MOLECULAR AND CELLULAR BIOLOGY, July 1996, p. 3381–3392 Vol. 16, No. 7
0270-7306/96/$04.00+0

The DNA-Binding and Enhancer-Blocking Domains of the *Drosophila suppressor of Hairy-wing* Protein

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Received 27 November 1995/Returned for modification 15 February 1996/Accepted 18 April 1996

Mutations in the *suppressor of Hairy-wing* [*su(Hw)*] gene of *Drosophila melanogaster* can cause female sterility and suppress mutations that are insertions of the gypsy retrotransposon. Gypsy binds the protein (SUHW) encoded by *su(Hw)*, and SUHW prevents enhancers promoter-distal to gypsy from activating gene transcription. SUHW contains 12 zinc fingers flanked by acidic N- and C-terminal domains. We examined the roles of each of the 12 zinc fingers in binding gypsy DNA and classified them into four groups: essential (fingers 6 through 10); beneficial but nonessential (fingers 1, 2, 3, and 11); unimportant (fingers 5 and 12); and inhibitory (finger 4). Because finger 10 is not required for female fertility but is essential for binding gypsy, these results imply that the SUHW-binding sites required for oogenesis differ in sequence from the gypsy-binding sites. We also examined the functions of the N- and C-terminal domains of SUHW by determining the ability of various deletion mutants to support female fertility and to alter expression of gypsy insertion alleles of the *yellow*, *cut*, *forked*, and *Ultrabithorax* genes. No individual segment of the N- and C-terminal domains of SUHW is absolutely required to alter expression of gypsy insertion alleles. However, the most important domain lies between residues 737 and 880 in the C-terminal domain. This region also contains the residues required for female fertility, and the fertility domain may be congruent with the enhancer-blocking domain. These results imply that SUHW blocks different enhancers and supports oogenesis by the same or closely related molecular mechanisms.

Several spontaneous mutations in *Drosophila melanogaster* are insertions of the gypsy retrotransposon into or next to a gene (42). Expression of the phenotypes of gypsy insertion alleles can be suppressed to wild-type or near wild-type levels by *suppressor of Hairy-wing* [*su(Hw)*] mutations (42, 54). Other than suppression of gypsy insertions, female sterility is the only known phenotype associated with *su(Hw)* mutations (38).

su(Hw) encodes a protein (SUHW) with 12 zinc finger motifs (44) that binds to a repeated consensus sequence downstream of the 5′ long terminal repeat (LTR) of gypsy (11, 61). Gypsy and the SUHW protein bound to gypsy can alter gene expression by multiple mechanisms. When gypsy is in a transcribed region of a gene and oriented in the same (parallel) direction, primary gene transcripts can be truncated and processed at the polyadenylation sites in the gypsy LTRs (13). Binding of SUHW to gypsy increases polyadenylation of gene transcripts in the 5′ LTR (11, 13). Truncation of gene transcripts in gypsy occurs in parallel gypsy insertions in the achaete-scute complex (3) and the *forked* gene (29). The gypsy LTR poly(A) sites may also explain why parallel gypsy insertions in an intron of the *Ultrabithorax* gene display stronger phenotypes than antiparallel insertions (47).

SUHW also alters gene expression by preventing enhancers and silencers from activating or repressing transcription (1, 2, 12, 19, 20, 28, 31, 32, 55). Only enhancers promoter distal to SUHW are blocked, while enhancers promoter proximal to SUHW still function (19, 28). Although enhancer blocking by SUHW is position dependent, it is distance independent, even at distances approaching 100 kbp (12, 32). SUHW enhancer blocking is also immediate and reversible (12), suggesting that SUHW does not block by inducing formation of quasistable and repressive heterochromatin-like structures. Therefore, when SUHW blocks an enhancer-promoter interaction, it does not prevent the enhancer from activating a second promoter on the opposite side (2, 55).

It seems likely that understanding how SUHW interferes with enhancer-promoter communication will help to elucidate how enhancers can regulate transcription at a distance. There is significant evidence that DNA bends or loops out to allow contacts between regulatory proteins (7, 14, 22, 49), and therefore it is conceivable that a protein that alters DNA bending could block enhancer-promoter interactions in a position-dependent manner. SUHW distorts DNA upon binding, and although the zinc fingers alone are sufficient to bind DNA, the distortion requires most of the flanking C-terminal domain (58). It is difficult to imagine, however, how a localized DNA distortion alone can block an interaction between an enhancer and promoter separated by approximately 90 kbp (12, 32). Analysis of various *su(Hw)* mutations have implicated a smaller region in the C-terminal domain of SUHW, which might be a site of interaction between SUHW and other proteins, in blocking enhancers in the *yellow* gene (24). Moreover, SUHW does not block interaction between an upstream activating sequence and a promoter in *Saccharomyces cerevisiae* (37), suggesting that DNA distortion is not sufficient to block enhancer-promoter interactions and that SUHW must interact with other proteins that are absent or unrecognizable in *S. cerevisiae.*

It is unknown if SUHW blocks different enhancers by the

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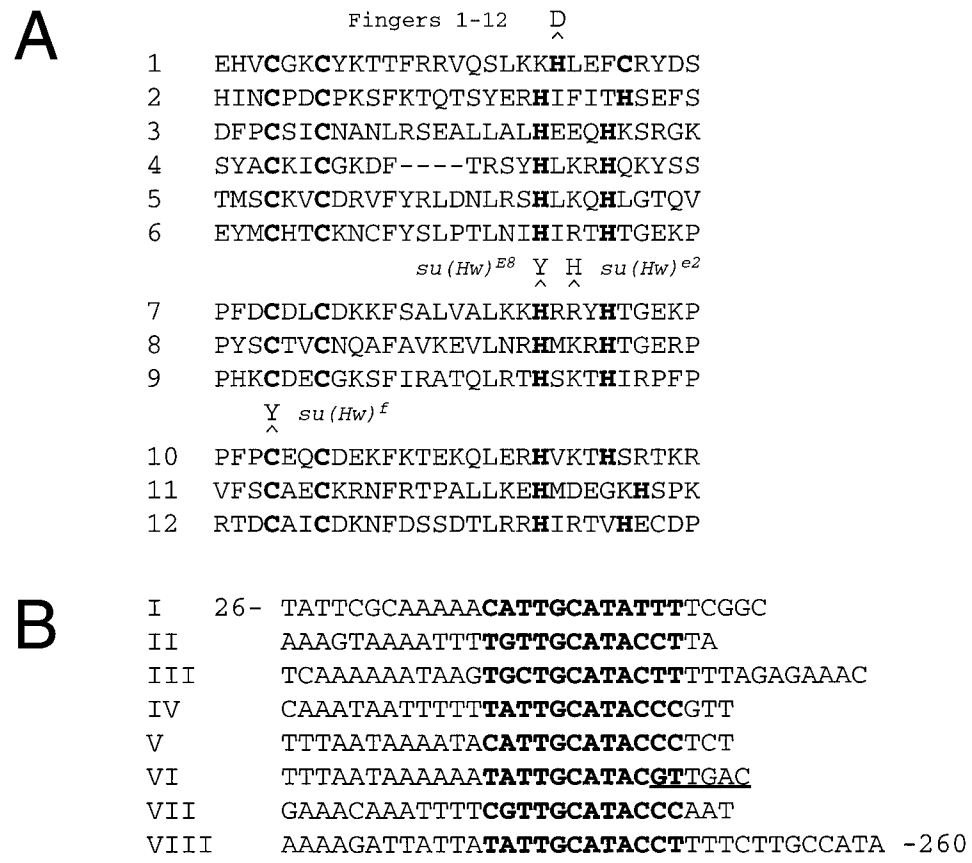

FIG. 1. SUHW zinc fingers and SUHW-binding sites in bx^{34e} gypsy. (A) The 12 zinc fingers are aligned, and the mutations created for these studies by oligonucleotide site-directed mutagenesis are indicated above the sequences. The first histidine in all fingers was converted to aspartic acid, and the zinc finger 7 mutations in the $su(Hw)^{E8}$ and $su(Hw)^{e2}$ alleles and the finger 10 mutation in the $su(Hw)^{f}$ allele (24) were recreated. The critical Zn^{2+}-coordinating residues are in bold letters. (B) Sequence of part of the BaBx fragment from the bx^{34e} gypsy element (numbered from the first nucleotide of the fragment). The eight SUHW-binding consensus repeats (bold letters) are aligned with each other. The *Hin*cII site used to generate the A and B fragments of BaBx is underlined.

same mechanism or if enhancer blocking is the SUHW activity required for oogenesis. It is feasible that DNA distortion is sufficient for blocking in some cases and that the SUHW activity needed for female fertility is distinct from the enhancer-blocking activity. In this report we explore these issues with a functional analysis of various SUHW domains. Although there are differences between the zinc finger requirements for binding gypsy DNA and to support female fertility, the same SUHW domain is most important for blocking enhancers in different genes, and it closely overlaps and may be congruent with the domain required for female fertility. Several SUHW mutants deficient in blocking enhancers retain the ability to distort DNA, suggesting that DNA distortion alone is not sufficient to block enhancers in the genes examined.

