***In situ* hybridizations:** The genotypes of the wild-type animals used for *in situ* hybridization experiments were either y^{59b} *z w f* or *y v f mal*. Both fly strains contain many copies of euchromatic gypsies. The *XhoI-XhoI* fragment of *gypsy* was used as a probe and was labeled with digoxygenin-dUTP as described in EPHRUSSI *et al.* (1991). For *gypsy* antisense RNA probes, the *Bgl*II-*Eco*RI fragment of *gypsy* was subcloned into the *Eco*RI-*Bam*HI sites of pgem-2 (Promega Corp., Madison, WI). *Gypsy* antisense RNA probes were synthesized with digoxygenin-UTP as described in the Boehringer-Mannheim "Genius" kit protocol.

In situ hybridization to whole-mount embryos, larval fat body and imaginal discs was performed using a *gypsy* riboprobe as described by TAUTZ and PFEIFLE (1989) with modifications by ROSEN and BEDDINGTON (1993). Briefly, embryos were rinsed, dechorionated and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), 1% DMSO (dimethyl sulfoxide) and heptane for 20 min at room temperature. The paraformaldehyde solution was removed, and methanol was added to devitellinize the embryos. The embryos were rehydrated, fixed again in 4% paraformaldehyde in PBS and dehydrated through a methanol series before storage at −70°. The embryos were rehydrated, washed with PBT(2 mg/ml bovine serum albumin, 0.1% Triton X in PBS) and permeabilized by incubating three times with RIPA (150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, 50 mM Tris, pH 8.0). The embryos were fixed for 20 min at room temperature in 4% paraformaldehyde and 0.2% glutaraldehyde in PBT. After fixation, the embryos were washed three times 5 min each in RIPA and then in PBT. The embryos were first washed in a 1:1 mix of hybridization solution to PBT and then in hybridization solution alone. The embryos were prehybridized in hybridization solution for 1 hr at 70°. Ten microliters of riboprobe were boiled for several minutes, chilled on ice and then added to 600 μl of hybridization solution. Hybridization was allowed to proceed overnight at 70°. The rest of the procedure was as described by ROSEN and BEDDINGTON (1993). Embryos were mounted in 70% glycerol and viewed under a Leitz microscope using Hoffmann optics. Embryos were staged according to CAMPOS-ORTEGA and HARTENSTEIN (1985).

For whole-mount *in situ* hybridization to larval tissues, third instar larvae were washed in PBS, cut in half with dissecting scissors and everted. Larval tissues were placed in small baskets made from Nytran and 1000-μl pipette tips (LANKENAU *et al.* 1994) and fixed in 4% paraformaldehyde in PBS that was kept on ice until all of the larvae had been dissected. The fixation step was then continued at room temperature for 20 min. Subsequent incubations were performed as described for whole-mount embryos. The larval tissues contained in baskets were transferred between solutions in 20-well culture plates. Stained imaginal discs and fat body were dissected and dehydrated through an ethanol series. They were briefly incubated in xylene and then mounted in Permount:xylene (16:9). Stained tissues were viewed under a Leitz microscope using Hoffmann optics.

For *in situ* hybridization to whole-mount ovaries, adult females (1 day old) were aged on yeasted vials for 3 days and dissected in 1× PBS. The ovarioles were gently teased apart and placed in 4% paraformaldehyde in PBS, 1% DMSO on ice until all of the ovaries had been dissected. The fixation step was then continued for 30 min at room temperature. The ovaries were transferred to small baskets and treated as described for embryos beginning with the RIPA permeabilization step. Before incubation in the 1:1 mix of PBT:hybridiza-

tion solution, the ovaries were incubated in 90% methanol:10% DMSO for 1 hr at −20° (EPHRUSSI *et al.* 1991) and then washed several times in PBT. The rest of the procedure was identical to that followed for whole-mount embryos. Stained ovaries were mounted in 50% glycerol:50% PBS and viewed under a Leitz microscope using Hoffmann optics.

In situ hybridizations to adult female frozen tissue sections were performed as follows. Newly enclosed adult females were washed in 1× PBS, embedded in Tissue-Tek OCT medium (Miles) and then frozen on dry ice as described in HAFEN and LEVINE (1987). Twelve-micrometer sections were cut on a Slee cryostat and hybridized with a *gypsy* DNA probe according to the protocol of TAUTZ and PFEIFLE (1989).

Plasmid constructions and *P* element–mediated germ-line transformations: Several different *P* element–containing plasmids were made. To construct pgypCaSpeR, the *Escherichia coli lacZ* gene was subcloned into the CaSpeR vector (PIRROTTA *et al.* 1985) as an *Eco*RI fragment from pDM66A (MISMER and RUBIN 1987; FORTINI and RUBIN 1990). The *gypsy-lacZ* fusion gene was generated by first subcloning the *HpaI-BstXI* fragment of *gypsy* into pUC18. The *gypsy* sequences were then subcloned as a *PstI-Bam*HI fragment into the CaSpeR plasmid containing *lacZ*. To construct the pryRP1 and pryRP2 plasmid, the *Xmn*I-*Bsp*1286I fragment of *gypsy* containing the *su(Hw)* binding region was initially subcloned into the *Sma*I site of pUC18. Both orientations of the *su(Hw)*-binding region were subcloned upstream of the *hsp70-lacZ* fusion gene of pryNHZ5 as *Xba*I-*Kpn*I fragments. The pryNHZ5 plasmid is a Carnegie 20 vector containing a basal *hsp70* promoter fused in frame to the *lacZ* gene (HIROMI and GEHRING 1987). The phs43RP1 and phs43RP2 plasmids were made by subcloning the *su(Hw)*-binding region as an *Eco*RI-*Bam*HI fragment into the CaSpeRhs43βgal plasmid (gift of V. PIRROTTA).

P element–mediated transformations were performed as described by RUBIN and SPRADLING (1982). The pgypCaSpeR, phs43RP1 and phs43RP2 plasmids were injected into *Df(1)w, y* w^{67c23} flies at a concentration of 400 μg/ml. Flies carrying the insertion were identified by rescue of the *white* phenotype. The pryRP1 and pryRP2 plasmids were injected into y^{-} ac^{-}; ry^{506} flies at a concentration of 400 μg/ml. Transformed flies were identified by rescue of the *rosy* phenotype. The helper plasmid pπ25.7 wc was used in all of the injections at a concentration of 100 μg/ml (KARESS and RUBIN 1984). Inserts in transgenic flies were made homozygous and mapped genetically by crossing to appropriately marked strains.

Histochemical analysis of β-galactosidase: Embryos from the phs43RP1- and phs43RP2-transformed lines were stained for β-galactosidase activity in the presence of 0.3% Triton as described in BELLEN *et al.* (1989). Embryos were mounted in 50% glycerol and examined under the light microscope. Third instar larvae and adults were dissected, fixed with glutaraldehyde and stained with X-gal (5-bromo-4-chloro-3-indoyl β-D-galactosidase) according to the method of GLASER *et al.* (1986). All samples shown within each figure were stained simultaneously and for the same length of time; larval tissues were stained overnight, whereas adult samples were stained for 5 hr. Tissues were mounted in 50% glycerol/ 50% PBS and photographed with Ektachrome T-160 film. Adult males and females (1 day old) were aged for several days (3–4 days) on yeasted vials to allow maturation of adult fat body and ovaries to occur. To control for position effects, three fly lines from either the pgypCaSpeR insertion or the phs43RP1 and phs43RP2 insertions were used in the analysis of *su(Hw)* mutations. Because the *su(Hw)* gene is located on the third chromosome, only those fly lines containing the pgypCaSpeR or the phs43RP1 and phs43RP2 insertions on either the *X* or the second chromosome were used. For the pgypCaSpeR construct, transformed lines *P[gypCaSpeR 5]*, *P[gypCaSpeR 68]* and *P[gypCaSpeR 55]* were used, whereas for the phs43RP1 and phs43RP2 constructs the lines used were *P[hs43RP1, 25.4]*, *P[hs43RP1, 37.2]* and *P[hs43RP2, 12.2]*. For the analysis of the $su(Hw)^{V}$ *P[CaSX/K 5.3]* and $su(Hw)^{E8}$ alleles, flies carrying these genotypes were crossed to flies of the genotype $su(Hw)^{V}$/ *TM6B*. Mutant larvae were selected as Tb^{+} and mutant adults were selected as Hu^{+}. For the analysis of the $su(Hw)^{\Delta 100}$, $su(Hw)^{NoAD}$ and $su(Hw)^{\Delta 283}$ alleles, mutant larvae and adults were selected as described above.

