DSP1, an HMG-like Protein, Is Involved in the Regulation of Homeotic Genes

M. Decoville, E. Giacomello,[1] M. Leng and D. Locker

Centre de Biophysique Moléculaire, CNRS UPR 4301, Conventionnée avec l'Université d'Orléans, 45071 Orléans, France

Manuscript received February 18, 2000
Accepted for publication September 20, 2000

ABSTRACT

The Drosophila *dsp1* gene, which encodes an HMG-like protein, was originally identified in a screen for corepressors of Dorsal. Here we report that loss of *dsp1* function causes homeotic transformations resembling those associated with loss of function in the homeotic genes *Sex combs reduced* (*Scr*), *Ultrabithorax* (*Ubx*), and *Abdominal-B*. The expression pattern of *Scr* is altered in *dsp1* mutant imaginal discs, indicating that *dsp1* is required for normal expression of this gene. Genetic interaction studies reveal that a null allele of *dsp1* enhances *trithorax*-group gene (trx-G) mutations and partially suppresses *Polycomb*-group gene (Pc-G) mutations. On the contrary, overexpression of *dsp1* induces an enhancement of the transformation of wings into halteres and of the extra sex comb phenotype of *Pc*. In addition, *dsp1* male mutants exhibit a mild transformation of A4 into A5. Comparison of the chromatin structure at the *Mcp* locus in wild-type and *dsp1* mutant embryos reveals that the 300-bp DNase I hypersensitive region is absent in a *dsp1* mutant context. We propose that DSP1 protein is a chromatin remodeling factor, acting as a trx-G or a Pc-G protein depending on the considered function.

THE family of HMG-box proteins, originally defined by the presence of a common DNA-binding domain called the HMG box, includes diverse regulatory proteins (Bianchi *et al.* 1992). The HMG box is a highly conserved basic motif, 70–80 amino acids in length, that adopts an L-shaped three-dimensional structure and is responsible for DNA-binding activity (Read *et al.* 1995). HMG-box proteins preferentially bind to curved microcircles or distorted DNA structures such as four-way junctions, cisplatin-modified DNA, and S-S tethered DNA. The HMG-box proteins are divided in two classes according to the sequence conservation and the number of their HMG boxes (Grosschedl *et al.* 1994). Proteins belonging to the first class are generally transcription factors that bind to specific DNA sequences. They contain only one HMG box and are expressed in restricted cell types. They are exemplified by the human sex-determining factor SRY (Sinclair *et al.* 1990), the lymphoid enhancer binding factor Lef1 (Travis *et al.* 1991), or the T-cell factor Tcf-1 (Waterman *et al.* 1991). The second class includes a larger number of nuclear proteins that contain two or more tandem HMG boxes and bind to DNA in a relatively sequence-aspecific manner. The archetype of this class are the mammalian HMG 1/2 proteins. *In vitro* studies have shown that these proteins are able to remodel chromatin and participate in DNA replication, nucleosome assembly, and transcription (Bonne *et al.* 1982; Bonne-Andrea *et al.* 1984; Tremethick and Molloy 1988; Singh and Dixon 1990). Recently, Calogero *et al.* (1999) have established that HMG1 is not essential for packaging DNA into chromosomes, or for embryonic and fetal development in mouse. Nevertheless, HMG1 is required for specific gene regulatory processes after birth. Despite intensive studies, the biological functions of these proteins still remain elusive.

Lehming *et al.* (1994) isolated a new HMG1-like protein from Drosophila as a corepressor of Dorsal protein that was named DSP1 (Dorsal switch protein). This protein contains two HMG boxes, a small acidic tail, and two N-terminal glutamine-rich regions. DSP1 is expressed throughout embryogenesis, ubiquitously during the first stages (cellular blastoderm and germ band extension) and then exclusively in the central nervous system during the last stages (stages 15–16). In adults, the protein is detected only in ovaries and in brain (Mosrin-Huaman *et al.* 1998). Lehming *et al.* (1998) have proposed that DSP1 could be part of a repressing chromatin complex containing SP100 and HP1, a component of Drosophila heterochromatin involved in position effect variegation (PEV; Eissenberg *et al.* 1990, 1992). PEV occurs when a euchromatic gene is transposed adjacent to a segment of heterochromatin. Expression of the transposed gene is repressed in some cells and not in others, producing a mosaic phenotype. Many mutations that enhance or suppress PEV have been isolated (Locke *et al.* 1988; Sinclair *et al.* 1989, 1992; Wustmann *et al.* 1989; Dorn *et al.* 1993), and most of them identified genes that encode nonhistone chromatin proteins. Some of these proteins share a region of sequence similarity with other chromatin regulators such as Pc-G or trx-G proteins that control the expression of homeo-