MATERIALS AND METHODS

Mutagenesis of SUHW zinc fingers and expression of mutant proteins in yeast cells. A yeast vector was constructed to facilitate site-directed mutagenesis of individual zinc fingers and expression of the mutant proteins. An f1 phage origin from pUG-f1 (Pharmacia Biotech), which allows isolation of single-stranded DNA for mutagenesis, was cloned into the pGEX-2T bacterial expression vector that allows fusion to glutathione *S*-transferase (GST) (60) between the *Eco*RI and *Sma*I sites, resulting in the pGEX-2T-f1 plasmid. A *Bsa*BI-to-*Eco*RI fragment of the *su(Hw)* cDNA, which contains primarily the zinc finger domain, was then cloned into the *Bam*HI site of pGEX-2T-f1, resulting in the pGEX-2T-ZF-f1 plasmid, with the GST domain fused to the zinc finger domain. A fragment of pGEX-2T-ZF-f1 containing the f1 origin and the *su(Hw)* cDNA fragment was then subcloned into the pSJ101 yeast expression vector (obtained from S. Johnson, University of Washington). The resulting plasmid, pSJ101-ZF-f1 was used as a template for oligonucleotide-mediated site-directed mutagenesis (39) of each of the individual zinc fingers. Each of the fingers was individually mutagenized to convert the first histidine residue to aspartic acid (Fig. 1). In addition, oligonucleotides were also used to recreate the exact lesions found in zinc finger 7 in the $su(Hw)^{E8}$ and $su(Hw)^{e2}$ alleles and the zinc finger 10 lesion found in $su(Hw)^{f}$ (24) (Fig. 1). All mutants were sequenced to confirm that they had the appropriate mutations.

The entire N-terminal domain and zinc fingers 1 through 3 were deleted from full-length SUHW by *Xba*I and *Stu*I restriction, blunting, and religation of the previously described yeast expression vector containing a full-length *su(Hw)* cDNA [pSJ-$su(Hw)^{+}$] (37, 58).

All mutant SUHW proteins were expressed in yeast cells by galactose induction, and extracts were prepared as previously described (37, 58). The amounts of the mutant proteins in the yeast extracts were determined by Western blots (immunoblots) with anti-GST antiserum (provided by M. Nussenzweig and Z. Misulovin, Rockefeller University).

Gel mobility shift assays and quantitation of DNA-binding activity. Gel mobility shift assays were performed with crude yeast extracts (37, 58), Schneider 2 cell nuclear extracts, and pupal nuclear extracts (11) with ^{32}P-labeled BaBx fragment of bx^{34e} gypsy containing eight consensus SUHW-binding repeats (Fig. 1), as described previously under conditions in which the total amount of SUHW-BaBx complex formed is linearly dependent on the amount of SUHW-binding activity (11, 37, 58). Because the BaBx fragment contains multiple binding sites, multiple complexes can be formed. The amounts of all complexes were quantitated with a Fuji PhosphorImager and summed to determine the total amount of binding. The amount of unbound BaBx DNA fragment was also quantitated and found in all cases to be reciprocally related to the total amount of the complex. To examine binding of the zinc finger mutants to individual binding sites, 46-bp double-stranded oligonucleotides containing each of the

eight consensus repeats in BaBx with their natural flanking sequences (Fig. 1) and *Xho*I and *Sal*I overhangs were subcloned into a modified pBend2 plasmid (58). Plasmid fragments (166 bp) were used for the binding experiments.

Full-length SUHW expressed in yeast cells was partially purified by phosphocellulose chromatography as previously described (58) to compare the binding of 166-bp plasmid fragments containing binding site VII or VIII to the 134-bp *Hin*cII B fragment of BaBx containing both sites VII and VIII. Full-length SUHW and the zinc finger 4 mutant expressed in yeast cells were also partially purified by phosphocellulose chromatography for DNase I footprinting experiments with the *Hin*cII A fragment of BaBx that were conducted as previously described (11, 58).

Construction of P elements expressing SUHW deletion variants. P elements expressing mutant SUHW proteins from the normal *su(Hw)* promoter were constructed by cloning a 1.3-kb *Pst*I-to-*Xho*I fragment containing the *su(Hw)* gene promoter and the *RpII215* gene generated by PCR (43) from Oregon R wild-type genomic DNA into the *Xho*I-*Pst*I sites of the CaSpeR P element vector (48) in which the polylinker *Xba*I site was converted to an *Xho*I site. The PCR primers were 5′-ATATCTCGAGAAATTCCAACACGAC-3′ and 5′-ATATCT GCAGTTTTCTCGCAATGA-3′. The blunted 0.95-kb *Hin*dIII fragment of the *su(Hw)* gene (44) containing the polyadenylation site was cloned into the blunted *Eco*RI polylinker site. A 3-kb *Xho*I-to-*Sma*I cDNA fragment containing the *su(Hw)* coding sequence from the pSJ-*su(Hw)*$^+$ yeast expression vector (37) was cloned into the *Xho*I and blunted *Bam*HI sites.

To make a SUHW protein lacking residues 782 to 944 (Δ782–944), a 2.4-kb *su(Hw)* cDNA *Dra*I-to-*Bam*HI fragment was used instead of the entire *su(Hw)* coding sequence. The *su(Hw)* cDNA clones encoding C-terminal truncation and internal deletion mutants are described elsewhere (37, 58), and fragments of these clones from the *Xba*I site in the *su(Hw)* coding sequences to a *Sma*I at the 3′ end of the coding sequences were substituted for the *Xba*I-to-*Bam*HI fragment of the Δ782–944 vector. The N-terminal deletion mutant and the N- and C-terminal truncation mutant cDNA clones are also described elsewhere (37, 58) and cloned as *Sal*I-to-*Sma*I fragments instead of the complete *su(Hw)* coding sequence. In some mutants, a few non-native amino acids replace deleted SUHW residues (37, 58).

P element transformations and genetic crosses. P element-mediated transformation (53) was used to integrate *su(Hw)* expression vectors into the genome of *y w*; *Ki P[ry*$^+$ *Δ2-3]{99B)* (51) flies. G_0 flies were crossed with In(1)FM6, y^{31d} w^{FM6} dm^+ ct^{83h} B flies, and transformed G_1 progeny were crossed to y^2 w^a ct^6 f^1; $su(Hw)^V$ bx^{34e} *er*/In(3LR)TM6, Hn^P ss^{P88} $su(Hw)^f$ bx^{34e} Ubx^{P15} flies. Flies homozygous for the P element inserts were generated by backcrosses between the G_2 progeny.

Determination of effects of mutant SUHW proteins on female fertility and gypsy insertion allele phenotypes. Newly enclosed $su(Hw)^V$ females containing P elements expressing the various mutant SUHW proteins from the normal *su(Hw)* promoter were aged 2 to 3 days, placed individually in vials with three wild-type males, and transferred every few days. Hatches were determined from the empty egg shells and the presence of larvae. For each SUHW protein, at least 10 females from three independent transgenic lines were scored for a week. Females were scored as fertile when they laid eggs that hatched and as sterile if they did not lay any eggs. In all cases, when a female laid eggs, most of the eggs hatched, and females from independent lines with the same protein always displayed the same fertility phenotype.