The genotypes of wild-type and *su(Hw)* mutant flies pictured in Figures 3–6 are as follows: $y^{1}w^{67c23}$ *P[gypCaSpeR 55]*; +; + *(wild-type)*; $y^{2}w^{67c23}$ *P[gypCaSpeR 55]*; +; $su(Hw)^{V}/su(Hw)^{V}$ *P[CaSX/K 5.3]*; $y^{2}w^{67c23}$ *P[gypCaSpeR 55]*; +; $su(Hw)^{E8}/su(Hw)^{V}$; $y^{2}w^{67c23}$ *P[gypCaSpeR 55]*; $P[su(Hw)^{\Delta 100}$; $su(Hw)^{V}$; $y^{2}w^{67c23}$ *P[gypCaSpeR 55]*; +; $su(Hw)^{J}$; $y^{2}w^{67c23}$ *P[gypCaSpeR 55]*; +; $P[su(Hw)^{NoAD}]$ $su(Hw)^{V}$ and $y^{2}w^{67c23}$ *P[gypCaSpeR 55]* $P[su(Hw)^{\Delta 283}]$; +; +. The genotypes of wild-type and *su(Hw)* mutant flies pictured in Figure 8 are as follows: $y^{1}w^{67c23}$ *P[hs43RP1 44.1]* (not mapped); y^{-} ac^{-}; *P[ryRP1 37.2]*; ry^{506} (wild-type salivary glands); y^{2}; *P[ryRP1 37.2]*; $su(Hw)^{V}/su(Hw)^{V}$ *P[CaSX/K 5.3]* and y^{2}; *P[ryRP1 37.2]*; $su(Hw)^{E8}/su(Hw)^{V}$.

RESULTS

***Gypsy* is expressed in a tissue-specific manner throughout development:** The *gypsy* retrotransposon encodes a 7.0-kb mRNA that is expressed at different stages of Drosophila development (PARKHURST and CORCES 1985, 1986). To examine the tissue-specific distribution of *gypsy* RNA, we carried out *in situ* hybridizations to *gypsy* transcripts in ovaries, embryos, third instar larvae and adults. Hybridization of a strand-specific *gypsy* RNA probe to whole mount ovaries shows that there may be some expression in the earlier stages of oogenesis, but the signal is barely detectable with this method. However, by stage 8 of oogenesis, *gypsy* RNA is expressed in the nurse cell cytoplasm and in the oocyte (Figure 1A). This pattern of expression continues through stage 9 (data not shown). In stage 10 egg chambers (Figure 1B), there is maximal *gypsy* RNA accumulation in the nurse cell cytoplasm, the oocyte and the follicle cells. The presence of *gypsy* RNA in the oocyte cannot be detected by *in situ* hybridization after stage 13 (Figure 1B). *Gypsy* RNA was not found during the early stages of embryogenesis when *in situ* hybridizations were performed with whole-mount embryos (data not shown). However, in the later stages of embryogenesis, *gypsy* RNA is expressed in the salivary glands, the foregut and the gonads (Figure 1, C and D). At this stage, expression can also be observed in the embryonic fat body in a pattern similar to that seen for the Drosophila GATA family member box-A binding factor (ABF) (ABEL *et al.* 1993).

In third instar larvae, *gypsy* RNA is localized to the eye-antenna, wing (Figure 1E), haltere and leg imaginal discs (Figure 1F) and in the larval fat body (Figure 1G). *Gypsy* RNA expression can be seen throughout the wing disc with seemingly more intense expression present in the anterior cells (Figure 1E). *Gypsy* RNA is uniformly

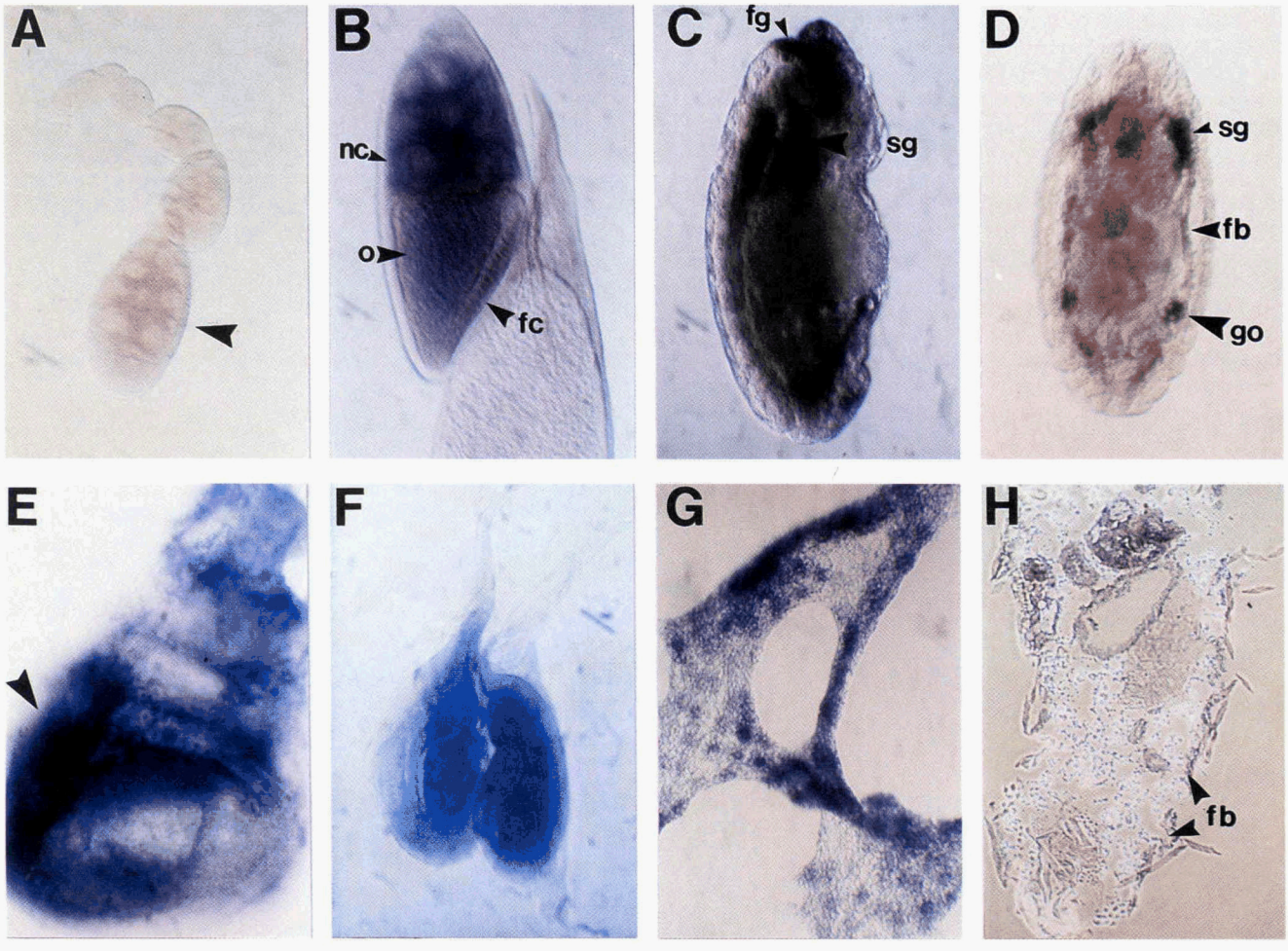

FIGURE 1.—Distribution of *gypsy* RNA during development. *In situ* hybridization to whole mount embryos, larval tissues and adult ovaries and to adult female frozen sections are shown. (A) Early stages of oogenesis. Stage 8 egg chamber is denoted by the arrowhead. (B) Late stages of oogenesis. Egg chambers at stages 10 (top) and 13 (bottom) are shown. Nurse cell (nc), oocyte (o), follicle cells (fc). (C) Lateral view of a late stage 14 embryo. Foregut (fg), salivary gland (sg). (D) Horizontal view of stage 14 embryo. Salivary gland (sg), fat body (fb), gonad (go). (E) Wing imaginal disc from a late third instar larva. The disc is oriented so that anterior is to the left, as indicated by the arrowheads. (F) Pair of leg discs from a third instar larva. (G) Fat body of a third instar larva. (H) Parasagittal section of adult female abdomen. Fat body (fb). The whole mount egg chambers, embryos and adult abdominal section are oriented so that anterior is up.

localized throughout the leg discs (Figure 1F) and the larval fat body (Figure 1G). A low amount of *gypsy* expression was also seen in a small percentage of the salivary glands probed with a *gypsy* riboprobe in the *in situ* hybridization experiments (data not shown), suggesting that *gypsy* expression in the larval salivary glands is very low and cannot be detected reproducibly. In addition to the expression of *gypsy* RNA in adult ovaries, *in situ* hybridization to frozen sections of newly enclosed adult females indicates that *gypsy* RNA also accumulates in the fat body (Figure 1H). Hybridization was not detected in any other adult tissues. These results indicate that *gypsy* RNA is transcribed in a tissue-specific manner during the embryonic, larval and adult stages of development.

