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Repression of *hsp70* Heat Shock Gene Transcription by the Suppressor of Hairy-Wing Protein of *Drosophila melanogaster*

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The suppressor of hairy-wing [*su(Hw)*] locus of *Drosophila melanogaster* encodes a zinc finger protein that binds a repeated motif in the gypsy retroposon. Mutations of *su(Hw)* suppress the phenotypes associated with mutations caused by gypsy insertions. To examine the mechanisms by which *su(Hw)* alters gene expression, a fragment of gypsy containing multiple *su(Hw)* protein-binding sites was inserted into various locations in the well-characterized *Drosophila hsp70* heat shock gene promoter. We found no evidence for activation of basal *hsp70* transcription by *su(Hw)* protein in cultured *Drosophila* cells but observed that it can repress heat shock-induced transcription. Repression occurred only when *su(Hw)* protein-binding sites were positioned between binding sites for proteins required for heat shock transcription. We propose that *su(Hw)* protein interferes nonspecifically with protein-protein interactions required for heat shock transcription, perhaps sterically, or by altering the ability of DNA to bend or twist.

Several spontaneous mutations at numerous loci in *Drosophila melanogaster* are insertions of the 7.5-kb gypsy retroposon (26). The mutant phenotypes of nearly all gypsy insertion alleles are made less severe (suppressed) by mutations in suppressor of hairy-wing [*su(Hw)*] (26, 36). The *su(Hw)* locus encodes a protein that contains 12 zinc fingers (29), a motif found in the DNA-binding domains of several nucleic acid-binding proteins (24). The *su(Hw)* protein binds a region of gypsy DNA located approximately 200 bp downstream of the 5′ long terminal repeat (LTR). This region contains multiple direct repeats of the consensus sequence YRYTGCATAYYY (Y, pyrimidine; R, purine). The repeat motif is part of the binding site (7, 41), and residues between repeats also contribute (7).

Binding of *su(Hw)* protein to DNA can alter gene expression in more than one way. When gypsy is situated in the transcribed region of a gene and oriented in the same transcriptional direction, most gene transcripts are polyadenylated in the 5′ LTR (3, 6). Binding of *su(Hw)* protein is required for maximal use of the LTR poly(A) site (7). Potentiation of upstream polyadenylation sites, however, is not the only mechanism by which *su(Hw)* protein alters gene expression. In the suppressible yellow^2 (y^2) allele, the gypsy insertion is upstream of the yellow transcription start site (2, 12). It has been proposed that *su(Hw)* protein activates gypsy transcription (12, 28, 29) and thereby decreases yellow transcription indirectly through promoter interference (12). It has also been proposed that *su(Hw)* protein could act more directly, by preventing upstream enhancers from activating the yellow promoter (14, 15, 29).

To explore the ability of *su(Hw)* protein to activate or repress transcription, we inserted the *su(Hw)* protein-binding region of a gypsy element (from the suppressible bx^{34e} allele) into various locations within the *Drosophila hsp70* heat shock gene promoter. This promoter was chosen because it is well characterized and responsive to several different transcription activators (10, 11, 17, 19, 20, 22, 32, 43). We found no evidence for activation of the *hsp70* promoter by *su(Hw)* protein in cultured *Drosophila* cells. Indeed, *su(Hw)* protein-binding sites repress heat shock-induced transcription but only when positioned between sites that bind proteins required for heat shock-induced transcription. We propose that *su(Hw)* protein interferes nonspecifically with interactions between other DNA-bound proteins.