The effects of the various SUHW proteins produced from homozygous expression vectors on the y^2, ct^6, f^1, and bx^{34e} gypsy insertion allele phenotypes in both $su(Hw)^V$ and $su(Hw)^V/su(Hw)^f$ flies were scored independently by two of the authors. Rare discrepancies were resolved by reexamination of the phenotypes. A fully suppressed phenotype was assigned a value of 0, a weak but detectable phenotype was scored 1, an intermediate-strength phenotype was scored 2, and a strong phenotype approaching that observed with wild-type *su(Hw)* was scored 3. For y^2, flies were aged 3 to 4 days prior to scoring. A weak phenotype was denoted by a body color slightly lighter than that of the wild type. An intermediate-strength phenotype was denoted by a muddy body color, most easily scored from the abdomens of males, while a strong y^2 phenotype was denoted by a very light color in the wings and bodies. The strength of the ct^6 phenotype was indicated as follows: weak, one to three nicks in the wing margins; intermediate, more than a few but less than 10 nicks per wing; and strong, 10 or more nicks per wing. The forked bristle phenotype is not fully suppressed in a null *su(Hw)* background. A weak f^1 phenotype was the presence of two to four forked dorsal thoracic bristles, an intermediate-strength phenotype was the presence of four to approximately a dozen forked bristles, and a strong phenotype was when most of the dorsal thoracic bristles were forked. The bx^{34e} phenotype was different in $su(Hw)^V$ and $su(Hw)^V/su(Hw)^f$ flies because the $su(Hw)^f$ chromosome carries Ubx^{P15} and bx^{34e}/Ubx^{P15} is stronger than homozygous bx^{34e}. In both cases, however, a weak phenotype was the presence of hypopleural bristles, an intermediate-strength phenotype was the presence of additional bristles on the capitellum and just dorsal to the haltere, and a strong phenotype was the presence of extra bristled cuticle between the thorax and abdomen and flattening of the haltere capitellum.

RESULTS

Roles of individual SUHW zinc fingers in binding gypsy DNA. The SUHW protein binds to a 12-bp consensus repeat sequence in gypsy (Fig. 1) and contacts approximately 30 bp, with the contacts extending into the flanking AT-rich sequences (11, 58, 61). The zinc finger domain of SUHW located in the middle of the protein contains 11 TFIIIA-like C_2H_2 zinc finger motifs and 1 C_2HC finger motif (44) (Fig. 1). A truncated SUHW protein consisting primarily of these 12 motifs binds gypsy with the same affinity as does full-length SUHW and generates a DNase I footprint identical to that of full-length SUHW (37, 58).

To determine which of the 12 zinc finger motifs are involved in binding gypsy, we tested the ability of the SUHW zinc finger domain with different zinc finger mutations to bind a bx^{34e} gypsy DNA fragment (BaBx) containing eight consensus repeats (11). For each finger, oligonucleotide site-directed mutagenesis (39) was used to convert the first histidine residue, critical for Zn^{2+} coordination, to aspartic acid (Fig. 1). In addition, the zinc finger lesions found in the $su(Hw)^{E8}$, $su(Hw)^{e2}$, and $su(Hw)^f$ alleles (24) (Fig. 1) were recreated to determine if they affect gypsy binding. In $su(Hw)^{E8}$, the first histidine of zinc finger 7 is converted to tyrosine, and in $su(Hw)^{e2}$, an arginine residue between the two histidine residues of finger 7 is converted to an extra histidine. The $su(Hw)^f$ lesion is conversion of the first cysteine of finger 10 to tyrosine.

The various zinc finger mutants were expressed in yeast cells, and their ability to bind BaBx was determined by gel mobility shift assays under conditions in which the amount of SUHW protein-BaBx complex is linearly dependent on the amount of DNA-binding activity (Fig. 2). Fresh extracts were prepared for each experiment and used immediately without storage. To ensure that differences in binding activity were not due to differences in the amounts of the mutant proteins, the amounts of the fusion proteins in the yeast extracts were determined by Western blots with anti-GST antibodies (data not shown). The levels of the different mutant proteins in the extracts, although they varied slightly from experiment to experiment, were always the same in extracts prepared at the same time. Yeast extracts do not contain other proteins that bind to BaBx or interfere with SUHW binding (37, 58) (Fig. 2). Although the BaBx fragment contains eight potential SUHW-binding sites, in most complexes only one or two of the binding sites are occupied by the wild-type zinc finger domain under the standard assay conditions (11) (Fig. 2).

The SUHW proteins with mutant zinc fingers vary dramatically in their binding activity. Conversion of the first histidine residue to aspartic acid in finger 6, 7, 8, 9, or 10 causes complete loss of detectable DNA-binding activity, while mutation of finger 5 or 12 has little or no effect on binding (Fig. 2). The same lesion in finger 1, 2, 3, or 11 reduces binding from 2- to 10-fold, while mutation of finger 4 actually increases binding approximately 2-fold in all of the several experiments conducted.

In some experiments, two- to threefold-lower amounts of binding activity were used, but the relative differences in binding activity between the various mutant proteins did not differ substantially. Because the experiments were performed with fresh crude yeast extracts, it was not possible to increase DNA-binding activity in the reactions sufficiently to determine if the same total amount of complex could be formed with the various mutant proteins at saturating levels. Attempts to purify the proteins by glutathione-affinity chromatography led to total loss of binding activity, and the mutant proteins partially purified by phosphocellulose chromatography displayed signifi-

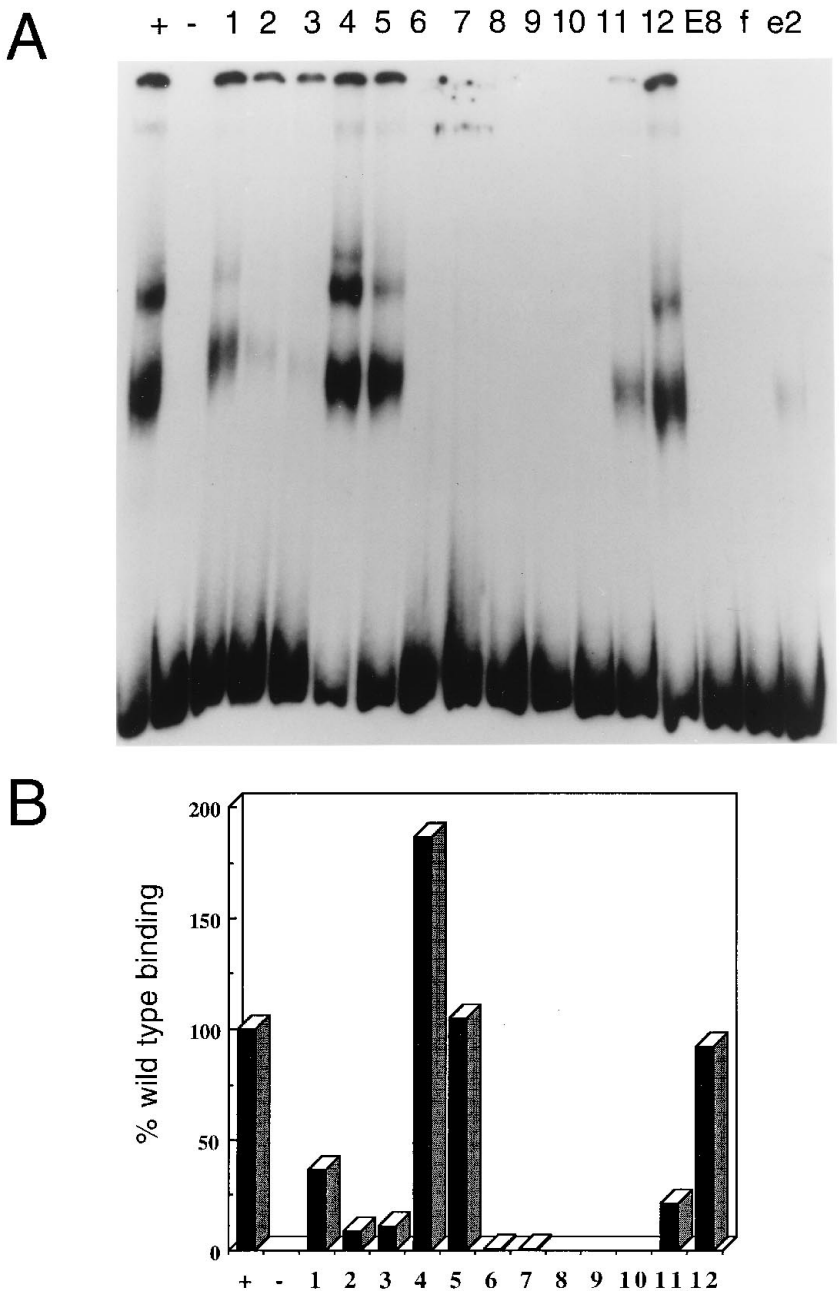

FIG. 2. Effects of zinc finger mutations on binding of SUHW zinc finger domain to a fragment containing multiple binding sites. (A) Autoradiogram of a mobility shift gel. Binding reactions were performed with the BaBx fragment of bx^{34e} gypsy (0.16 μg/ml) containing multiple binding sites as described previously (11, 37, 58) with extracts (800 μg/ml) from yeast cells expressing the following GST-SUHW zinc finger domain (residues 204 through 672) fusion proteins: +, all wild-type zinc fingers; 1 through 12, conversions of the first histidine to aspartic acid in fingers 1 through 12, respectively (Fig. 1); E8, a histidine-to-tyrosine lesion in finger 7 present in the $su(Hw)^{E8}$ allele (Fig. 1); f, a cysteine-to-tyrosine lesion in finger 10 present in the $su(Hw)^{f}$ allele; and e2, an arginine-to-histidine lesion in finger 7 present in the $su(Hw)^{e2}$ allele. All extracts contained equal amounts of the respective mutant proteins as determined by Western blots with anti-GST antibodies. The lanes labeled "−" contain extract from yeast cells with an expression plasmid lacking an *su(Hw)* cDNA insert. The band at the bottom of each lane is the unbound BaBx fragment, and all bands migrating more slowly are GST-SUHW zinc finger domain-BaBx complexes. (B) The binding activities of the individual zinc finger mutants are expressed as the percent of wild-type binding activity as determined by PhosphorImager quantitation. The amounts of all complexes were summed to determine the total binding activity, and the values shown for each SUHW protein are the averages of at least three independent experiments.

cant and variable instability when stored overnight. Therefore, it was not possible to conduct more quantitative assays to determine the actual dissociation constants.