Corresponding author: M. Decoville, CBM, CNRS, rue Charles Sadron, 45071 Orléans cedex 2, France. E-mail: decovil@cnrs-orleans.fr

[1] *Present address:* Institut Curie, U.M.R. 144, 26 rue d'Ulm, 75248 Paris cedex 05, France.

tic genes. For example, Pc shares a region of sequence similarity with *Su(var)205*, which encodes HP1 (Eissenberg *et al.* 1990; Paro and Hogness 1991), and ***Su(var)-309*** shares a domain with ***E****nhancer of zeste* [*E(z)*], a Pc-G gene, and with ***trx*** (SET domain; Tsiersch *et al.* 1994). Pc-G and trx-G genes may act by modifying chromatin structure. Several groups have found that some Pc-G or trx-G genes act as suppressors or enhancers of PEV. Examples are *Enhancer of Polycomb* [*E(Pc)*; Sinclair *et al.* 1998a], *cramped* (Yamamoto *et al.* 1997), *Asx* (Sinclair *et al.* 1998b), and *Trl* (Farkas *et al.* 1994). These results have prompted us to investigate a possible function of *dsp1* in homeotic gene regulation.

Homeotic genes encode transcriptional factors that specify the identities of body segments in Drosophila. They are clustered in two complexes, the Antennapedia and Bithorax complexes (ANT-C and BX-C; Duncan 1987; Kaufman *et al.* 1990). In early embryos, the initial boundaries of homeotic gene transcription are controlled by segmentation genes. Later in development, the pattern of homeotic gene transcription is maintained by two groups of regulatory proteins, the *Polycomb*-group of repressors (Pc-G) and the *trithorax*-group of activators (trx-G; reviewed in Kennison 1995; Simon 1995; Pirotta 1997). Mutations in Pc-G genes cause homeotic transformations due to the ectopic expression of ANT-C and BX-C genes, resembling gain-of-function mutations of the BX-C and ANT-C. In contrast, mutations in trx-G genes cause homeotic transformations similar to loss-of-function mutations in BX-C and ANT-C, due to the failure to maintain expression of homeotic genes. It was proposed that Pc proteins package inactive homeotic genes into inaccessible complexes in the early embryo, therefore preventing their expression. Biochemical studies have demonstrated physical interactions between different members of Pc-G proteins. Genetic studies have suggested that transcription of homeotic genes is regulated by interaction between trx-G proteins. Three complexes containing trithorax group proteins have been identified (Papoulas *et al.* 1998). One of them, the BRM complex, is composed of at least seven major polypeptides, four of which are related to subunits of the yeast chromatin remodeling complexes SWI/SNF (Dingwall *et al.* 1995) and RSC (Cairns *et al.* 1996).

Here, we report the phenotype of a loss-of-function mutant of *dsp1* (named $dsp1^{1}$). We show that lack of *dsp1* product causes homeotic transformations. Results of genetic interactions with BX-C and ANT-C mutants suggest that DSP1 is involved in the regulation of several homeotic genes. $dsp1^{1}$ mutation suppresses the homeotic transformations observed in *Pc* heterozygotes and on the contrary enhances the trx-G mutant phenotype. Overexpression of *dsp1* results in enhancement of the *Pc* phenotype. Finally, analysis of the chromatin structure at the *Mcp* locus suggests that DSP1 could act as a chromatin remodeling factor. These results support the idea that *dsp1* could function as an activator or repressor, depending on the considered function.

MATERIALS AND METHODS

***In situ* hybridization:** *Scr* expression was monitored by whole mount embryo *in situ* hybridization using digoxygenin-labeled riboprobes. Probes were prepared according to the manufacturer's directions (Boehringer Mannheim Biochemicals, Mannheim, Germany). Prehybridization and hybridization conditions were based on the protocol described by Tautz and Pfeifle (1989) and conditions for embryos and imaginal discs were based on Masucci *et al.* (1990). The *Scr* riboprobe was generated from pGEM3Zf(+) containing a 1011-bp DNA fragment [nucleotides (nt) 1833–2844] obtained by PCR.