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MATERIALS AND METHODS

The *hsp70-cat* construct and its derivatives (B10, N6, B296, B304, and -HSE) are described elsewhere (5). Cloning of the 326-bp *Bal*I-*Bst*XI fragment of bx^{34e} gypsy containing *su(Hw)* protein-binding sites (BaBx) as a *Sal*I fragment into pGEM-1 was described previously (7). The B10-Gp and B10-Ga forms of *hsp70-cat* were made by blunting the *Sal*I BaBx fragment and cloning it into the *Kpn*I site of B10 with linkers. BaBx orientation was determined by restriction; the orientation in which the sense strand of gypsy was in the sense strand of *hsp70* was designated B10-Gp, and the opposite orientation was designated B10-Ga. N6-Gp, N6-Ga, -HSE-Gp, and -HSE-Ga were constructed by cloning into the unique *Kpn*I sites. AC-Gp and AC-Ga were constructed by cloning BaBx as an *Acc*I fragment into a *Cla*I site adjacent to the *Eco*RI site used to clone the *hsp70* promoter fragment in *hsp70-cat* (5). The entire B10-Gp *hsp70-cat* gene was recloned as a blunted *Hin*dIII-*Xho*I fragment into the unique *Hpa*I site of the Carnegie 20 (35) P-element transformation vector. The *hsp82-cat* (CAT82SVSX) cotransfection control gene is described elsewhere (6).

Transfection of Schneider 2 (S2) cells, germ line transformation, genetic crosses, heat shock, RNA isolation, and Northern (RNA) blot hybridization were performed as described elsewhere (6). An antisense CAT RNA probe was made from a pGEM-1 plasmid template containing a 795-bp bacterial sequence with the entire chloramphenicol acetyltransferase (CAT)-coding sequence (6). Transcript levels were quantitated by an exposure ratio procedure (6). Briefly, autoradiograms were made by exposing preflashed Kodak XAR X-ray film to Northern blots with the aid of an intensifying screen at −85°C. Several autoradiograms dif-

fering in exposure time were made from each blot. Relative amounts of transcript were estimated by comparing the exposure times for the two radioactive bands to give the same intermediate film density. This procedure is accurate for radioactive bands giving intermediate film densities in 2 to 72 h. Signals requiring significantly shorter or longer exposure times are roughly estimated.

RESULTS

We reasoned that if *su(Hw)* protein were a transcriptional activator, it might increase basal *hsp70* promoter transcription in the absence of heat shock, and that if it were a repressor, it might reduce the level of heat shock-induced transcription. Furthermore, because the protein-binding elements of the *hsp70* promoter and the protein-protein interactions required for maximal heat shock transcription are relatively well characterized, clues to the mechanism of activation or repression by *su(Hw)* protein might be obtained.

The *hsp70* gene requires two copies of a heat shock element sequence (HSE) for maximal heat shock transcription (1, 4, 8, 30, 39, 40, 42). The HSEs are located upstream of the transcription start site (−62 to −47 and −87 to −70) and are binding sites for heat shock transcription factor (HSF) (44, 46). In the current view, the promoter-proximal HSE (I) is an array of three perfect and one imperfect 5-bp units (NGAAN) and the upstream HSE (II) is an array of one perfect and three imperfect 5-bp units (47). HSF is thought to bind as a trimer, each monomer subunit contacting a 5-bp unit (31). HSF binding occurs after heat shock in vivo (45) and promotes transcription in vitro (27, 44). HSF binds cooperatively to the two HSEs in vitro (38, 44), indicating interactions between the protein molecules bound to the adjacent sites. Heat shock transcription shows a periodic dependence on the distance between the two HSEs in vivo (5). The period of ~10.5 bp is equivalent to a helical turn, indicating that protein-protein interactions are required for maximal transcription (5). Similar periodic dependence on the distance between the HSEs and downstream elements in vivo (5) indicates that interactions between HSF and other promoter-bound proteins (27, 45) are also required. When pBR322 DNA fragments on the order of 300 bp are inserted into these intervals, transcription no longer displays a dependence on the number of helical turns between the promoter elements, indicating that long DNA stretches can twist enough to allow the rotational alignment required for protein-protein interactions (5).