Correlation of effects of zinc finger mutations on in vitro DNA binding and SUHW activity in vivo. Although the instability of the partially purified proteins prohibited quantitative comparisons of the dissociation constants for the various mutant proteins, the relative binding levels determined by gel mobility shift experiments appear to accurately reflect the in vivo activities of at least the finger 7 and finger 10 mutants. Like the histidine-to-aspartic-acid conversions, the $su(Hw)^{E8}$ lesion in finger 7 and the $su(Hw)^{f}$ lesion in finger 10 both

abolish binding, consistent with the strong suppression of gypsy insertions observed with both of these alleles and the loss of female fertility with *su(Hw)*^{E8}. The correlation between the ability to bind gypsy and in vivo activity extends to the *su(Hw)*^{e2} lesion in finger 7. This lesion does not affect one of the Zn^{2+}-coordinating residues, and the mutant protein retains 5 to 10% wild-type binding activity in the gel mobility shift assay (Fig. 2). This finding is consistent with the observations that in contrast to *su(Hw)*^{E8}, *su(Hw)*^{e2} only weakly suppresses *bx*^{34e} and various gypsy insertions in *cut* and supports female fertility (12).

Alteration of SUHW protein-DNA interaction by finger 4 mutation. The observation that the finger 4 mutation increases DNA binding was unexpected. However, the DNase I footprint obtained with a partially purified finger 4 mutant protein on site III in fragment of BaBx fragment displays more hypersensitive sites than the footprint obtained with the partially purified wild-type protein (Fig. 3). Furthermore, the finger 4 mutant protein displays additional hypersensitive sites in similar positions next to sites II, IV, and V in the BaBx fragment, although significant protection from DNase I digestion is seen only with site II (Fig. 3). Although the presence of additional hypersensitive sites does not necessarily indicate an increase in binding affinity, it confirms that the protein-DNA interactions have been altered significantly. The other mutant proteins, including the finger 5 and 12 mutants with wild-type binding activity, were too unstable after partial purification to obtain footprints.

Effects of zinc finger mutations on binding to individual binding sites. The binding studies described above were conducted with the BaBx fragment of *bx*^{34e} gypsy that contains eight consensus SUHW-binding repeats. We considered the possibility that the roles of the individual zinc fingers might vary with the individual binding sites. However, the results obtained were similar to those with BaBx. Double-stranded oligonucleotides (46 bp) recreating each of the eight individual consensus repeats and flanking sequences were individually subcloned into a plasmid vector, and 166-bp plasmid fragments containing the cloned sites were used in gel mobility shift experiments. Unexpectedly, the binding affinity to single sites is severalfold lower than that with the complete BaBx fragment. Indeed, with five of the eight sites (sites I, IV, V, VI, and VIII [Fig. 1]) there is little binding even with the wild-type protein. With the exception of site VIII (Fig. 4), the zinc finger 4 mutant displays significant binding to these same sites, confirming that the finger 4 mutation increases binding to individual sites as it does for the BaBx fragment. As expected, the finger 6 to 10 mutants do not bind any of the three individual sites (II, III, and VII) that display significant binding to the wild type. Binding of the finger 5 and 12 mutants is similar to that seen with the wild-type protein, the finger 4 mutant increases binding, and the finger 1 and 11 mutants display levels of binding that are the same as or lower than that of the wild-type protein, while the finger 2 and 3 mutants have very little or no detectable binding activity with any of the individual sites. An example of the binding of mutant proteins to site VII is shown in Fig. 4. Because the level of binding observed for individual sites is already severalfold-lower than that for BaBx, the lack of detectable binding of the finger 2 and 3 mutants to the individual sites is expected. We conclude, therefore, that within the sensitivity of the assay, the roles of the individual fingers do not vary significantly between the individual gypsy-binding sites.

Interactions between SUHW-binding sites. The relatively poor binding of SUHW to single sites was unexpected because binding of SUHW to the BaBx fragment does not appear to be cooperative. Under the conditions used, only a single site is bound in most BaBx-SUHW complexes, and SUHW probably binds as a monomer. For example, mixing different deletion mutants in a binding reaction mixture does not generate single-site complexes with intermediate mobilities (57), and the number of zinc fingers involved in binding is sufficient for a single molecule of SUHW to contact an entire single site. With a fragment of BaBx containing sites I through VI, the SUHW footprint covering site III extends into site II (11, 58) (Fig. 3), raising the possibility that SUHW may even interfere with binding to neighboring sites. Indeed, with fragments containing two neighboring copies of site III, complexes with wild-type full-length SUHW bound to both sites cannot be detected even with levels of DNA binding activity that form higher-order complexes with BaBx (57).

Even more curious than the generally low level of binding to single sites is that SUHW binds a single site VII much better than it binds a single site VIII (Fig. 4). With a fragment of BaBx containing only these two sites, DNase I footprints and methylation interference experiments have demonstrated that site VIII is occupied and that site VII is not (see Fig. 2 in

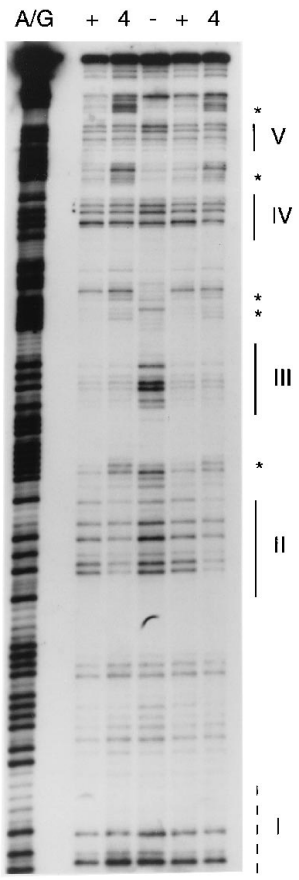

FIG. 3. Effect of finger 4 mutation on SUHW DNase I footprint. The autoradiogram shows the DNase I footprints obtained with wild-type full-length SUHW (lanes labeled "+") and the GST-SUHW zinc finger domain fusion protein with the finger 4 mutation (lanes 4). The reactions were conducted as previously described (11, 58) and contained end-labeled *Hin*cII A BaBx fragment (0.16 μg/ml) and yeast extracts (120 μg/ml) partially purified by phosphocellulose chromatography. The center lane (labeled "−") is a no-extract control and is flanked by duplicate reactions. The first lane (labeled A/G) is an A+G Maxam-Gilbert reaction. Locations of the 12-bp consensus repeats in binding sites I through V are indicated by bars on the right (only part of site I is shown), and the locations of additional hypersensitive sites obtained with the finger 4 mutant are indicated by asterisks (*). The wild-type zinc finger domain footprint (data not shown) is identical to the wild-type full-length protein footprint (58).