Drosophila strains and crosses: Flies were raised on standard medium at 22°. All mutations and chromosome aberrations are described in Lindsley and Zimm (1992) unless otherwise noted. Isolation of the *dsp1* null mutant was described previously (Mosrin-Huaman *et al.* 1998). $Ubx^{bx\text{-}83kb}/TM1$, $Pc^{11}/TM3$, $Ubx^{bx34e}/TM1$, $Antp^{D43}/TM$, and *Df(3R)P9/Dp(3;3)P5* were obtained from the Umea Drosophila Stock Center. $Df(1)19$, $f^{1}/C(1)RM$, $y^{1}\ shi^{1}\ f^{1}$; $Dp(1;Y)shi^{+3}$, y^{+}, $Scr^{4}/TM3$, $Scr^{s}/TM3$, $trx^{1}/TM1$, $trx^{E2}/TM6$, $trx^{E2}brm^{2}/TM6$, $ash2^{1}/TM6$, and *w*[*]; *P{w[+mC] = Gal4-HSP70.PB}2/CyO* were obtained from the Bloomington Fly Stock Center. $ash2^{x2}/TM3$, $ash1^{vw183}/TM3$, and w^{118} strains were kindly provided by A. Shearn and B. Limbourg-Bouchon, respectively. Oregon-R was used as wild-type reference strain.

Overexpression of *dsp1*: A *dsp1* transgenic strain was obtained by cloning a fragment of 1.3 kb spanning the whole *dsp1* open reading frame and obtained by reverse transcriptase (RT)-PCR into pUAST vector (generous gift from B. Limbourg-Bouchon). *P*-element-mediated germ-line transformation was done using standard procedures (Spradling and Rubin 1982a,b). The mini-*white* transformation marker in the pUAST transformation vector was designed to allow detection of transformants by the rescue of the *white* mutation in the recipient strain (w^{118}). The transgenic strain was controlled by PCR and Southern assays. Chromosomal linkage of construct was determined by segregation with respect to the balancer chromosomes *CyO* and *TM3*. A homozygous transgenic strain was established and maintained at 22°.

Virgin homozygous *dsp1* transgenic flies were mated to *w*[*]*; P{w[+mC] = Gal4-HSP70.PB}2/CyO* males. *w*[*];*P{w[+mC] = Gal4-HSP70.PB}2* virgin females were recovered and submitted to heat-shock treatment (3 heat shocks at 36° for 20 min, with equivalent recovery times at room temperature). Then, the females were crossed with $Pc^{11}/TM3$ males at 22°, and the progeny were recovered at different times after laying (0/24 hr, 24/40 hr, 40/48 hr, 48/72 hr, and >72 hr after heat shock). The same results were obtained for 0/24 hr and 24/40 hr.

Chromatin studies: Nuclei were prepared from 0–12-hr mass-collected embryos as described (Jowett 1986). The nuclei were incubated for 3 min at 25° with different concentrations of DNase I. The DNA was then purified by proteinase K treatment and phenol extraction and digested with *Eco*RI. After electrophoresis on an agarose gel, and blotting to nitrocellulose, the DNA was hybridized with a *Mcp* probe (PCR product corresponding to nt 8–2475).

RESULTS

***dsp1* mutant strain:** We have obtained by *P* mutagenesis a loss-of-function allele of *dsp1*. Molecular analysis has revealed a deletion of the *dsp1* open reading frame

that does not affect another transcription unit. This mutant does not produce detectable RNA or protein (Mosrin-Huaman *et al.* 1998). We have named this allele $dsp1^1$ and we use this nomenclature hereafter. $dsp1^1$ was isogenized with wild-type Oregon-R chromosomes by recombination around the *dsp1* locus and was maintained as a homozygous strain at 22°. Reynolds and Tanouye (1998) have proposed that *dsp1* is allelic to *bang senseless* (*bss*). *bss* mutants become paralyzed for several minutes following a vibration of the culture vial. Surprisingly, we do not observe this phenotype in $dsp1^1$ adults. To resolve this discrepancy we performed a complementation experiment between bss^1 and $dsp1^1$. Heterozygotes for bss^1 are distinguishable from homozygotes or hemizygotes by the length of time they remain paralyzed. The phenotype of $bss^1/dsp1^1$ heterozygotes and $+/bss^1$ was the same, indicating that bss^1 and $dsp1^1$ do complement. We observed the same result with other alleles of the *bss* gene. We concluded from this experiment that *bss* and *dsp1* are not alleles. To avoid confusion between the two genes, we propose that *dsp1* be named only *dsp1* and not *dsp1/bss*.