To examine the influence of *su(Hw)* protein on these promoter elements, we inserted the 326-bp *su(Hw)*-binding region of bx^{34e} gypsy (denoted BaBx) in both orientations into three locations in a cloned *hsp70* gene marked with bacterial CAT-coding sequences (*hsp70-cat*; 5): 150 bp upstream of the promoter-distal HSE (AC-Gp and AC-Ga; Fig. 1), between the two HSEs (B10-Gp and B10-Ga), and between the proximal HSE and the TATA box (N6-Gp and N6-Ga). The length of the BaBx gypsy DNA insertion, which contains eight copies of the consensus *su(Hw)*-binding repeat (Fig. 1), is sufficient to allow proper rotational alignment of the *hsp70* promoter elements, regardless of the number of helical turns. Two particular copies of the repeat bind *su(Hw)* protein with significantly greater affinity than do the others in vitro (7), but binding to other repeats is observed with high protein concentrations (7, 41). Insertions between the *hsp70* promoter elements were made with versions of *hsp70-cat* that contained *Kpn*I linker insertions. B10 (Fig. 1; 5) contains a 10-bp linker between the HSEs, and N6 (Fig. 1; 5) contains a 6-bp insertion between the proximal HSE and TATA box. Transcription of B10 is equivalent to that of *hsp70-cat*; transcription of N6 is reduced because the linker does not contain an integral number of helical turns (5). Insertions of appropriate lengths into the N6 linker restore transcription (5). BaBx was also inserted 51 bp upstream of the TATA box in an *hsp70-cat* gene lacking HSEs (-HSE in Fig. 1; 5). Transcription of the -HSE *hsp70-cat* gene is very low (5).

Drosophila S2 cultured cells were cotransfected with the constructs described above and a *hsp82-cat* gene (CAT-82SVSX; 6) as a quantitative control. DNA sequences containing *su(Hw)* protein-binding sites have been shown to potentiate polyadenylation sites in S2 cells (6, 7), and the repeat-binding activity partially purified from S2 nuclei (7) has been unambiguously identified as *su(Hw)* protein (unpublished observations) on the basis of the fact that it reacts with affinity-purified anti-*su(Hw)* protein antibody (41; kindly provided by Carl Spana). Two days posttransfection, half of each culture was subjected to heat shock (37°C for 20 min) prior to isolation of total cellular RNA (6). Transcripts were quantitated by Northern blot hybridization as described previously (6) with an antisense RNA probe directed against the CAT sequence present in the *hsp70-cat* constructs and the *hsp82-cat* control gene. The *hsp70-cat* transcript levels were normalized to the levels of the *hsp82-cat* control. Each of the values in Fig. 1 is the result of at least three independent experiments. Representative Northern blots are presented in Fig. 2. Because the constructs differed only upstream of the transcription start site, we assume that transcript levels reflect differences in transcription.

Binding sites for *su(Hw)* protein do not enhance basal *hsp70* promoter transcription in S2 cells. In the absence of heat shock, *hsp70-cat* (B10, which contains a 10-bp linker insertion between the HSEs) gave a reproducible basal level of transcript (Fig. 1, B10; Fig. 2, lanes 1 and 15). None of the BaBx insertions gave higher levels of basal transcript. Indeed, transcript was not detected when BaBx was inserted in either orientation, between the HSEs (Fig. 1, B10-Gp and B10-Ga; Fig. 2, lanes 4 and 5) or between the proximal HSE and the TATA box (Fig. 1, N6-Gp and N6-Ga; Fig. 2, lanes 17 and 18). The level was reduced three- to sixfold when BaBx was inserted upstream of both HSEs (Fig. 1, AC-Gp and AC-Ga; Fig. 2, lanes 6 and 7). Insertions of 296 or 304 bp of DNA derived from pBR322 between the HSEs (5) actually increased the level of basal transcript two- to threefold (Fig. 1, B296 and B304; Fig. 2, lanes 2 and 3).

These observations indicate that *su(Hw)* protein does not activate *hsp70* transcription in S2 cells. We considered the possibility that the HSEs might interfere, but the BaBx constructs lacking HSEs also did not give detectable transcript levels (Fig. 1, -HSE-Gp and -HSE-Ga; Fig. 2, lanes 21 and 22).