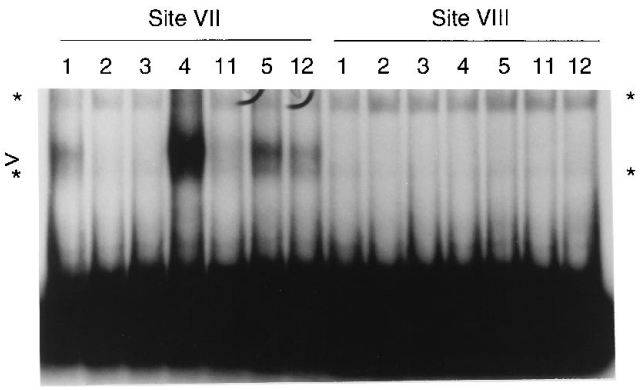

FIG. 4. Binding of selected zinc finger mutants to single sites VII and VIII. Shown is an autoradiogram of a mobility shift gel. Binding reactions were carried out as described in the legend to Fig. 2 with crude yeast extracts (1600 μg/ml) containing GST-SUHW zinc finger domain fusion proteins with mutations in the fingers indicated by the numbers above the lanes (fingers 1, 2, 3, 5, 11, and 12) and end-labeled 166-bp fragments (0.16 μg/ml) containing either site VII or VIII (Fig. 1) and the flanking sequences. Asterisks (*) indicate contaminants in the DNA fragment preparations, and the caret indicates the mutant zinc finger domain-oligonucleotide complexes. Note that the lanes for the finger 5 and finger 11 mutants are reversed for site VII. The finger 6 to 10 mutants display no binding to these sites, and the amount of complex formed with the wild-type zinc finger domain is similar to that seen with the finger 5 and 12 mutants (data not shown).

reference 11). At higher DNA concentrations, low levels of binding activity of a complex between wild-type SUHW and a single site VIII can be detected (Fig. 5). However, the level of binding of SUHW to the fragment of BaBx containing both sites VII and VIII is approximately sixfold higher and very similar to that with site VII alone (Fig. 5) even though site VIII is the one occupied in the fragment with two sites (11). The sequences flanking the single sites are identical to those in the fragment of BaBx. A possible explanation for these results is that SUHW first recognizes site VII and then diffuses along the DNA to site VIII. Site VII may be more easily recognized by SUHW while binding to site VIII is more stable. It is also possible, on the basis of this argument, that SUHW displays stronger binding to site III in the BaBx fragment than to a single site III because the unbound sites provide more entry points.

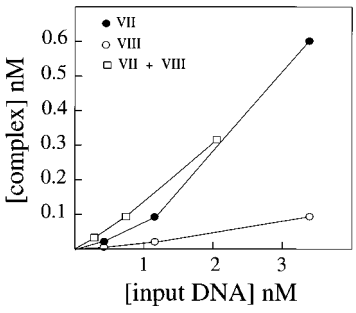

FIG. 5. Comparison of binding of SUHW to single sites VII and VIII and a fragment containing both. Gel mobility shift assays were conducted with a constant amount of partially purified yeast extract containing wild-type full-length SUHW (180 μg/ml) and the indicated concentrations of end-labeled 134-bp *Hin*cII B fragment of BaBx containing sites VII and VIII or 166-bp fragments containing either site VII or VIII. Complexes and free DNA were quantitated with a PhosphorImager. Input DNA and complex concentrations were calculated on the basis of PhosphorImager quantitation of known quantities of each fragment. Although the curve for the fragment containing only site VII more closely resembles the curve for the fragment containing both sites VII and VIII, only site VIII is occupied in the complexes formed with the fragment containing both sites (11).

Mapping of SUHW protein domains involved in blocking enhancers and female fertility. To determine which parts of the N- and C-terminal domains of SUHW that flank the zinc fingers are involved in enhancer blocking and female fertility, we constructed transgenic flies producing mutant SUHW proteins with deletions in the N- and C-terminal domains. The abilities of the mutant SUHW proteins to block enhancers were assessed with four different gypsy insertion alleles to determine whether the same SUHW domains are required to block different enhancers. The y^2, ct^6, f^1, and bx^{34e} gypsy insertion alleles also differ significantly in the location and orientation of the gypsy insertion within the gene. y^2 has an antiparallel gypsy with 12 consensus repeats of the SUHW binding site between the promoter-proximal wing and body enhancers and the promoter of *yellow* (20). Accordingly, y^2 displays a pigmentation deficiency only in the wing and body. In ct^6, a gypsy element with 12 repeats is close to and blocks a promoter-distal wing margin enhancer nearly 90 kbp upstream of the promoter in *cut* (12, 30, 32), producing a cut wing phenotype. The f^1 allele of *forked* is a parallel gypsy with 12 repeats in an intron (29) that produces a forked bristle phenotype. Transcription control elements have not been mapped in *forked*, but transcripts are truncated in the gypsy 5′ LTR (29), indicating that poly(A) site potentiation is at least part of the mechanism by which SUHW alters *forked* expression. The bx^{34e} allele is an antiparallel gypsy with eight repeats in an intron of the *Ultrabithorax* (*Ubx*) gene that causes thoracic homeotic transformations. It is likely that the primary mechanism by which the bx^{34e} gypsy alters *Ubx* expression is by blocking the *bithorax* region enhancer (50) and enhancers in the *anterobithorax* region (59), both of which are several kilobase pairs downstream of the promoter.

The activities of the mutant SUHW proteins were assessed in two different *su(Hw)* mutant backgrounds. Homozygous $su(Hw)^V$ and the $su(Hw)^V/su(Hw)^f$ heterozygous combination both strongly suppress the gypsy insertion alleles. The $su(Hw)^V$ allele is a deletion and is usually homozygously lethal because an RNA polymerase subunit gene (*RpII215*) just upstream of *su(Hw)* is also affected (25). However, when *RpII215* is supplied transgenically, $su(Hw)^V$ flies are viable and female sterile (25). The P element used to express mutant *su(Hw)* cDNAs in these experiments contains *RpII215*, allowing the ability of the mutant SUHW proteins to restore fertility of homozygous $su(Hw)^V$ females to be determined.

Several independent transformant lines were examined for each mutant protein, and it was observed that when a mutant SUHW protein altered expression of a gypsy insertion allele, the strength of the phenotype could vary between individual transformant lines. Because the ability or inability to support female fertility was the same in all transformant lines that were tested, it appears either that the gypsy insertion allele phenotypes are more sensitive to differences in the level of SUHW or that germ line expression of the SUHW P elements is less subject to position effects than is somatic expression. To compensate for this variability, an average of 23 individual transformant lines were examined for each construct, with a minimum of 8 and a maximum of 39 (Fig. 6). This allowed the activity of a mutant SUHW protein to be assessed by both the proportion of the independent transgenic lines that display a particular gypsy insertion allele phenotype and by the strongest phenotype observed in any of the independent lines. A fully suppressed phenotype was assigned a value of 0, a weak phenotype was 1, an intermediate-strength phenotype was 2, and a strong phenotype was 3 (see Materials and Methods for how