Sequences within the 5′ LTR and the transcribed, untranslated region are required for proper tissue-specific expression of *gypsy*: To determine how the *su(Hw)* protein affects the expression of a single *gypsy* element, we constructed a plasmid containing *gypsy* sequences and a reporter gene that could mimic *gypsy* expression *in vivo*. To this end, a *Hpa*I-*Bst*XI fragment of *gypsy* extending from nucleotides 1 to 1077 was fused to the *E. coli lacZ* gene in a *P*-element vector (Figure 2). This 1.1-kb region should contain most of the transcriptional regulatory sequences, because it includes the 5′ LTR, which contains the *gypsy* promoter (MARLOR *et al.* 1986; JARRELL and MESELSON 1991), and the 5′ transcribed untranslated leader, which contains the *su(Hw)*-binding region and a palindromic sequence homologous to the *lac*-operator of *E. coli* (MAZO *et al.* 1989). The 3′ end of the *Hpa*I-*Bst*XI *gypsy* sequence ends immediately upstream of the translation start site. This construct was named pgypCaSpeR and injected into *y* w^{67c23} flies; eight transformed fly lines were obtained.

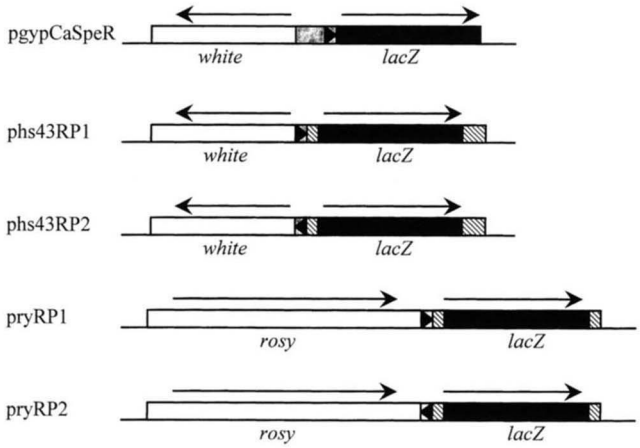

FIGURE 2.—Diagrams of *gypsy-lacZ* constructs. The constructs used to generate the transgenic flies discussed in the text are diagrammed below. □, either the *white* or *rosy* gene as indicated. ▨, *gypsy* sequences located in the 5′ end of the retrotransposon. These sequences include the 5′ LTR, the transcribed untranslated region containing 12 *su(Hw)* binding sites in pgypCaSpeR and the *su(Hw)*-binding region in the rest of the plasmids. This region is represented by an arrow head indicating its orientation in the plasmid with respect to its orientation in the *gypsy* element. ■, *E. coli lacZ* reporter gene. ▧, *hsp70* promoter and SV40 polyadenylation sequences used in the phs43RP1, phs43RP2, pryRP1 and pryRP2 plasmids. *P*-element sequences are denoted by thin horizontal lines flanking each plasmid. The horizontal arrows located above the schematic diagrams of the plasmids indicate the direction of transcription of the respective genes.

We carried out a developmental analysis using a histochemical assay for β-galactosidase activity with third instar larvae and adults of transformed lines carrying a wild-type *su(Hw)* background. All three transformants examined showed the same pattern of β-galactosidase staining, and only slight quantitative differences were observed between different transformed lines. These differences were consistent in the different tissues and developmental stages examined. The β-galactosidase expression pattern obtained with third instar larvae homozygous for the pgypCaSpeR insertions is very similar to the expression pattern of *gypsy* RNA. As shown in Figures 3 and 4, β-galactosidase activity can be seen in the fat body, the imaginal discs and, to a lesser extent, in the salivary duct and glands. Because only very low levels of *gypsy* RNA can be detected in salivary glands by *in situ* hybridization, the higher level of *gypsy* expression in the salivary glands detectable in fly lines transformed with pgypCaSpeR might be because the stability of the β-galactosidase protein.

We also determined whether adult flies transformed with pgypCaSpeR showed a β-galactosidase expression pattern similar to the *gypsy* RNA pattern observed in adults as detected by *in situ* hybridization. Both adult males and females were dissected, fixed and stained for *lacZ* expression. β-galactosidase activity was found in the ovaries (Figure 5) and fat bodies (Figure 6) of 3–5-day-old adult females carrying a wild-type *su(Hw)* background. No β-galactosidase activity was detected in males, suggesting that the regulation of *gypsy* expression in adult fat bodies is sex-specific (data not shown). These results correlate well with data obtained from the *in situ* hybridization experiments because *gypsy* RNA expression was detected in the same tissues (Figure 1, A, B and H). Thus, the 1.1-kb fragment of *gypsy* present in the pgypCaSpeR construct contains the *cis*-elements required for the proper spatial and temporal expression of this retrotransposon.

Effect of *su(Hw)* mutations on *gypsy* expression during larval development: To study the role of specific structural domains of *su(Hw)* protein on *gypsy* expression, several *su(Hw)* mutations were crossed into three different fly lines homozygous for pgypCaSpeR insertions. The effects observed were the same for all three transformants examined and only results from one of them will be shown here. To show that the observed β-galactosidase expression pattern was dependent on the presence of a functional *su(Hw)* protein, *lacZ* expression was analyzed in transformed flies containing the $su(Hw)^{V}$ and $su(Hw)^{E8}$ backgrounds. The $su(Hw)^{V}$ allele is caused by a deletion of the N-terminus of the *su(Hw)* gene and the adjacent *RpII15* gene encoding a subunit of RNA polymerase II (Figure 7) (HARRISON *et al.* 1992); flies carrying this mutation do not accumulate detectable levels of the *su(Hw)* RNA or protein (HARRISON *et al.* 1993). The $su(Hw)^{E8}$ mutation is caused by a C to T transition in the seventh zinc finger, resulting in the replacement of a histidine with a tyrosine residue and the inability of the protein to bind DNA (Figure 7) (HARRISON *et al.* 1993). When flies homozygous for $su(Hw)^{V}$ are stained for β-galactosidase expression, a dramatic decrease in enzyme activity can be seen in the larval fat body (Figure 3) and in the imaginal discs and salivary glands (Figure 4). These results indicate that the *su(Hw)* protein is necessary for the activation of *gypsy* expression during larval development.

The *su(Hw)* protein has 12 copies of the zinc finger motif. Mutations in this domain of the protein disrupt the ability of *su(Hw)* to bind DNA (HARRISON *et al.* 1993). To test whether this domain is also important for activation of *gypsy* transcription, we analyzed the effect of the $su(Hw)^{E8}$ allele on the β-galactosidase staining pattern in larvae. No *lacZ* expression was observed in larvae containing a $su(Hw)^{E8}/su(Hw)^{V}$ background (Figures 3 and 4). Thus, binding of the *su(Hw)* protein to *gypsy* DNA is necessary for proper regulation of *gypsy* expression. The amount of β-galactosidase staining in the fat body of larvae containing a $su(Hw)^{E8}/su(Hw)^{V}$ genotype is lower than that seen in the $su(Hw)^{V}$ mutant (Figure 3). The basal amount of *lacZ* expression present in the fat body of the $su(Hw)^{V}$-transformed line shown in Figure 3 is probably because of position effects in