Current information regarding basal *hsp70* transcription is insufficient to interpret the negative effects of *su(Hw)* protein-binding sites on basal transcript levels. Most basal transcription appears not to be stress induced and HSF dependent because basal and heat shock transcription respond differently to insertions. For example, insertion of *su(Hw)* protein-binding sites at −155 reduced basal transcript levels three- to sixfold without reducing heat shock-induced transcripts (B10 versus AC-Gp and AC-Ga; Fig. 1 and 2). In contrast, the linker insertion in N6 reduced heat shock transcripts more that fivefold while reducing basal transcripts only twofold (B10 versus N6; Fig. 1 and 2). The

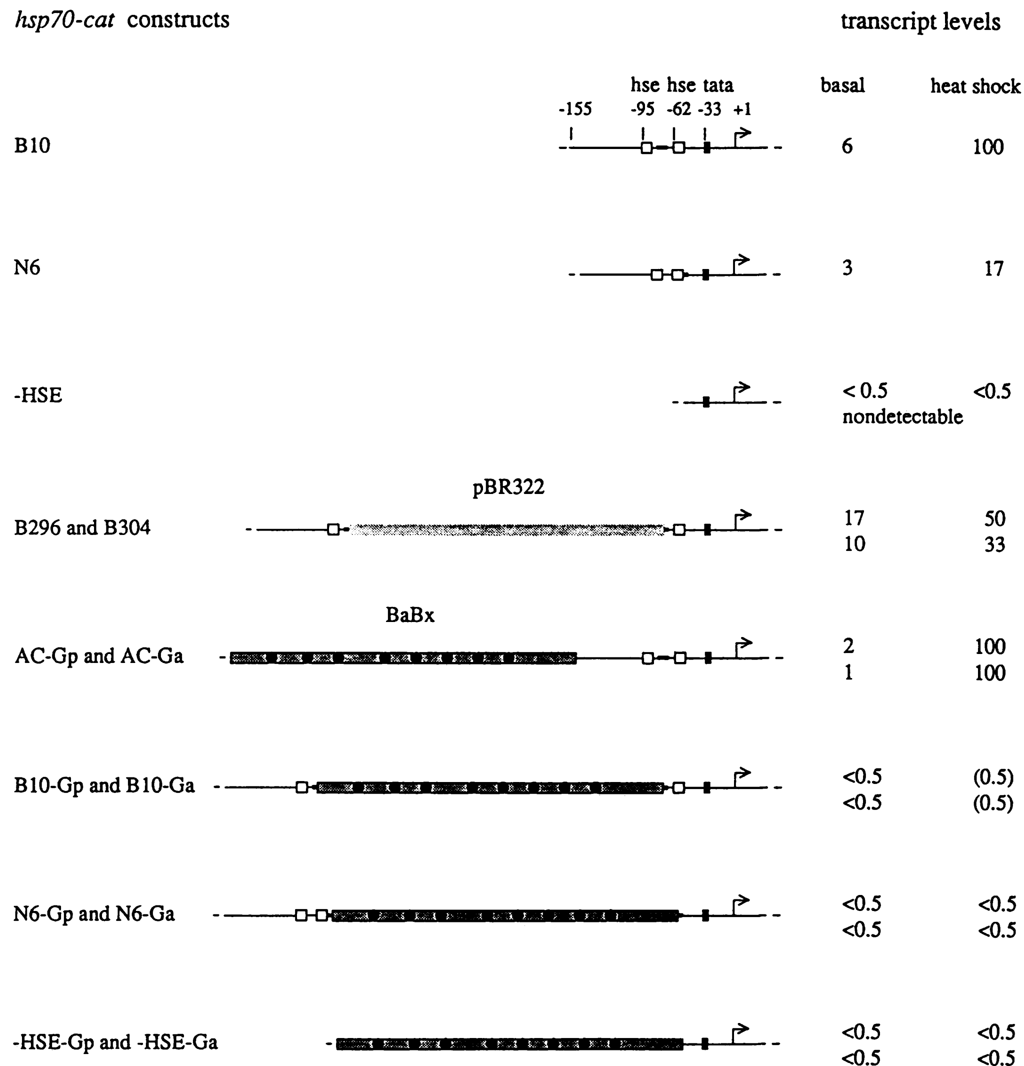

FIG. 1. Structures and transcript levels of *hsp70-cat* heat shock gene constructs. The diagrams display the 5′ regions of the various *hsp70-cat* constructs; numbers on the right indicate the relative levels of basal and heat shock-induced transcript obtained after transfection of S2 cells. Transcript levels were quantitated by the exposure ratio procedure described elsewhere (6) and normalized to the cotransfected *hsp82-cat* gene transcript level (see Fig. 2). The *hsp70-cat* transcript level obtained with the B10 gene was designated 100. All values are based on at least three independent experiments and varied less than 25% between experiments. The parentheses around the heat shock-induced levels for the B10-Gp and B10-Ga constructs indicate that these values are approximations. For simplicity, only the 5′ region of *hsp70-cat* (5) is shown. The diagrams are drawn to scale; +1 is the transcription start site. The open boxes (at −95 and −62 bp relative the start of transcription in B10) represent the HSEs, and the filled box (at −33 in B10) is the TATA box. Thick lines indicate linker insertions, stippled gray boxes represent pBR322 insertions, and stippled gray boxes with borders represent BaBx gypsy fragment insertions. Potential *su(Hw)* protein-binding sites are represented by filled circles.