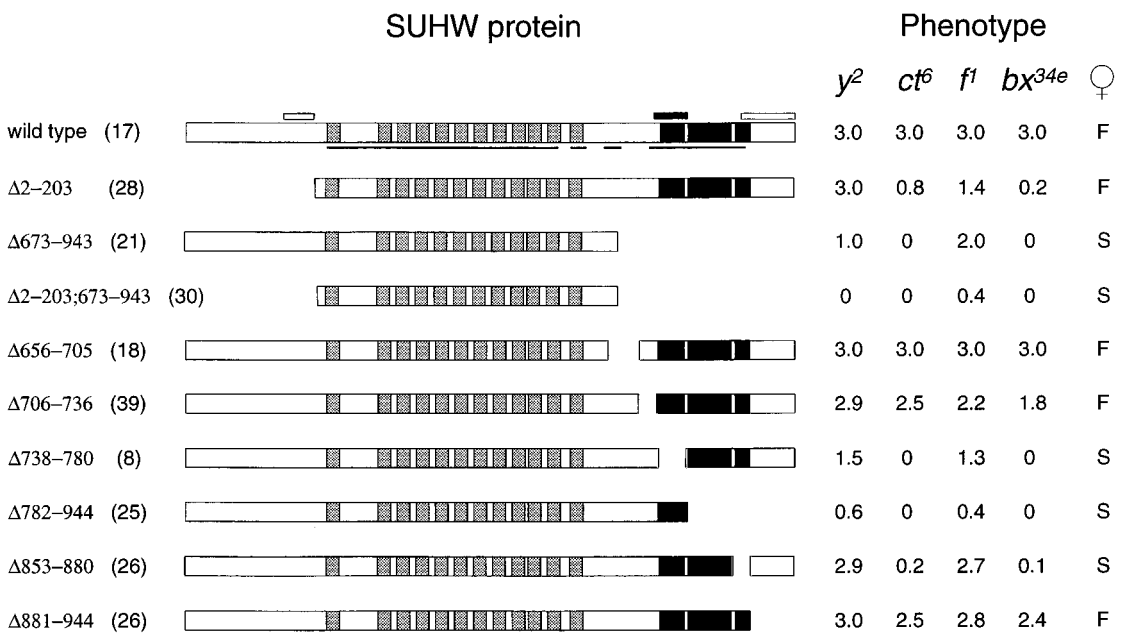

FIG. 6. Maps of SUHW proteins and their effects on gypsy insertion allele phenotypes and female fertility. The SUHW proteins shown were expressed from P element insertions with the native *su(Hw)* promoter. The stippled boxes represent zinc fingers, and the closed boxes represent C-terminal regions determined to be involved in enhancer blocking. The bars above the diagram of the wild-type protein represent previously noted (24) highly acidic domains (open bars) and a heptad repeat (closed bar). The lines below the wild-type protein diagram indicate regions in which the majority of residues are conserved between three species of *Drosophila* (24). The residues deleted from each of the mutant proteins are indicated on the left, with the number of independent transformant lines examined indicated in parentheses. The effects of each of the proteins on gypsy insertion allele phenotypes in y^2 ct^6 f^1; $su(Hw)^V$ bx^{34e}/In(3LR)TM6, $su(Hw)^f$ Ubx^{P15} flies and the fertility of y^2 ct^6 f^1; $su(Hw)^V$ bx^{34e} females are indicated on the right. Female fertility and phenotype values were determined as described in the text. The maximum phenotype strength is 3, indicating strong enhancer blocking, and 0 indicates a suppressed phenotype and a lack of detectable enhancer blocking. F indicates fertile females, and S indicate sterile females. Representative examples of ct^6 cut wing phenotypes are shown in Fig. 7.

phenotypes were scored for each allele). To integrate the two measurements and simplify comparisons, the enhancer-blocking activity of a SUHW mutant protein is expressed as the proportion of lines displaying a phenotype multiplied by the strength of the maximum phenotype observed. These values range from 0 (completely suppressed phenotype and no detectable enhancer blocking) to 3 (maximum phenotype and strong enhancer blocking) and are presented in Fig. 6. In practice, the cut wing phenotype of ct^6 proved to be the most quantitative. Representative ct^6 phenotypes observed with various SUHW proteins are shown in Fig. 7.

As expected, a control construct lacking *su(Hw)* cDNA does not alter expression of the gypsy insertion alleles (data not shown), while a construct producing wild-type SUHW induces strong phenotypes for all alleles (Fig. 6). $su(Hw)^V$ females expressing the N-terminal truncation (Δ2–230) are fertile, and enhancer blocking is significantly reduced with the N-terminal truncation for all alleles except y^2 (Fig. 6). The reduction is greatest with bx^{34e}, which appears to be the most sensitive to losses in enhancer-blocking activity. However, phenotypes are still observed for all of the gypsy insertions. Therefore, the N-terminal domain is not required to alter expression of gypsy insertion alleles or for female fertility.

Deletion of the C-terminal domain has more dramatic effects than deletion of the N-terminal domain. Loss of residues 673 to 943 diminishes the frequency and strength of y^2 and f^1 phenotypes and abolishes the visible effects on ct^6 and bx^{34e} (Fig. 6 and 7). The ability to restore fertility to $su(Hw)^V$ females is also abolished. Therefore, the C-terminal domain of SUHW is more important than the N-terminal domain for blocking enhancers and is essential for female fertility.

The ability of SUHW to alter expression of y^2 is lost only when both the N- and C-terminal domains are deleted (Fig. 6). The double truncation also reduces, but does not abolish, the frequency and strength of the f^1 phenotype.

Because the C-terminal domain has profound effects on gene expression and female fertility, smaller deletions were tested to better define the critical domains. Deletion of residues 655 to 736 or residues 881 to 944 has little effect on enhancer blocking and female fertility (Fig. 6 and 7), indicating that the important residues for both are between 737 to 880. Indeed, deletion of residues 738 to 780 or residues 782 to 944 abolishes the visible effects on ct^6 and bx^{34e} and the ability to rescue fertility and strongly diminishes the effects on y^2 and f^1. While deletion of residues 853 to 880 has little effect on y^2 and f^1 phenotypes, it greatly diminishes the ability to induce ct^6 and bx^{34e} phenotypes and prohibits rescue of female fertility. Therefore, the 737-to-852 region is the most important for blocking enhancers, and the adjacent 853-to-880 region makes a significant contribution.

With all the mutant proteins, the ability to alter expression of ct^6 and bx^{34e} is reduced more than the ability to alter expression of y^2 and f^1 (Fig. 6). However, deletions that abolish the visible effects on ct^6 and bx^{34e} have dramatic effects on y^2 and f^1, and mutations that only diminish the effects on ct^6 and bx^{34e} have minimal effects on y^2 and f^1 expression. Therefore, although the absolute magnitudes of the effects differ, the same SUHW domains are involved in altering the expression of all four genes. It is possible that it is more difficult to block enhancers in ct^6 and bx^{34e}, which have enhancers several kilobase pairs from the promoter, than in y^2, in which the enhancers are close to the promoter. It is also possible that a smaller decrease in y^2 or f^1 expression is required to produce a visible phenotype.

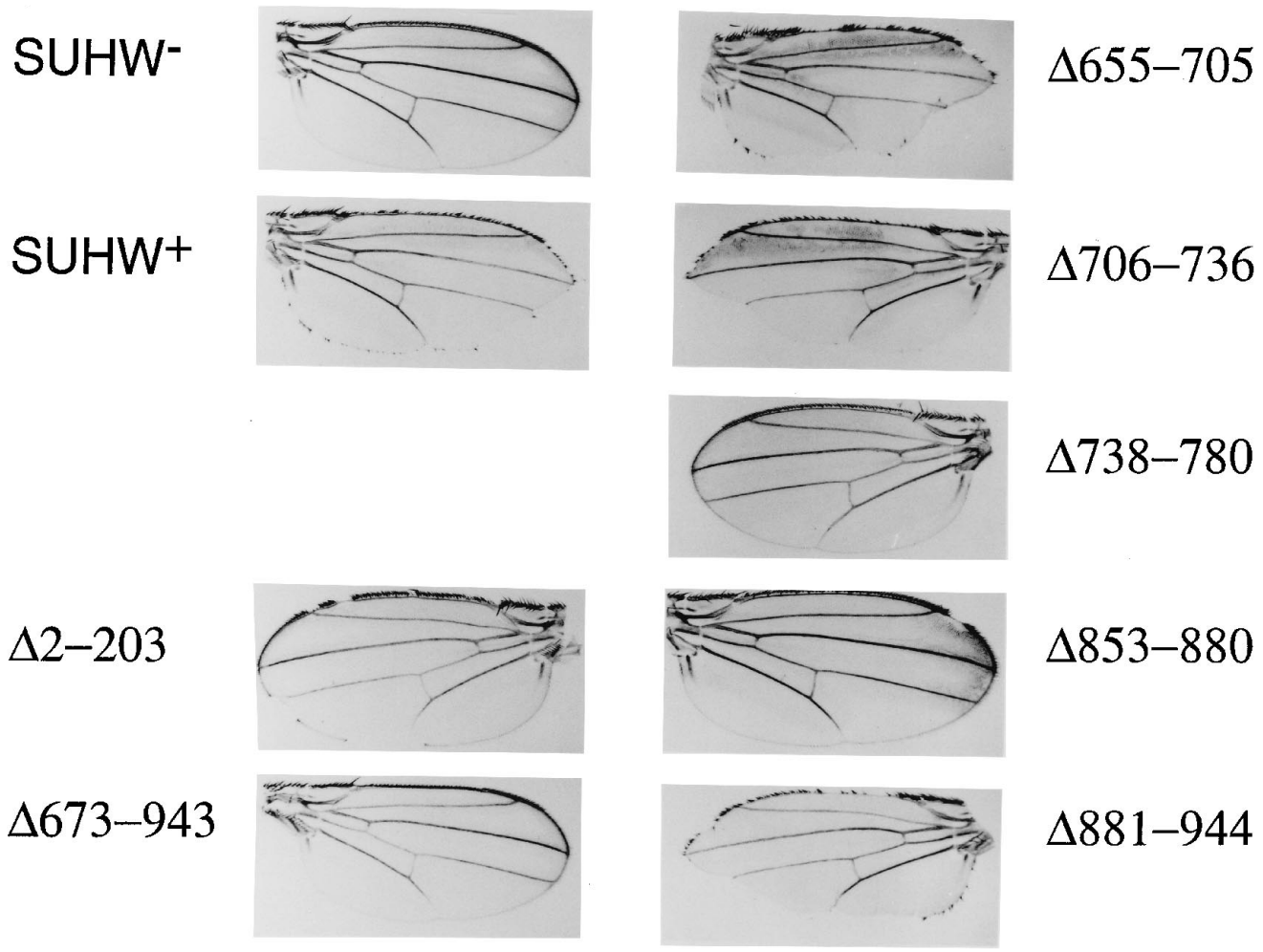

FIG. 7. Representative examples of ct^6 cut wing phenotypes induced by various SUHW proteins. For each of the indicated SUHW proteins, a wing from a y^2 ct^6 f^1; $su(Hw)^V$ bx^{34e}/In(3LR)TM6, $su(Hw)^f$ Ubx^{P15} fly expressing that protein is shown. See Fig. 6 for maps of the proteins. A suppressed (0 strength) phenotype is observed in the SUHW$^-$ control that does not produce SUHW, and a strong (strength of 3) phenotype is observed in the SUHW$^+$ control producing wild-type protein. The wing from a fly expressing the Δ2–203 mutant protein with approximately five nicks in the margin has as an intermediate-strength (value of 2) phenotype, and the wing with a single nick from the Δ853–880-expressing fly has a weak (value of 1) phenotype.