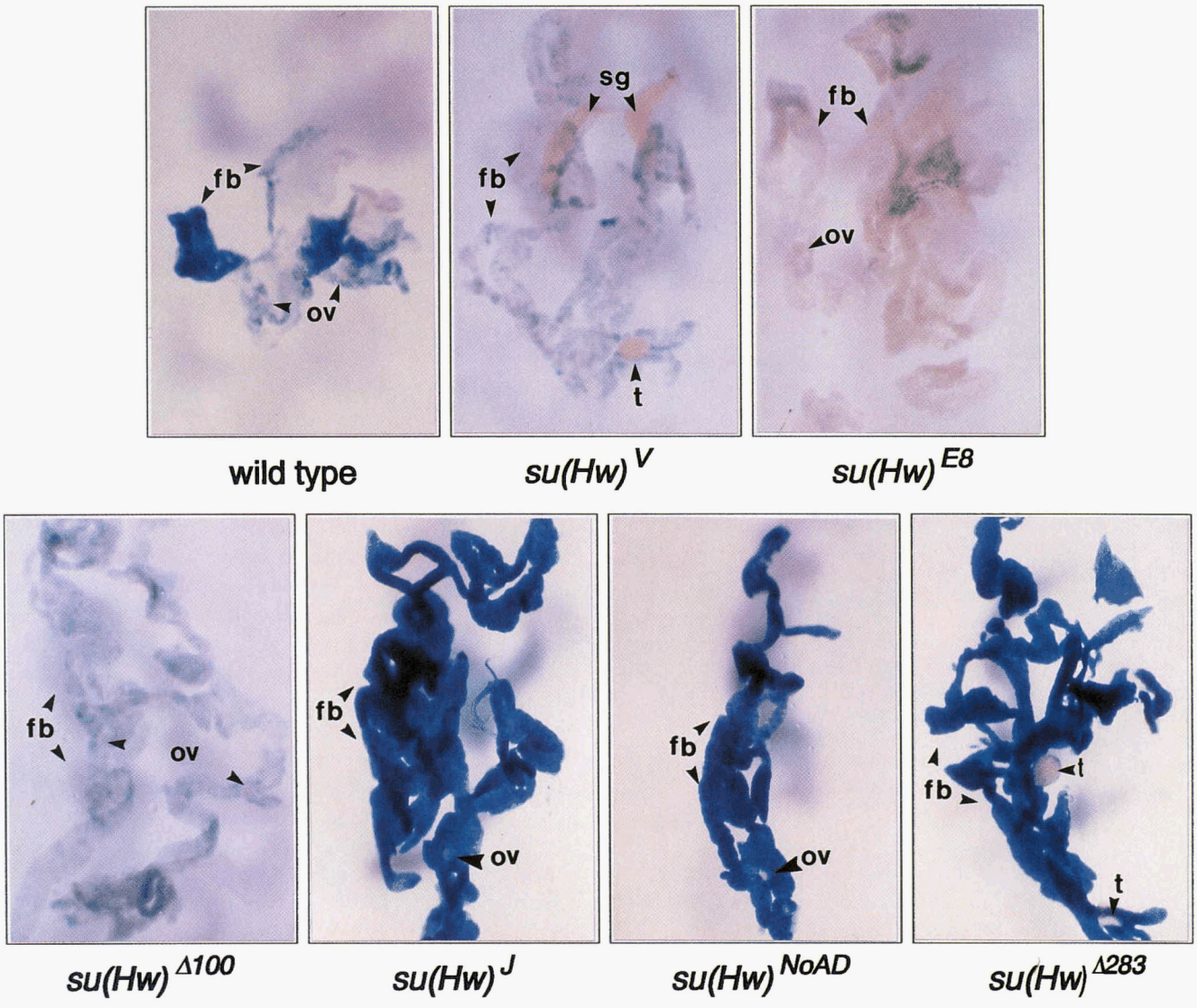

FIGURE 3.—β-galactosidase expression in fat bodies from wild-type and mutant *su(Hw)* larvae. Third instar larvae transformed with pgypCaSpeR were dissected, fixed and stained with X-gal. Fat bodies (fb) dissected from wild-type larvae and larvae containing *su(Hw)* mutant backgrounds are shown. The specific *su(Hw)* allele that was used is indicated below each panel. Background staining in the pericardial cells can be seen in some cases. Salivary glands (sg), larval ovaries (ov) and testes (t) can be seen in some of the panels; none of these tissues are stained with X-gal.

this particular transformant, because the other two pgypCaSpeR lines analyzed showed no β-galactosidase expression in a homozygous $su(Hw)^V$ background (data not shown).

Interestingly, the *su(Hw)* protein is ubiquitously expressed in nuclei throughout development (HARRISON *et al.* 1993). This suggests that *gypsy* is expressed in a tissue-specific manner because the *su(Hw)* protein is in a functionally active form in a subset of tissues or because it interacts with other proteins that are localized to specific tissues. In fact, the *su(Hw)* protein contains domains that are involved in protein-protein interactions. For example, this protein contains a region showing strong homology to the helix 2-coiled coil region of bHLH-Zip proteins (HARRISON *et al.* 1993). To test the role of this region of *su(Hw)* in *gypsy* expression, we analyzed the effect of the $su(Hw)^{\Delta 283}$ mutant in which 19 of 32 amino acid residues have been deleted from the leucine zipper motif. When larvae of the genotype $su(Hw)^{\Delta 283}$ containing the pgypCaSpeR insertion are assayed for β-galactosidase, there is a dramatic increase in activity in the fat body, a slight increase in the salivary glands, but no substantial increase in expression can be seen in the imaginal discs (Figures 3 and 4). Typically, leucine zipper domains are thought to mediate protein dimerization (LANDSCHULZ *et al.* 1988; O'NEIL *et al.* 1991) but because the *su(Hw)* protein migrates as a monomer in gel filtration columns (HARRISON *et al.*

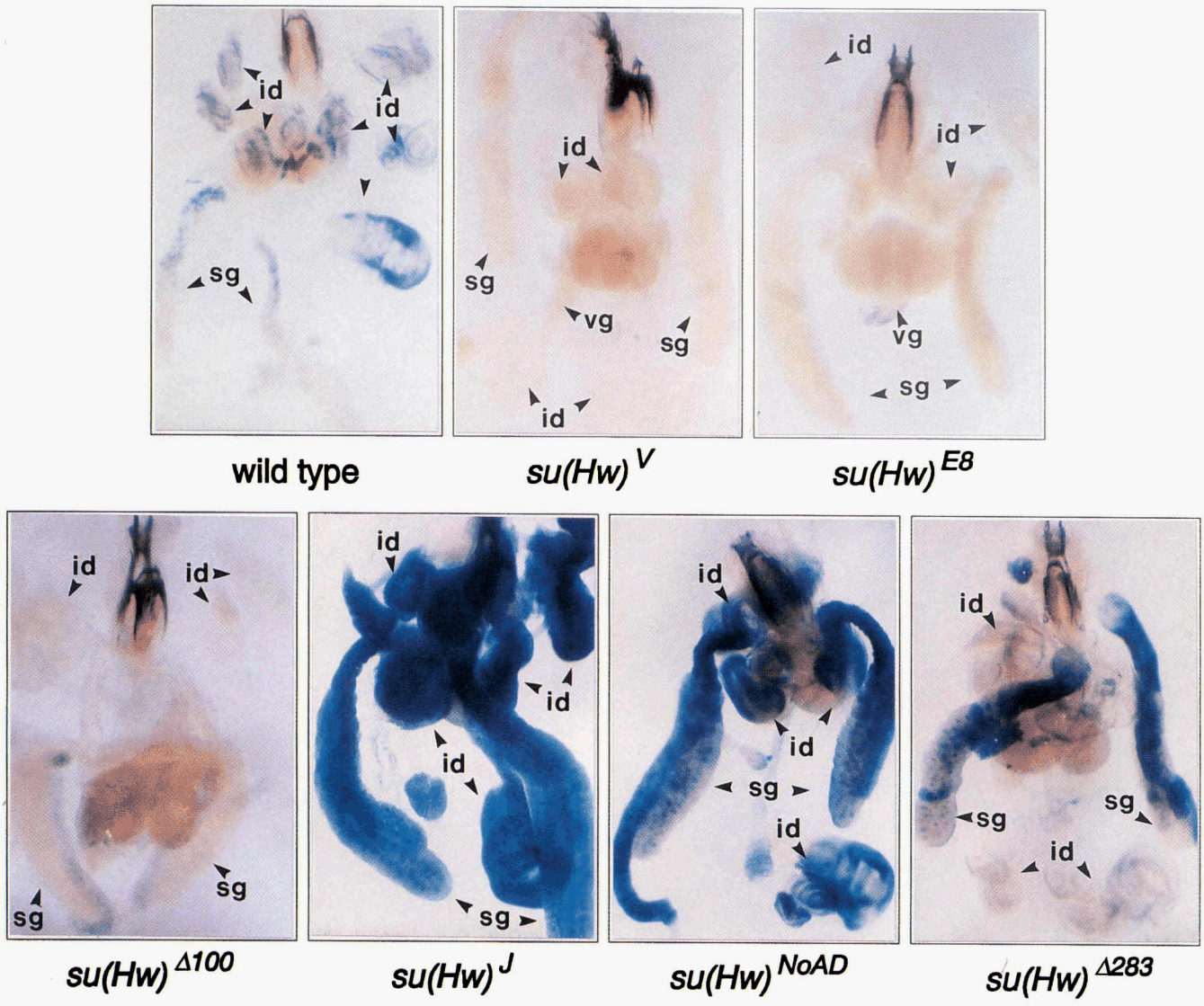

FIGURE 4.—β-galactosidase staining pattern in imaginal discs and salivary glands from wild-type and mutant *su(Hw)* larvae. Imaginal discs (id) and salivary glands (sg) from the same larvae transformed with pgypCaSpeR from which fat bodies were obtained in Figure 3 were dissected, fixed and stained with X-gal. The allelic state of *su(Hw)* is indicated below each panel.