$dsp1^1$ homozygotes or hemizygotes died prematurely and exhibited very low fertility. The same phenotypes were observed in $dsp1^1/Df(1)19$ flies bearing a deletion including *dsp1*. Inactivation of *dsp1* also led to a reduction of the size of the sex comb in males. This phenotype was suppressed in $dsp1^1/shi^+$ *Y* males bearing on the Y chromosome a translocation of *13F* to *14F* X region. To confirm that the phenotypes observed were a result of a lack of *dsp1* function rather than an effect of other loci, a phenotype rescue test was performed by introducing an extra copy of the wild-type *dsp1* gene into the $dsp1^1$ background. The wild-type copy of *dsp1* rescued all phenotypes.

Lack of *dsp1* function induces homeotic transformations: Inspection of adults homozygous or hemizygous for the $dsp1^1$ allele has revealed various homeotic transformations. The first one corresponded to a T1 to T2 transformation. Adult males hemizygous for the $dsp1^1$ allele showed a reduced sex comb, with an average of 6 teeth instead of the 11 normally found in the wild type. The size of the sex comb in $dsp1^1/Y$ males never exceeded 9 teeth and was always reduced whatever the mother (homozygous or heterozygous for $dsp1^1$), suggesting that this phenotype is the result of an absence of *dsp1* function in the zygote. This phenotype mimics, to some extent, loss-of-function mutations in the homeotic gene *Sex combs reduced* (*Scr*). We have studied interactions of $dsp1^1$ with Scr^4, a loss-of-function mutation (Pattatucci *et al.* 1991). $dsp1^1/Y$; $Scr^4/+$ males showed an increase in the severity of the *Scr* phenotype; the size of the sex comb was greatly reduced and the average number of teeth was 4 in the double mutant *vs.* 6 in the Scr^4 or $dsp1^1$ single mutants. We have also studied interactions of $dsp1^1$ with Scr^S, a gain-of-function mutation corresponding to a transposition that does not disrupt any of the identified *Scr* loci and is sensitive to mutation in *Pc* (Pattatucci *et al.* 1991). $dsp1^1/Y$; $Scr^S/+$ males exhibited a less severe phenotype than the *Scr* mutant alone (Table 1): the number of T3 legs with a sex comb was highly diminished (30 *vs.* 100%) and the size of the sex comb on the T2 legs was reduced (2.5 *vs.* 4.5 teeth). These results prompted us to study the expression of *Scr* in $dsp1^1$ embryos and imaginal discs of third instar larvae (Figure 1). No difference was observed in wild-type and $dsp1^1$ embryos (Figure 1, A and B; only stage 17 is shown). In contrast, *Scr* expression in $dsp1^1$ T1 imaginal discs was severely lowered (Figure 1, C and D). In particular, the strong expression of *Scr* in cells between the central knob and peripheral margin of the disc was not detected in $dsp1^1$ imaginal discs. These cells are the progenitors of the adult anterior tarsus and tibia, including the sex combs of adult males (Bryant 1978). These data strongly suggest that *dsp1* participates in activation of *Scr* in imaginal discs.

The second homeotic transformation corresponded to a T3 to T2 transformation. Adults homozygous or hemizygous for the $dsp1^1$ allele showed partial homeotic transformations of metathoracic into mesothoracic structures, mainly in the anterior compartment. Generally, only one haltere was affected and the transformations included, to various extents, dorsal development of wing tissue in place of haltere or mesonotal tissue in place of metanotum. This phenotype resembles the one obtained for loss of function in the *Ubx* gene, especially with *bx* alleles. This led us to study the interactions of $dsp1^1$ with two *bx* alleles, bx^{34e} and bx^{83kd}. These two alleles correspond to insertions of transposable elements and show reductions of Ubx protein expression. In $dsp1^1/Y$; $bx/+$ males, the frequency of transformation of halteres into wings was enhanced by a factor >15 (Table 1). A similar result was obtained with each of the two *bx* alleles. The enhancement was dramatically reduced but not

TABLE 1

Interactions of the $dsp1^1$ allele with *Ubx*, *Scr*, and *Antp* mutations

	Females		
Males	Oregon-R	$\frac{dsp1^1}{dsp1^1}$	$\frac{dsp1^1}{dsp1^+}$
$dsp1^1/Y$; +/+	—	1202/4	—
$dsp1^+/Y$; $Ubx^{34e}/+$	787/0	407/14	600/2
$dsp1^+/Y$; $Ubx^{83kd}/+$	314/0	208/8	—
$dsp1^+/Y$; $Scr^S/+$	200/192	235/70	356/124
$dsp1^+/Y$; $Antp^{D43}/+$	152/30	200/4	—

Homozygous female genotypes were crossed to male genotypes. Resulting male progeny were examined for homeotic transformations such as haltere to wing (*Ubx*), third legs transformed to first leg (*Scr*), and antennae transformed to legs (*Antp*). Data are presented as number of flies examined/number of flies showing homeotic transformations.