On the basis of the abilities of the mutant SUHW proteins to restore fertility of $su(Hw)^V$ females, we conclude that C-terminal residues between 738 and 780 and between 853 to 880 are required for oogenesis. Because the 853-to-800 region is included in the 782-to-944 deletion, it cannot be determined whether the 801-to-852 region is also required for fertility. However, the 738-to-780 region has profound effects on gypsy insertion alleles, and deletion of the 853-to-880 region significantly reduces enhancer blocking in the ct^6 and bx^{34e} alleles. Therefore, the fertility and enhancer-blocking domains closely overlap, and it is possible that they are congruent (Fig. 6).

The C-terminal domain of SUHW is not required for stability, nuclear localization, or DNA binding. The conclusion that SUHW residues between 737 to 880 are involved in enhancer blocking and oogenesis assumes that proteins lacking residues in this region are stable, enter the nucleus, and bind DNA. All the mutants, except that lacking both the N- and C-terminal domains, affect expression of y^2 and f^1, indicating that they do enter the nucleus and bind DNA to some extent. Furthermore, the zinc finger domain alone enters the nucleus in yeast cells and binds DNA as well as does wild-type SUHW (37, 58).

To confirm that the N- and C-terminal domains of SUHW are more important for enhancer blocking than for protein stability, nuclear entry, or DNA-binding activity, SUHW DNA-binding activity in nuclear extracts of pupae from selected representative transformant lines was examined with a gel mobility shift assay. The DNA-binding activity present in *su(Hw)* mutant pupae producing SUHW protein lacking the N terminus (Δ2–203) is a third of that in pupae with wild-type *su(Hw)* alleles (Fig. 8). The transformant line used for this experiment displays intermediate-strength ct^6 and bx^{34e} phenotypes and strong y^2 and f^1 phenotypes. By itself the threefold reduction in DNA-binding activity is not sufficient to explain the reduced strength of the ct^6 and bx^{34e} phenotypes. Thus, $su(Hw)^{e2}$ mutants display strong ct^6 and bx^{34e} phenotypes (12) even though SUHW DNA-binding activity is reduced 10- to 20-fold by the $su(Hw)^{e2}$ mutation (Fig. 2). Therefore, the intermediate-strength ct^6 and bx^{34e} phenotypes in the transformant line with the N-terminal truncation result, at least in part, from a reduction in enhancer-blocking activity. Because the zinc finger domain alone binds DNA with same affinity as full-length SUHW (58), the threefold reduction in DNA binding in the transformant line is likely to reflect less protein accumulation in the

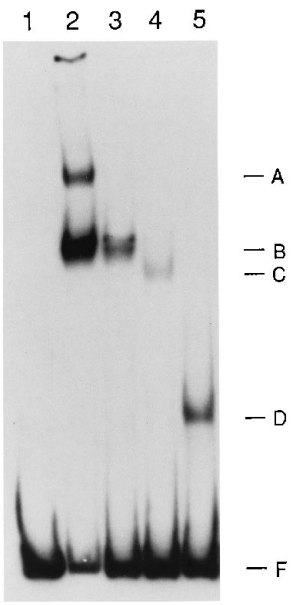

FIG. 8. SUHW DNA-binding activity in Schneider 2 cell and pupal nuclear extracts. Binding activity was assayed by gel mobility shift with the BaBx fragment of bx^{34e} gypsy. The amount of SUHW-DNA complex formed is proportional to the amount of DNA-binding activity with the extract concentration used (240 μg/ml). Lanes: 1, no extract; 2, Schneider 2 cell extract; 3, Canton S (wild-type) pupal extract; 4, extract of y^2 ct^6 f^1; $su(Hw)^V$ bx^{34e}/In(3LR)TM6, $su(Hw)^f$ Ubx^{P15} and y^2 ct^6 f^1; $su(Hw)^V$ bx^{34e} pupae producing SUHW lacking the N terminus (Δ2–203); 5, extract of y^2 ct^6 f^1; $su(Hw)^V$ bx^{34e}/In(3LR)TM6, $su(Hw)^f$ Ubx^{P15} and y^2 ct^6 f^1; $su(Hw)^V$ bx^{34e} pupae producing SUHW lacking the C terminus (Δ673–943). Band F at the bottom of the gel is free DNA. Wild-type SUHW is bound to two sites in complex A and to one site in complex B (11). One site is bound by SUHW lacking the N terminus in complex C, and one site is bound by SUHW lacking the C terminus in complex D. Complex D has much higher mobility because the Δ673–943 mutant protein does not alter the DNA structure (58). The complexes were quantitated with a PhosphorImager, and the amounts of all complexes in a lane were summed to determine the total binding. The Schneider 2 extract contains approximately five times the level of SUHW binding activity as that in the Canton S pupal extract, consistent with previous observations (11). The Δ673–943 and Δ2–203 pupal extracts have approximately the same level as and one-third of the binding activity present in the Canton S extract, respectively.

nucleus instead of a reduction in the ability of SUHW to bind DNA.

Wild-type levels of DNA-binding activity are present in *su(Hw)* mutant pupae producing SUHW protein lacking most of the C-terminal region (Δ673–943) (Fig. 8). This transformant line displays completely suppressed ct^6 and bx^{34e} phenotypes and intermediate-strength y^2 and f^1 phenotypes. Thus, although the level of SUHW DNA-binding activity in the nucleus is equivalent to wild-type levels, truncation of the C-terminal domain severely reduces enhancer-blocking activity.

The complexes formed with truncated proteins have higher gel mobilities than wild-type complexes as previously observed (37, 58), and consistent with previous observations (11), the level of SUHW DNA-binding activity in wild-type pupal extracts is fivefold lower than that in Schneider 2 cell extracts (Fig. 5).

DISCUSSION

Various domains of SUHW were mutagenized to explore the question of whether SUHW blocks different enhancers in different genes and supports oogenesis by the same or similar mechanisms. As discussed below, the results suggest that SUHW uses different zinc fingers to bind to different DNA sequences and that the molecular mechanisms by which SUHW blocks different enhancers and supports oogenesis are the same or closely related.

Roles of individual zinc fingers of SUHW. The 12 zinc finger motifs of SUHW fall into four classes with regard to binding gypsy DNA: essential (fingers 6 to 10), beneficial but nonessential (fingers 1 to 3 and 11), unimportant (fingers 5 and 12), and inhibitory (finger 4). There are other zinc finger proteins in which the different fingers vary in their importance. Only two of the five fingers of the PRDI-BF1 repressor (34), and four of the seven fingers of the *Evi-1* protein (9), are required for sequence-specific DNA binding. Similarly, the nine fingers of the TFIIIA protein vary in their contributions to DNA-binding energy (6, 8, 41).

It is tempting to suggest that the essential fingers in SUHW (fingers 6 to 10) make base-specific contacts in the 12-bp core consensus repeats in gypsy and that the helpful but nonessential fingers make nonspecific contacts in the flanking AT-rich sequences. Mutations in the core repeat can abolish SUHW binding (11), but the flanking sequences vary considerably in sequence. Furthermore, multiple mutations in the flanking sequences of a strong binding site reduce binding only two- to threefold (56, 61). However, methylation of particular G residues in the flanking sequences can prevent SUHW binding (11, 61), so SUHW does make at least some base-specific contacts in the flanking regions. Indeed, if fingers 6 through 10 contact base triplets in the major groove as do the fingers in the crystallized Zif268-DNA complex (45), then they should contact a minimum of 15 bp in a sequence-specific manner.