1993), this region may be used to interact with other proteins to regulate *gypsy* expression in the larval fat body and salivary glands. This domain does not seem to be essential for imaginal disc expression, suggesting a different requirement for the leucine zipper in different tissues, perhaps due to the tissue-specific localization of proteins that interact with this domain of *su(Hw)*.

The *su(Hw)* protein contains a large acidic domain in the amino terminal region and a second minor one in the C-terminus (PARKHURST *et al.* 1988; HARRISON *et al.* 1993). The acidic domain present in the C-terminus is not present in the *su(Hw)* protein from other Drosophila species, suggesting that it might not play an essential role in *su(Hw)* function (HARRISON *et al.* 1993). Acidic domains have been shown to be important for transcriptional activation (MA and PTASHNE 1987; HOPE *et al.* 1988; CRESS and TRIEZENBERG 1991). We have used the $su(Hw)^{\Delta 100}$ allele to address the question of whether the amino-terminal acidic domain of *su(Hw)* is involved in the regulation of *gypsy* expression. The $su(Hw)^{\Delta 100}$ mutation contains an in-frame deletion of the 48 amino acids that constitute the amino-terminal acidic domain (Figure 7) (HARRISON *et al.* 1993). Flies homozygous for the pgypCaSpeR insertion and the $su(Hw)^{\Delta 100}$ allele show a large decrease in β-galactosidase expression in the fat body (Figure 3) and in the imaginal discs and salivary glands (Figure 4). This result suggests that the N-terminal acidic domain of *su(Hw)* plays an important role in the activation of *gypsy* transcription.

To probe the function of the carboxy-terminal domain of the *su(Hw)* protein in *gypsy* expression, the

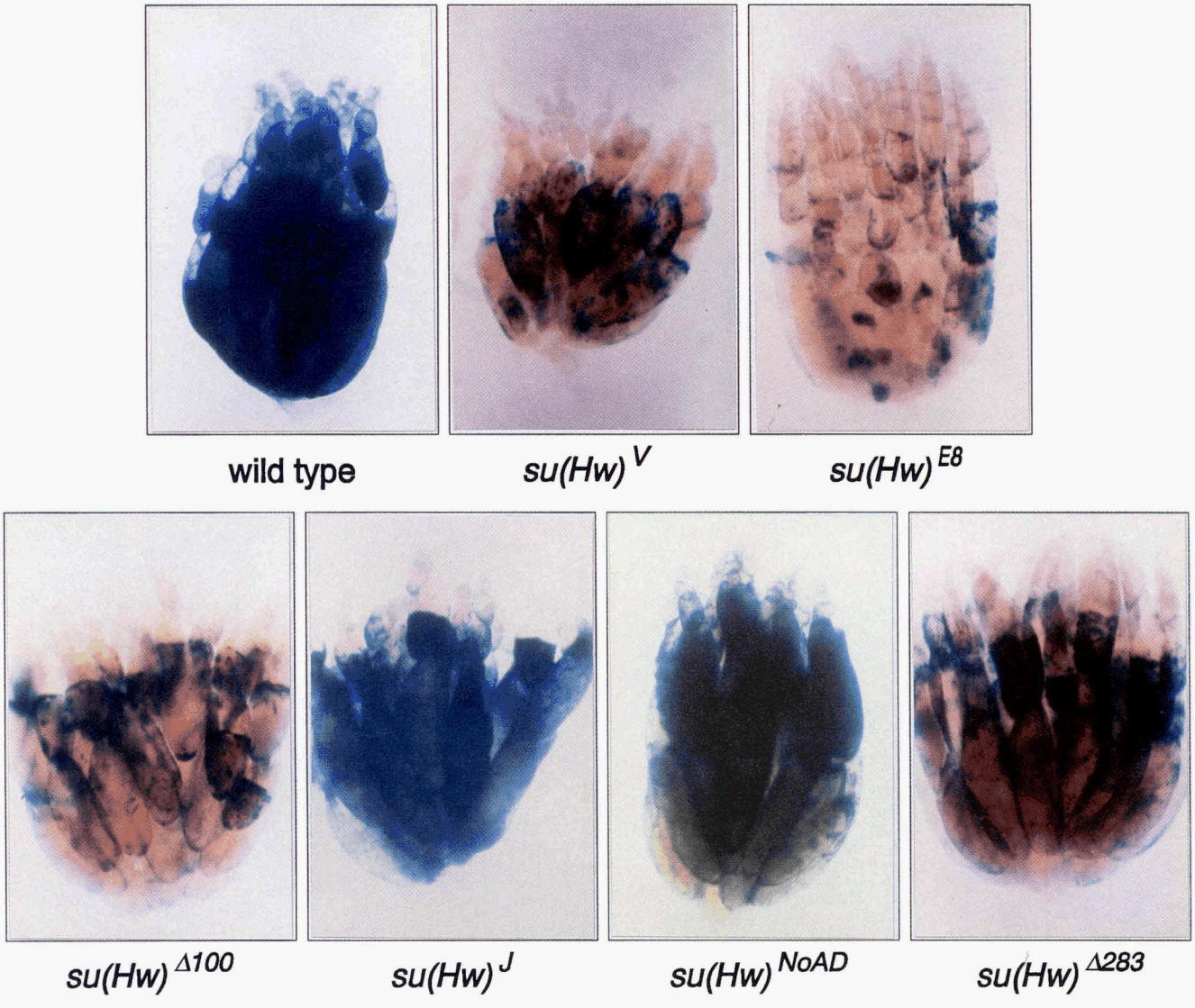

FIGURE 5.—β-galactosidase staining pattern in ovaries from wild-type and mutant *su(Hw)* adult females. Whole ovaries stained with X-gal were dissected from the pgypCaSpeR-transformed females displayed in Figure 6. The allelic state of *su(Hw)* is indicated below each panel. The ovaries are oriented such that the anterior regions of the egg chambers are at the top and the posterior regions of the egg chambers are at the bottom.

$su(Hw)^J$ allele was crossed into flies homozygous for the pgypCaSpeR insertion. The *su(Hw)* protein encoded by this allele lacks the terminal 149 residues, including the carboxy-terminal acidic domain, and a large hydrophobic region adjacent to the leucine zipper domain that is very well conserved among the *su(Hw)* proteins from different Drosophila species (Figure 7) (HARRISON *et al.* 1993). In agreement with results obtained from the deletion of the neighboring leucine zipper, there is a large increase in β-galactosidase expression in the fat body, imaginal discs and salivary glands when the carboxy-terminal region of *su(Hw)* is deleted (Figures 3 and 4). This result suggests that the carboxy-terminal portion of the *su(Hw)* protein might participate with the leucine zipper region in interactions with other proteins that negatively regulate *gypsy* expression. Simultaneous deletion of both the amino and carboxy terminal regions of the protein in the $su(Hw)^{NoAD}$ mutation results in an increase in the amount of β-galactosidase activity in the fat body, salivary glands and imaginal discs (Figures 3 and 4), but the increase is lower than that seen in the $su(Hw)^J$ background. This result suggests an additive effect of the amino- and carboxy-terminal deletions on the regulation of *gypsy* RNA expression.