-HSE construct (Fig. 1) gave undetectable basal transcript levels, indicating that *hsp70* sequences between −155 and −49 are important, but this finding does not rule out potential involvement of plasmid vector sequences. These uncertainties make it difficult to evaluate the negative effects of *su(Hw)* protein-binding sites.

***su(Hw)* protein-binding sites repress heat shock transcription when they are positioned between heat shock promoter elements.** The effect of *su(Hw)* protein-binding sites on heat shock-induced transcription varied dramatically with the site of insertion. Insertion upstream of the HSEs had no detectable effect on heat shock transcript levels (Fig. 1, B10 versus AC-Gp and AC-Ga; Fig. 2, lanes 8 versus lanes 13 and 14 and lane 23 versus lane 27), while insertion between *hsp70* promoter elements drastically reduced transcript levels. Insertion between the HSEs reduced heat shock transcript levels at least 180-fold (Fig. 1, B10-Gp and B10-Ga; Fig. 2, lanes 11 and 12), and transcript was not detectable with BaBx insertions between the proximal HSE and TATA box (Fig. 1, N6-Gp and N6-Ga; Fig. 2, lanes 25 and 26). Reduction of heat shock transcription by BaBx insertions cannot be attributed solely to increased distance between promoter elements because pBR322 insertions of similar lengths between the HSEs reduced the level of transcript only two- to threefold (Fig. 1, B10 versus B296 and B304; Fig. 2, lane 8 versus lanes 9 and 10), confirming previous observations by others (5). Although pBR322 DNA insertions between the proximal HSE and the TATA box (into the linker in N6)