If fingers 6 through 10 contact neighboring triplets in the consensus repeat, the linker sequence between them is likely to be important. Of all the fingers in SUHW, only fingers 6 through 9 are tightly spaced and separated by a conserved linker sequence (TGEK/RP) found in several other zinc finger proteins. Mutagenesis experiments have revealed that this linker sequence is important in high-affinity DNA binding of TFIIIA (3a, 6), and it has been suggested that it accommodates wrapping of neighboring fingers around the DNA. It is also intriguing that finger 10, the one finger essential for binding gypsy that is not associated with this linker, is also not required for female fertility.

X-ray crystallography of a complex between the GLI multifinger protein revealed fingers that make contacts only with the phosphate backbone (46). Certain fingers in the TFIIIA protein extend across the minor groove instead of making contacts in the major groove (5, 26, 27). It is possible that the beneficial but nonessential fingers in SUHW (fingers 1 to 3 and 11) contribute to binding by these type of contacts. It is also possible that mutations in some of the nonessential fingers affect binding by changing protein conformation. Some of the nonessential fingers in SUHW may also be involved in recognizing different sequences. Although *su(Hw)* mutants do not display obvious phenotypes other than female sterility and suppression of gypsy insertion alleles, SUHW binds to several sites on polytene chromosomes that do not contain gypsy (24, 62).

Enhancer-blocking domains of SUHW. A question that remains unresolved is how SUHW blocks enhancers once it has bound to gypsy. Previous studies on a series of mutant *su(Hw)* alleles and some SUHW deletion mutants have indicated that a heptad repeat similar to leucine zippers (40) in the C-terminal domain is important for blocking enhancers in the *yellow* gene (24). The SUHW C-terminal domain, however, is also involved in altering DNA structure (58), and changes in DNA

structure could interfere with DNA looping involved in enhancer-promoter interactions.

If SUHW blocked different enhancers by different mechanisms, it might be expected that the protein domains involved would differ. With all four of the gypsy insertion alleles tested, however, the region located in the C terminus of SUHW between residues 737 and 880 appears to be the most important for enhancer blocking. The simplest interpretation is that the 737-to-880 region contains a single enhancer-blocking domain that functions by the same or very similar mechanism in all four genes. Consistent with this idea, the longest continuous stretch of conserved amino acids in SUHW (outside of the zinc finger domain) between *D. melanogaster*, *Drosophila ananassae*, and *Drosophila virilis* extends from residue 721 through residue 865 (24). The lesser role of the N-terminal domain of SUHW in enhancer blocking is consistent with the lower evolutionary conservation of the N-terminal domain between three species.

What is the biochemical function of the major enhancer-blocking domain? How the major enhancer-blocking domain of SUHW functions remains unknown. It overlaps but is not congruent with the C-terminal domain required to alter DNA structure (58). Many mutant proteins that only weakly block enhancers, such as the mutant lacking residues 738 to 780, distort DNA (58), indicating that distortion is not sufficient to block enhancers. We cannot rule out, however, the possibilities that DNA distortion contributes to enhancer blocking or that small changes in the extent or type of distortion that are difficult to detect may lead to loss of enhancer-blocking activity.

It appears most likely that the enhancer-blocking domain of SUHW, which is primarily acidic in nature, interacts and interferes with proteins required for enhancer function. These interactions, however, are unlikely to involve a leucine zipper because the heptad repeat stretching from residues 730 to 775 is not congruent with the major enhancer-blocking domain. A protein lacking the first two critical residues of the heptad repeat (Δ706–736) displays intermediate-to-strong blocking in all four gypsy insertions examined. Therefore, the enhancer-blocking domain begins within, and is significantly larger than, the heptad repeat. Although the latter part of the heptad repeat may be functional, it is also unlikely to be a leucine zipper because it is interrupted near the middle by two consecutive proline residues in the *D. ananassae* homolog and by a single proline in the same position in the *D. virilis* homolog (24).

SUHW may interact with the transcription factor-like products of the *E(var)3-93D* gene, which positively regulate expression of homeotic genes (10). The *mod(mdg4)*^{u1} mutation is a Stalker element insertion into *E(var)3-93D* (17, 18). Unlike null alleles, *mod(mdg4)*^{u1} is recessive viable, and when it is homozygous, it enhances the y^2 gypsy insertion, leading to loss of gene activity in all tissues (17, 18). However, *mod(mdg4)*^{u1} suppresses other gypsy insertions, such as ct^6, and has no effects on others such as f^1 (17). Intriguingly, *mod(mdg4)*^{u1} even suppresses the sc^1 gypsy insertion in some tissues and enhances it in others. *su(Hw)* mutations still suppress y^2 in the presence of the *mod(mdg4)*^{u1} mutation, and therefore the repressive effects of *mod(mdg4)*^{u1} on y^2 appear to require SUHW (18). However, the gene and tissue dependence of the effects of the *mod(mdg4)*^{u1} mutation on gypsy insertions imply that the *E(var)3-93D* gene products are not targets of the major enhancer-blocking domain of SUHW. If they were, the *mod(mdg4)*^{u1} mutation would not have opposite effects on different gypsy insertions.

It is feasible that the SUHW enhancer-blocking domain interacts and interferes with factors that form chromatin structures that bring distant enhancers into physical proximity of the promoter. The position dependence of SUHW enhancer blocking is similar to that observed for the scs and scs′ elements from a heat shock gene cluster (35, 36, 63, 64), the *Mcp* and *Fab-7* insulators from the bithorax complex (16, 23, 33), and an insulator element from the chicken β-globin gene complex (4). Like many of these elements, SUHW protects a mini-*white* gene in a P element from chromosome position effects thought to be mediated by enhancers and silencers flanking the insertion sites (52). Because the *scs*, *scs'*, *Mcp*, *Fab-7*, and chicken insulators are correlated with differences in chromatin structure, it is feasible that SUHW alters chromatin structure. However, the possibilities that SUHW interferes with particular transcription factors or even components of the nuclear matrix that facilitate long-distance enhancer-promoter interactions cannot be ruled out.

Role of SUHW in oogenesis. The regions in SUHW required for female fertility overlap and may be congruent with the major enhancer-blocking domain (Fig. 3), suggesting that the SUHW activity that blocks enhancers is required for oogenesis. In homozygous *su(Hw)*^{2} mutants, oogenesis is blocked prior to yolk accumulation in the oocyte (21, 38) and after nurse cell polytene chromosome decondensation (21). Oogenesis may be blocked slightly earlier in *su(Hw)*^{V} mutants, which also occasionally display fused egg chambers (24). *su(Hw)* mRNA accumulates at approximately 10-fold-higher levels in the ovaries than in the rest of the body (21), and expression of a wild-type *su(Hw)* cDNA clone from a female germ line-specific promoter (15) restores fertility to *su(Hw)*^{2} homozygous females without inducing somatic gypsy insertion phenotypes (21). Together these observations suggest that SUHW regulates expression of one or more genes in the female germ line.

It is possible that the sites which SUHW binds to regulate oogenesis differ in sequence from the gypsy-binding sites. The *su(Hw)*^{E8} allele, a mutation in finger 7 (24) that blocks binding to gypsy DNA, causes female sterility, while the *su(Hw)*^{e2} mutation in finger 7 only reduces binding to gypsy DNA and does not interfere with oogenesis. The correlation between the in vitro binding activity of the finger 7 mutants and their ability to support oogenesis suggests that DNA binding is important for female fertility. However, the *su(Hw)*^{f} finger 10 mutation, which abolishes binding to gypsy and strongly suppresses gypsy insertions, retains the ability to support oogenesis. The simplest explanation is that the DNA sequences important for female fertility differ in sequence from the gypsy repeats such that SUHW does not require finger 10 to bind. Alternatively, it is possible that DNA binding is not required for female fertility and that the finger 7 mutations disrupt other interactions such as protein-RNA or protein-protein interactions important for oogenesis that the finger 10 mutation does not. If DNA binding is important for female fertility, however, determination of which of the other 10 fingers are essential for female fertility may allow in vitro-binding site selection experiments to isolate a consensus sequence for the sites critical for oogenesis.

ACKNOWLEDGMENTS

J.K. and B.S. contributed equally to this work.

We thank Len Freedman, Beth Blackwood, and Pam Geyer for helpful discussions; Steve Henikoff and Claude Desplan for insightful comments on the manuscript; and Michel Nussenzweig and Ziva Misulovin for providing antisera. We also thank an anonymous reviewer for information on published work indicating the role of the conserved linker sequence in DNA binding.

This research was supported by ACS Research Grant NP-715 to D.D. and NIH Cancer Center Support Grant NCI-P30-CA-08748 to the Memorial Sloan-Kettering Cancer Center.

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