The acidic domain and leucine zipper region are essential for *gypsy* expression in the adult ovaries: We have also analyzed the effect of various structural domains of *su(Hw)* on the expression of the pgypCaSpeR reporter gene in ovaries. Very little β-galactosidase expression was observed in ovaries dissected from 3–5-

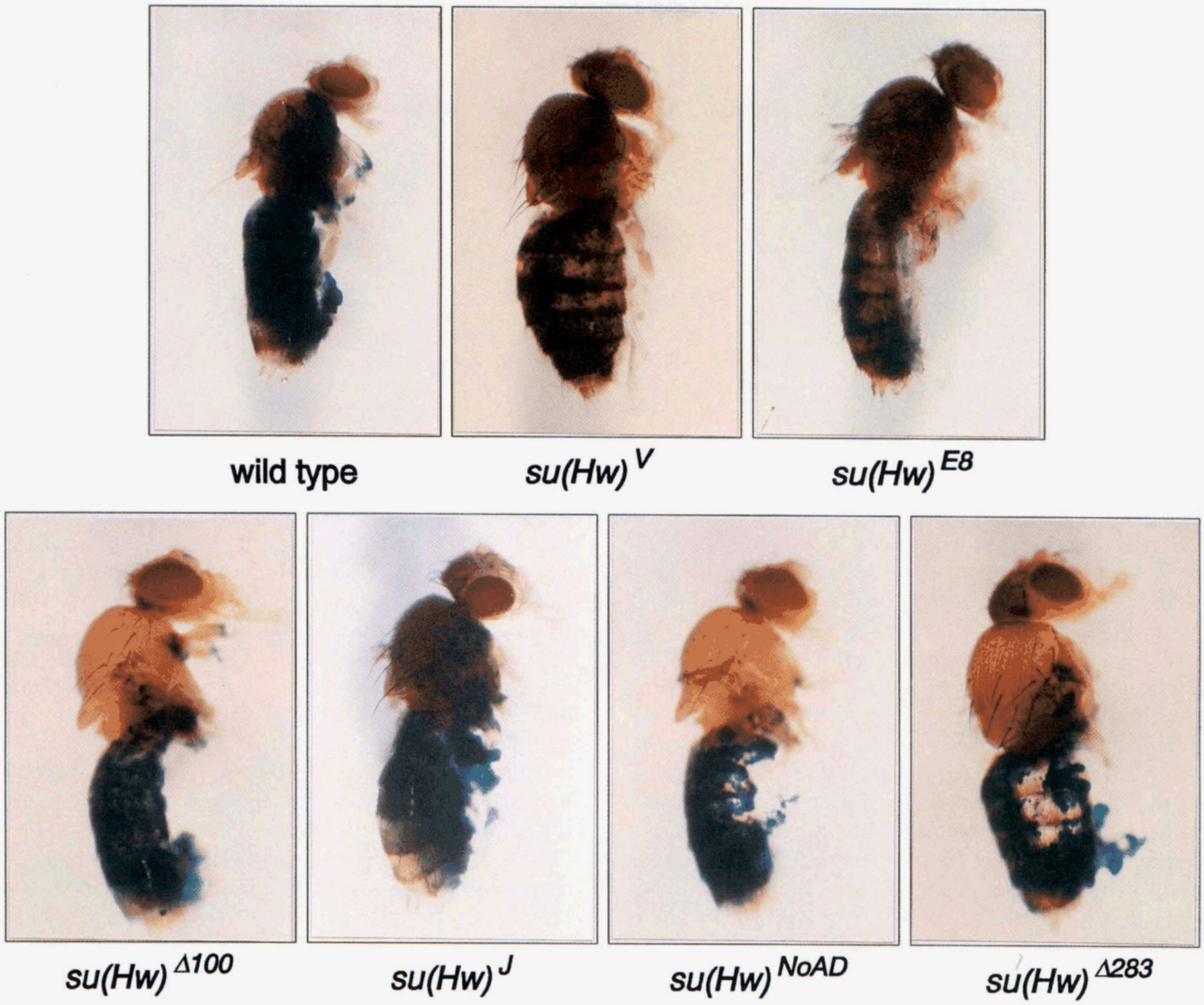

FIGURE 6.—β-galactosidase expression in fat bodies from wild-type and mutant *su(Hw)* adult females. Three- to 5-day-old adult females transformed with the pgypCaSpeR plasmid were dissected, fixed and stained with X-gal. The females contain either a wild-type or a mutant *su(Hw)* background as indicated below each panel. β-galactosidase expression can be seen in the fat body present in the abdomen and thorax.

day-old females homozygous for the null allele $su(Hw)^V$ (Figure 5). A similar situation was seen with ovaries from $su(Hw)^{E8}/su(Hw)^V$ females (Figure 5), indicating that *gypsy* expression is dependent on the *su(Hw)* protein and its ability to bind to DNA. Ovaries obtained from transformed pgypCaSpeR flies carrying a homozygous $su(Hw)^{\Delta 100}$ mutation showed a large decrease in *lacZ* activity (Figure 5). This result indicates that the amino-terminal acidic domain also plays an important role in transcriptional activation of *gypsy* expression in ovaries, perhaps through interactions with other proteins present in the transcription complex. However, when the carboxy-terminal region is missing as in the $su(Hw)^J$ allele, there is a very slight decrease in the amount of expression in ovaries obtained from flies homozygous for this mutation (Figure 5). Unlike the results obtained with larvae, the carboxy-terminal domain does not play an important role in *gypsy* expression in ovaries. There is a decrease in *lacZ* expression in ovaries obtained from flies homozygous for the $su(Hw)^{NoAD}$ allele (Figure 5). Because the absence of the carboxy-terminal region has no effect on *gypsy* expression, the reduction observed in $su(Hw)^{NoAD}$ flies is presumably due to the deletion of the amino-terminal acidic domain. Finally, ovaries obtained from flies transformed with pgypCaSpeR carrying the $su(Hw)^{\Delta 283}$ mutation, in which a portion of the leucine zipper has been deleted, show a decrease in β-galactosidase activity comparable to that seen with ovaries from females homozygous for $su(Hw)^{\Delta 100}$ (Figure 5). Thus, the leucine zipper

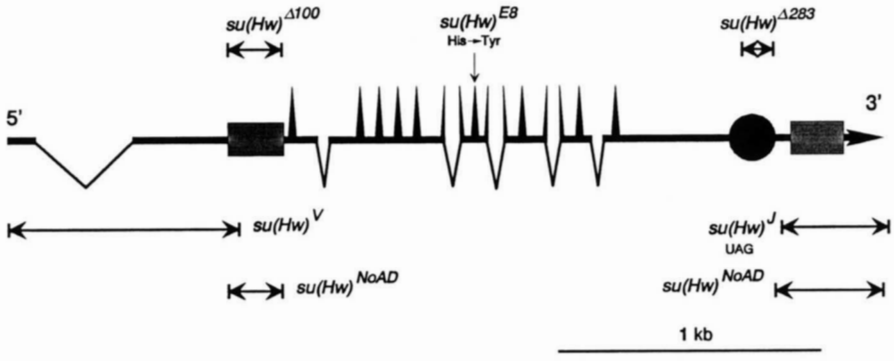

FIGURE 7.—Diagram of *su(Hw)* mutations. The structure of the *su(Hw)* gene and the lesions defining the *su(Hw)* mutations described in the text are shown. The solid spikes represent the zinc fingers. ■, position of the acidic domains; ●, leucine zipper. The horizontal arrows above and below the schematic diagram of the *su(Hw)* transcript indicate those regions missing from the encoded protein in the specified mutation. The region deleted in the $su(Hw)^{V}$ allele includes the portion of the *su(Hw)* gene indicated and extends 1.7 kb into the distal sequences (HARRISON *et al.* 1993). The vertical arrow indicates the position and nature of the missense mutation in the $su(Hw)^{E8}$ allele.

domain is important for the proper expression of *gypsy* in the ovaries, but it mediates activation of *gypsy* transcription, rather than the repression observed in larval tissues.

The *su(Hw)* protein regulates *gypsy* expression in the adult fat body: To determine the effect of the different structural domains of the *su(Hw)* protein on *gypsy* expression in the adult fat body, 3–5-day-old females from a strain carrying the pgypCaSpeR construct were dissected, fixed and stained for β-galactosidase activity. Analysis of *gypsy* expression in the fat body was complicated by the fact that there seems to be little adult fat body in transformed females carrying the genotypes $su(Hw)^{V}$ and $su(Hw)^{E8}/su(Hw)^{V}$. The lack of this tissue in adult females could be due to a direct effect of *su(Hw)* protein on fat body development. The fat body that is present is very lightly stained (Figure 6). Thus, the presence of a functional *su(Hw)* protein capable of binding to DNA might be necessary for the development of the fat body in adult females, and the fat body that develops in its absence accumulates very low levels of *gypsy* RNA.