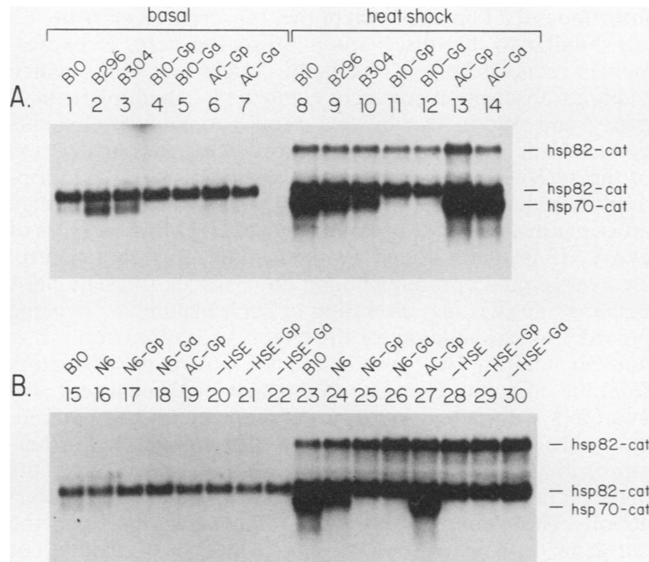

FIG. 2. Northern blot hybridization of *hsp70-cat* transcripts from S2 cells. Panels A and B are autoradiograms of Northern blots from two independent transfection experiments. Each lane contained 5 μg of total cellular RNA isolated from cells before (lanes 1 to 7 and 15 to 22) or after (lanes 8 to 14 and 23 to 30) heat shock. The lanes contain RNA from S2 cells cotransfected with 1 μg of *hsp82-cat* control gene (CAT82SVSX; 6) per ml and 2 μg of the indicated *hsp70-cat* genes per ml. The *hsp70-cat* transcript is 1.6 kb, and the *hsp82-cat* transcripts are 1.8 kb (spliced) and 3.4 kb (unspliced). For panel A, the X-ray film was preflashed and exposed for 46 h at −85°C with an intensifying screen; for panel B, the exposure was 20 h under the same conditions.

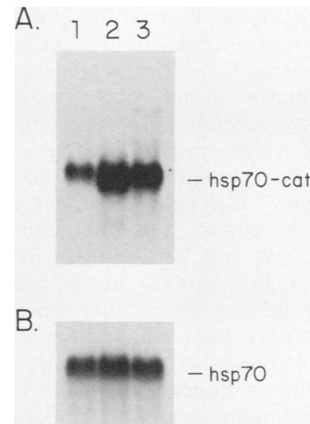

FIG. 3. Northern blot hybridization of *hsp70-cat* transcripts from pupae with the B10-Gp *hsp70-cat* gene (K18 chromosome) and different *su(Hw)* alleles. The panels are autoradiograms of the same Northern blot hybridized to antisense RNA probes specific for *hsp70-cat* (A) and endogenous *hsp70* (B) transcripts. The lanes contained 2.5 μg of total cellular RNA isolated from heat-shocked pupae with the following genotypes: lane 1, y^2 w^a ct^6 f^1; K18; lane 2, y^2 w^a ct^6 f^1; K18; $su(Hw)^2/su(Hw)^f$; lane 3, y^2 w^a v^1; K18; $su(Hw)^{f3}$. For panel A, the X-ray film was preflashed and exposed for 18 h at −85°C with an intensifying screen; for panel B, the film was exposed for 30 min at room temperature without a screen.

were not examined in this study, they have also been observed to reduce the level of heat shock transcript twofold relative to the *hsp70-cat* level (5). We postulate, therefore, that repression of *hsp70* transcription by BaBx inserts between promoter elements is due in part to the *su(Hw)* protein-binding sites.

Maximal repression of *hsp70-cat* transcription in transformed pupae requires wild-type *su(Hw)* alleles. To confirm that the negative effects of BaBx insertions on heat shock transcription were dependent on *su(Hw)* protein, flies were transformed with a Carnegie 20 P-element vector containing an *hsp70-cat* gene with BaBx between the HSEs (B10-Gp; Fig. 1). In one of the six transformed lines obtained (K18), the insertion was on chromosome 2 and homozygous viable. Integrity of the insertion in K18 was confirmed by restriction enzyme digests and Southern blot hybridization analysis of genomic DNA (not shown). Because *su(Hw)* is on chromosome 3, K18 was used construct stable fly stocks with different *su(Hw)* genotypes and with suppressible gypsy insertion alleles on the X chromosome to confirm the *su(Hw)* genotype. The level of *hsp70-cat* transcript in heat-shocked pupae was observed to be three- to fourfold higher, when normalized to the endogenous *hsp70* gene transcript level, in pupae with mutant *su(Hw)* alleles (Fig. 3, lanes 2 [y^2 w^a ct^6 f^1; K18; $su(Hw)^2/su(Hw)^f$] and 3 [y^2 w^a v^1; K18; $su(Hw)^{f3}$]) than in flies with wild-type *su(Hw)* (Fig. 3, lane 1 [y^2 w^a ct^6 f^1; K18]). This result is consistent with the 3- to 10-fold effect of these leaky (29) *su(Hw)* alleles on the level of RNA polyadenylated in the 5′ LTR of gypsy in pupae (6) and on the 4- to 6-fold effect on the level of *su(Hw)* DNA-binding activity in pupal nuclear extracts (7). We conclude that *su(Hw)* protein is required for maximal repression of *hsp70-cat* transcription.