A decrease in the amount of fat body was also seen in transformed females carrying the $su(Hw)^{\Delta 100}$ and $su(Hw)^{NoAD}$ alleles, but the amount of β-galactosidase expression in the fat body of these females is similar to that of wild type (Figure 6). Females carrying the $su(Hw)^{J}$ mutation contain an amount of fat body and β-galactosidase expression comparable to that of wild type (Figure 6). These results suggest that neither the amino- nor the carboxy-terminal regions of the *su(Hw)* protein are important for the expression of *gypsy* in the adult female fat body. This conclusion is supported by the fact that the amount of β-galactosidase expression in pgypCaSpeR-transformed flies carrying the $su(Hw)^{NoAD}$ allele is similar to wild type. A similar situation was seen with fat body from flies homozygous for the pgypCaSpeR insertion and the $su(Hw)^{\Delta 283}$ allele. The level of β-galactosidase activity in the fat body was similar to that seen in transformed flies carrying a wild-type *su(Hw)* chromosome, but the amount of fat body appeared lower in $su(Hw)^{\Delta 283}$ flies (Figure 6). This indicates that the leucine zipper domain is not important for *su(Hw)* function in the expression of *gypsy* in the adult fat body.

The *su(Hw)*-binding region acts as a tissue-specific enhancer in embryos and in larvae: Because binding of the *su(Hw)* protein to DNA is necessary for the expression of the pgypCaSpeR construct, we decided to investigate whether the *su(Hw)*-binding site by itself would be sufficient to elicit the proper tissue-specific pattern of *gypsy* expression. In fact, the *su(Hw)*-binding region contains several copies of a sequence homologous to the octamer motif present within mammalian transcriptional enhancers (SPANA *et al.* 1988). If this region of *gypsy* could act as a tissue-specific enhancer, it would suggest that the *su(Hw)* protein acts at the level of transcription initiation to regulate *gypsy* expression. To determine whether this was the case, the *su(Hw)*-binding region was cloned in both possible orientations upstream of an *hsp70* promoter fused to the *E. coli lacZ* reporter gene (Figure 2). These reporter genes were cloned into either the CaSpeR vector (PIRROTTA *et al.* 1985) to give rise to plasmids phs43RP1 and phs43RP2 or the Carnegie 20 vector (RUBIN and SPRADLING 1983) to give rise to plasmids pryRP1 and pryRP2. All four plasmids were then introduced into the Drosophila germ line by *P*-element–mediated transformation. A total of 17 independent fly lines were obtained with the phs43RP1 and phs43RP2 constructs, and 19 fly lines were obtained with the pryRP1 and pryRP2 plasmids.

Embryos transformed with phs43RP1 and phs43RP2 were stained for *lacZ* activity. As shown in Figure 8A, embryos show β-galactosidase expression in the salivary

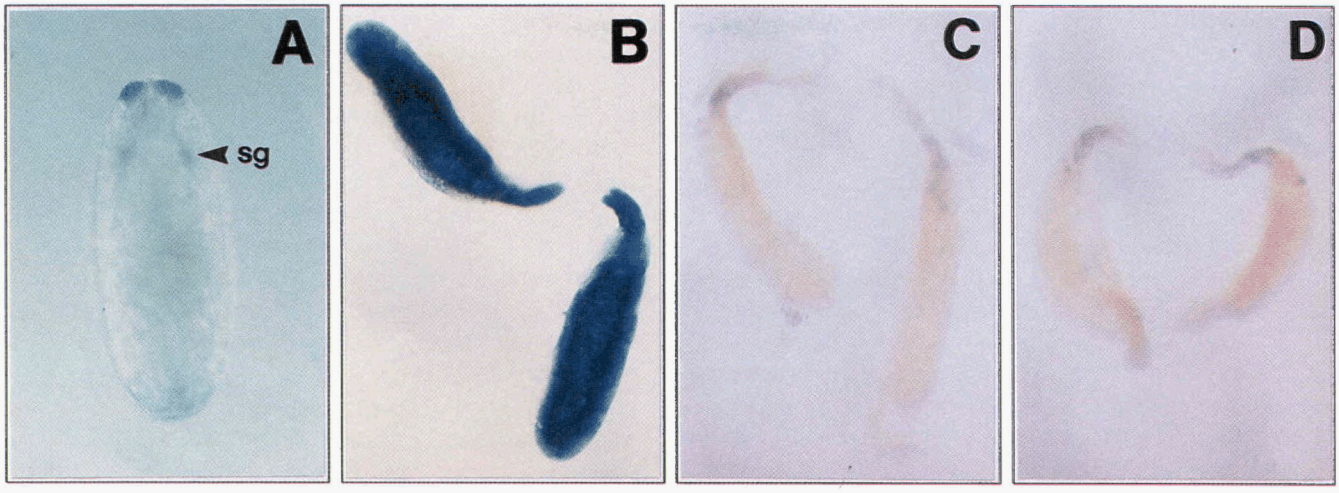

FIGURE 8.—Tissue-specific enhancer activity of *su(Hw)*-binding region. Embryos and larvae transformed with constructs in which the *su(Hw)*-binding region was cloned upstream of an *hsp70-lacZ* fusion were stained with X-gal. (A) Horizontal view of a late stage whole mount embryo stained with X-gal. The location of the salivary glands (sg) is indicated by an arrowhead. Anterior is up. There is additional staining in the posterior spiracles and in the maxillary regions flanking the mouth hooks. (B) Salivary glands dissected from a transformed third instar larva with a wild-type *su(Hw)* genotype. (C) Salivary glands dissected from a transformed third instar larva with a homozygous $su(Hw)^{V}$ genotype. (D) Salivary glands dissected from a transformed third instar larva with a $su(Hw)^{E8}/su(Hw)^{V}$ genotype.

glands similar to that seen in *in situ* hybridization experiments during the later stages of embryogenesis (Figure 1, C and D). The same results were obtained regardless of the orientation of the *su(Hw)*-binding region within the plasmid. β-galactosidase expression was also seen in the salivary glands of third instar larvae transformed with either the phs43RP1 and phs43RP2 plasmids (data not shown) or the pyrRP1 and pryRP2 plasmids (Figure 8B). However, no expression was seen in either the fat body or the imaginal discs (data not shown). To show that the β-galactosidase expression pattern in larvae is dependent on the presence of a functional *su(Hw)* protein, the $su(Hw)^{V}$ and $su(Hw)^{E8}$ mutations were crossed into fly lines homozygous for the pryRP1 and pryRP2 insertions. β-galactosidase activity was absent from the salivary glands of transformed third instar larvae containing a homozygous $su(Hw)^{V}$ background (Figure 8C). The binding of *su(Hw)* protein to this *gypsy* sequence is necessary for activation, because no β-galactosidase activity can be seen in salivary glands from transformed larvae carrying a $su(Hw)^{E8}/su(Hw)^{V}$ background (Figure 8D). Finally, no tissue-specific expression was seen in either the adult fat body or ovaries of flies transformed with the *su(Hw)* binding region *hsp70-lacZ* fusion constructs (data not shown). These data indicate that the *su(Hw)*-binding region acts as a tissue-specific enhancer in embryos and in larvae and suggests that the *su(Hw)* protein acts at the level of transcription initiation. However, this sequence cannot by itself reproduce the spatial localization of *gypsy* RNA in every stage of development, implying that additional *gypsy* sequences are required for expression in the imaginal discs, the larval and adult fat body and adult ovaries.

DISCUSSION

Here we describe experiments that were initiated in an effort to determine the role of *su(Hw)* in *gypsy* transcription and, ultimately, in the expression of other cellular genes. Northern analysis indicates that mutations in *su(Hw)* result in a 25-fold decrease in the accumulation of *gypsy* full-length RNA (PARKHURST and CORCES 1986). This result could be due to an effect on transcription initiation or on RNA stability. It has also been speculated that the effect of *su(Hw)* on *gypsy* expression might result from premature termination of *gypsy* transcription due to a possible role of *su(Hw)* in the use of the polyadenylation site located in the 5′ LTR of *gypsy* (DORSETT *et al.* 1989; DORSETT 1990). Our results are most consistent with an effect of *su(Hw)* on transcription initiation. If the *su(Hw)* protein was involved in the premature termination of *gypsy* transcription, we would not expect to detect expression of the *lacZ* reporter constructs in a wild-type *su(Hw)* background. Likewise, we would expect to see an increase in *lacZ* expression from the reporter constructs in a mutant *su(Hw)* background.