Transcript of the *hsp70-cat* gene containing *su(Hw)* protein-binding sites between the HSEs (B10-Gp; Fig. 1) was reduced at least 180-fold and barely detectable in transfected S2 cells (Fig. 2, lane 11) yet was easily detected in transformed pupae, even in the presence of wild-type *su(Hw)* alleles (Fig. 3, lane 1). This was true for the six transformed lines obtained (not shown). Although *hsp70-cat* transcripts may be more stable in pupae, we believe that because S2 cell nuclei contain more *su(Hw)* DNA-binding activity than wild-type pupal nuclei (7), repression of *hsp70-cat* transcription is less in pupae than in S2 cells. We estimate the specific activity of *su(Hw)* DNA-binding activity in S2 nuclear extracts to be at least 10-fold higher than in wild-type pupal nuclear extracts (7; unpublished observations).

DISCUSSION

To test whether the zinc finger protein encoded by the *Drosophila su(Hw)* locus can influence transcription, we inserted a fragment of the bx^{34e} gypsy element (BaBx) containing multiple binding sites for *su(Hw)* protein (7) into various positions in the 5′ control region of a cloned *hsp70* heat shock gene promoter. The insertions did not activate *hsp70* transcription in S2 cells and repressed heat shock transcription only when inserted between protein-binding elements required for maximal heat shock transcription. Repression of heat shock transcription due to a BaBx insertion between the heat shock transcription factor-binding sites (HSEs) was at least partially alleviated in pupae with *su(Hw)* mutations.

These observations do not support the hypothesis that *su(Hw)* protein is a transcription activator. The *hsp70* promoter is sensitive to a variety of upstream activators (10, 11, 17, 19, 20, 22, 32, 43); even certain pBR322 DNA insertions can activate transcription in the absence of heat shock (Fig. 1, B296 and B304, Fig. 2, lanes 2 and 3). If *su(Hw)* protein is

an activator, therefore, it must be promoter specific or require a coactivator not present in S2 cells. It also possible that the high level of *su(Hw)* protein in S2 cells (7) is sufficient to sequester a significant fraction of a required factor and thereby squelch (16) transcription.

The ability of *su(Hw)* protein-binding site insertions between *hsp70* promoter elements to repress *hsp70* transcription can be considered in light of the current knowledge of the heat shock promoter. It is postulated that the DNA between the HSEs, and between the HSEs and TATA element, bends to allow protein-protein contacts (5), similar to bending proposed to occur in other genes (9, 34). Because short DNA insertions that rotationally misalign the protein-binding elements cannot twist sufficiently to allow realignment, transcription is dependent on the number of helical turns between promoter elements (5). Helical periodicity is not observed with insertions approximately 300 bp in length, indicating that longer insertions do allow sufficient twist (5). In the absence of other considerations, the BaBx fragment containing *su(Hw)* protein-binding sites is long enough to allow rotational alignment of the heat shock promoter elements. Indeed, significant transcription was observed with the *su(Hw)* protein-binding insertion between the HSEs in pupae with *su(Hw)* mutations (Fig. 3, lanes 2 and 3). The *su(Hw)* protein binding to the inserted DNA, therefore, must hinder the protein-protein interactions required for heat shock transcription. It could interact specifically with one or more of the proteins required, preventing it from interacting with its proper partner, or it could interfere nonspecifically by either sterically hindering protein interactions or placing constraints on the bending or twisting of DNA.

If the *su(Hw)* protein interacted specifically with one or more of the heat shock transcription factors, one would predict that insertions of *su(Hw)* protein-binding sites upstream of the HSEs would repress heat shock transcription. Insertion of BaBx in either orientation upstream of the HSEs did not interfere. Furthermore, BaBx insertions 0.5 kb downstream of the *hsp82* gene transcription start site do not interfere with *hsp82* heat shock gene transcription (6, 7). These observations contrast with active repression by the engrailed homeodomain protein, which apparently interacts specifically with other proteins and represses transcription when bound upstream of control elements (22a). It is likely, therefore, that *su(Hw)* protein interferes nonspecifically with the protein-protein interactions required for *hsp70* transcription.