We have found that *gypsy* is expressed in a remarkably consistent tissue-specific pattern during Drosophila development. RNA for this transposable element is essentially confined to the gonads, salivary glands and fat body during all developmental stages. This complex pattern of expression must arise under the control of different transcription factors that interact with *gypsy* sequences located in the 5′ LTR and transcribed untranslated region. The requirement for factors other than *su(Hw)* is suggested by the finding that the *su(Hw)*-

binding region can only induce expression of a reporter gene in a subset of the tissues and developmental stages where *gypsy* is normally expressed. The fact that *su(Hw)*-binding sites can activate transcription in an orientation-independent and tissue-specific manner when placed upstream of a heterologous promoter is indicative of the role of this sequence as a transcriptional enhancer (ATCHINSON 1988) and implies the involvement of the *su(Hw)* protein in the initiation of transcription of the *gypsy* retrotransposon. The specificity of this effect is underscored by the requirement of a functional *su(Hw)* protein for proper activation of the reporter gene; no expression of the reporter gene is seen in genetic backgrounds containing either no *su(Hw)* protein or a protein that cannot bind to DNA. Although the *su(Hw)*-binding region cannot induce transcription of the reporter gene in all the tissues and times of development when *gypsy* is normally expressed, additional sequences located in the 5′ LTR and transcribed untranslated region of *gypsy* accomplish this effect. This result suggests that other proteins, in addition to *su(Hw)*, interact with *gypsy* sequences to give rise to the complex pattern of temporal and spatial expression of this retrotransposon. This may also explain why *gypsy* is transcribed in a specific pattern even though the *su(Hw)* protein is ubiquitously expressed (HARRISON *et al.* 1993). Thus, the *su(Hw)* protein is necessary but not sufficient for the proper tissue-specific expression of *gypsy*.

Because binding of the *su(Hw)* protein to DNA is necessary for the expression of the reporter gene constructs, it seems likely that proteins might interact directly with *su(Hw)* to induce *gypsy* expression. This conclusion is supported by the nature of the domains of *su(Hw)* necessary for *gypsy* transcription. Specific regions of the protein thought to be crucial for protein-protein interactions are important for the accurate tissue-specific expression of *gypsy*. For example, results obtained with the $su(Hw)^{\Delta 283}$ mutation indicate that a region homologous to the helix 2-coiled coil region of bHLH-Zip proteins is critical for the proper expression of *gypsy* in larvae and in adults. Similarly, the leucine zipper domain was shown to be essential for the repression of *yellow* wing and body enhancers as assayed by the effect of *su(Hw)* mutations on the y^2 phenotype (HARRISON *et al.* 1993). Leucine zipper domains have been shown to be important for protein dimerization (LANDSCHULZ *et al.* 1988; O'NEIL *et al.* 1991). Because the *su(Hw)* protein migrates as a monomer on gel filtration columns (HARRISON *et al.* 1993), it may interact with other proteins that contain leucine zipper domains instead of dimerizing with itself.

Acidic domains function in transcriptional activation and are regions of protein-protein interactions (GILL and PTASHNE 1988; HOPE *et al.* 1988; BERGER *et al.* 1990; CRESS and TRIEZENBERG 1991). The amino-terminal acidic domain of the *su(Hw)* protein is crucial for *gypsy* expression in larval tissues and in adult ovaries. Presumably, proteins required for the activation of *gypsy* expression in these tissues interact with *su(Hw)* through this region of the protein. This result is in contrast to the fact that the amino-terminal acidic domain plays no role in the repression of *yellow* enhancer function (HARRISON *et al.* 1993). We have found that the carboxy-terminal region of *su(Hw)*, however, is required only during larval development. Results obtained with the $su(Hw)^{J}$ mutation suggest that the carboxy-terminal region interacts with proteins that negatively regulate *gypsy* expression. It is not clear whether this effect is due to the loss of the C-terminal acidic domain or rather to the loss of the 66-amino acid hydrophobic region highly conserved among Drosophila species.

None of the *su(Hw)* mutations analyzed in this study affected the pattern of tissue-specific expression of this retrotransposon. Rather, only positive or negative effects on expression in those tissues in which *gypsy* is normally transcribed during the larval and adult stages were observed. One interesting finding of these studies is that particular *su(Hw)* mutations have different effects in larval versus adult tissues. For example, results obtained with the $su(Hw)^{\Delta 283}$ mutation suggest that the leucine zipper domain may interact with a protein that negatively regulates *gypsy* transcription in the larval fat body, whereas results obtained with adult ovaries indicate that the same region of *su(Hw)* interacts with a protein that positively regulates *gypsy* expression in this tissue. These observations may be explained by the fact that *su(Hw)* interacts with different proteins in the two tissues. The particular proteins that interact with *su(Hw)* may be defined by their tissue-specific localization. Another possibility is that separate enhancers exist within the transcribed untranslated region of *gypsy* for the larval salivary glands and fat body and the adult fat body and ovaries. Binding of tissue-specific transcription factors to these enhancers might require the previous binding of *su(Hw)* to its adjacent target sequence. This is suggested by the fact that the *su(Hw)*-binding region acts as a larval salivary gland enhancer but cannot increase transcription in the larval or adult fat body or the adult ovaries.

It has been previously shown that the *su(Hw)* protein plays a role in mediating *gypsy*-induced phenotypes by interfering with the ability of transcriptional enhancers located further from the promoter than the *su(Hw)*-binding sites present in *gypsy* (CORCES and GEYER 1991; JACK *et al.* 1991). The precise mechanism by which *su(Hw)* represses enhancer function has not been determined, but several models have been proposed to explain these results. One possibility is that *su(Hw)* interacts directly with transcription factors bound to enhancer sequences or interferes with either their ability to track down the DNA toward the promoter or

their capacity to loop out intervening sequences in the process of interacting with the transcription complex (GEYER and CORCES 1992). A second explanation proposes that the repressive effect of *su(Hw)* on the expression of adjacent genes is attributable to alterations in chromatin structure due to the establishment of boundaries between higher order domains of chromatin structure (ROSEMAN *et al.* 1993). In either case, the repressive effect of *su(Hw)* on the expression of nearby genes is opposite to the effects that we have observed on *gypsy* expression.

There are precedents for other eucaryotic proteins that act both as activators and repressors of gene expression. For example, the *dorsal (dl)* morphogen of Drosophila activates transcription of genes, such as *twist (twi)* and *snail (sna)*, required in the ventral portion of Drosophila embryos. The same protein represses transcription of genes, such as *zerknullt* (*zen*) and *decapentaplegic* (*dpp*), that are expressed in the dorsal region of the embryo (JIANG *et al.* 1993; KIROV *et al.* 1993). A second well-characterized activator-repressor is the protein encoded by the MCM1 gene in yeast. This protein is a non-cell-specific factor that binds to the promoters of **a**-specific genes in **a** cells and activates their expression. When the α-cell-specific α2 homeodomain protein forms a complex with Mcm1 at the α2-Mcm1 operator, the expression of **a**-specific genes is silenced (KELEHER *et al.* 1988, 1989). Two other yeast proteins, RAP1 and SIN4, have been also shown to be activators and repressors of gene expression and play a role in this process that might be similar to that of *su(Hw)*. RAP1 is a sequence-specific DNA-binding protein that also plays a role in telomere elongation. RAP1 interacts with RIF1 at silencers and telomeres, and the resulting complex then recruits the SIR proteins that alter the chromatin structure at these sites (HARDY *et al.* 1992). SIN4 has also been shown to be an activator and a repressor of gene expression. In SIN4 mutants, plasmids show a decrease in superhelical density, suggesting that the SIN4 protein alters chromatin structure (JIANG and STILLMAN 1992). The *su(Hw)* protein may play a global role in gene expression, activating or repressing transcription by mechanisms similar to those described above. Because the *su(Hw)* protein is ubiquitously expressed throughout development (HARRISON *et al.* 1993), this sequence-specific DNA-binding protein might recruit tissue-specific transcription factors that will then directly interact with the basal transcriptional machinery. The resulting effect on transcription may solely depend on the nature of the proteins that bind to *su(Hw)*.

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