Detailed knowledge of the protein structures is necessary to determine whether *su(Hw)* protein can sterically block interactions between heat shock transcription factors. The available information, however, does not favor this possibility. The closest potential *su(Hw)* protein-binding site and HSE in any of the *hsp70-cat* constructs would leave a 30-bp gap between the DNase I footprints of *su(Hw)* and HSF, as determined from published data (7, 42). It is very unlikely, therefore, that bound *su(Hw)* protein interferes sterically with HSF binding or with interactions between bound HSF and other proteins. A modified z2 homeodomain protein (18) can bind between a glucocorticoid response element and a TATA box without sterically blocking activation even though the homeodomain protein-binding site is only 40 bp downstream of the glucocorticoid response element and 14 bp upstream of the TATA box (22a). We prefer the hypothesis, therefore, that *su(Hw)* protein represses by placing constraints on DNA bending or twisting.

We also favor this hypothesis on the basis of evidence from the yellow and cut loci. In the y^2 allele, a gypsy insertion is 0.7 kbp upstream of the transcription start site (2, 12). Similar to the observations presented here, y^2 expression is reduced only in the body cuticle and wing, which require transcription enhancer elements located upstream of gypsy and not in tissues that require enhancer elements downstream of gypsy (13). Mutation of *su(Hw)*, or deletion of the *su(Hw)* protein-binding sites (14), restores transcription dependent on the upstream elements, even though they are separated from the promoter by several kilobase pairs of gypsy. If proteins bound to the yellow upstream control elements contact proteins bound downstream through DNA bending and twisting, alteration of such bending or twisting provides an explanation for the effects of *su(Hw)* on y^2. It is unlikely that *su(Hw)* protein blocks movement of factors from the upstream elements to the promoter because it also interferes with activation of a yellow gene on the homologous chromosome by the upstream elements (15). Furthermore, the distance separating the yellow control elements makes it unlikely that *su(Hw)* protein sterically blocks interactions. An even more extreme example is provided by the cut gene, in which a transcription enhancer activating cut expression in the wing margin is located 80 kbp upstream of the region known to be transcribed (5a), yet gypsy insertions at various positions in the 80-kbp intervening region block wing margin expression in a *su(Hw)*-dependent fashion (21).

Previously observed repression mechanisms include competition between the repressor and an activator for binding sites; quenching, in which repressor interferes with the function of a bound activator; and direct repression, in which the repressor blocks the activity of the basal transcription apparatus (reviewed in reference 25). The mechanism proposed for the *su(Hw)* protein belongs to the quenching class, although in most previously described examples, specific interactions between the repressor and activator have been postulated (18, 23, 37). It may be advantageous for gypsy to repress transcription of genes into which it inserts; if this is the case, a mechanism that does not require specific protein-protein interactions would be valuable.

The *su(Hw)* protein has been shown to potentiate upstream polyadenylation sites (7) and now to repress transcription of the *hsp70* promoter. These activities are sufficient to explain the mutagenic effects of gypsy element insertions in both transcribed and nontranscribed regions. The *su(Hw)* protein, however, binds to 100 to 200 sites in salivary gland polytene chromosomes, in a stock containing only a few gypsy elements (41). On the basis of the observed activities of *su(Hw)* protein, we find it reasonable to speculate that a function of *su(Hw)* protein is to functionally separate genes in close physical proximity and to prevent them from interfering with each other. For example, by potentiating poly(A) sites, it can reduce readthrough transcription (7) and thereby reduce promoter occlusion in which transcription from an upstream promoter interferes with transcription from a downstream promoter (33). Repression of transcription by interfering nonspecifically with other protein-protein interactions could prevent inappropriate activation (or repression) of a promoter by protein-binding elements of a neighboring gene. This hypothesis is consistent with lack of a particular phenotype in *su(Hw)* mutants, as *su(Hw)* protein would not be required for expression of particular genes but rather would be required to ensure that some genes are expressed more distinctly.

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