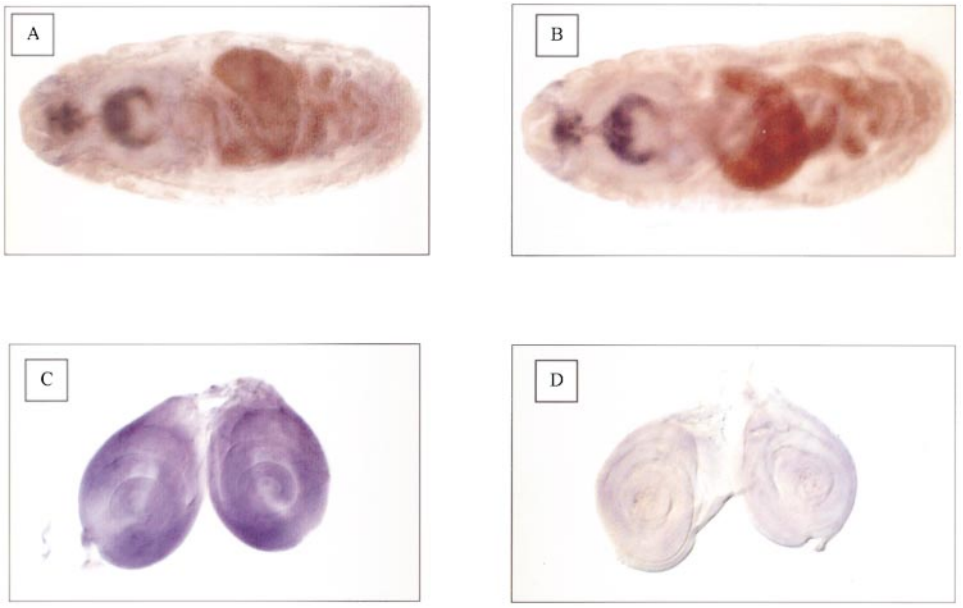

FIGURE 1.—Pattern of *Scr* expression in embryos and prothoracic imaginal discs. (A and C) Wild-type Oregon-R; (B and D) *dsp1*^{1} mutant. (A and B) Ventral views of embryos at approximately stage 17 of development (the anterior is on the left). (C and D) Prothoracic (T1) imaginal discs.

suppressed if females were heterozygous for *dsp1*^{1}, indicating that maternal DSP1 function is involved in a concentration-dependent manner.

The third homeotic transformation corresponded to an A6 into A5 transformation. About 25% of males hemizygous for the *dsp1*^{1} allele showed bristles on the A6 sternite, some of them bearing more than six bristles (Figure 2). As this phenotype is reminiscent of mutations in the *iab-6* regulatory region of *AbdB*, we studied the interaction between the *dsp1*^{1} allele and *Df(3R)P9*, a deletion of BX-C. About 90% of *dsp1*^{1}*/Y; Df(3R)P9/+* males exhibited bristles on the A6 sternite compared to 25% in *dsp1*^{1} or *Df(3R)P9* single mutants. This observation strongly suggests that DSP1 is involved in the regulation of the *iab-6* locus. In addition to the A6 to A5 transformation, we observed in ~50% of males patches of pigmentation on the A4 tergite, suggesting a partial transformation of A4 into A5 (Figure 2). This point is discussed later.

To know whether *dsp1* is involved in the expression of other homeotic genes, we looked at the interaction between *dsp1*^{1} and *Antp*^{D43}, a gain-of-function mutation of *Antennapedia* (Table 1). Analysis of the *dsp1*^{1} male progeny heterozygous for *Antp*^{D43} revealed that the number of homeotic transformations of antennae into legs was strongly reduced (~10 times). In addition, in the female progeny heterozygous for *dsp1*^{1} and *Antp*^{D43}, the frequency of homeotic transformations was also reduced (2 times). On the contrary, when the *Antp*^{D43} mutant was crossed with a *Pc*^{11} mutant, ~100% of the *Antp*^{D43}*/Pc*^{11} progeny exhibited transformation of antennae into leg. This result suggests that *dsp1* is also involved in expression of *Antennapedia* and acts in a concentration-dependent manner as it does for *Ultrabithorax*.

***dsp1* genetically interacts with trithorax-group and Polycomb-group genes:** The results described above suggest that *dsp1* is involved in the expression of several homeotic genes. Two groups of genes are known to control homeotic gene expression: the trx-G and the Pc-G genes. We studied genetic interactions between *dsp1*^{1} and various mutations of trx-G or Pc-G genes (Ta-

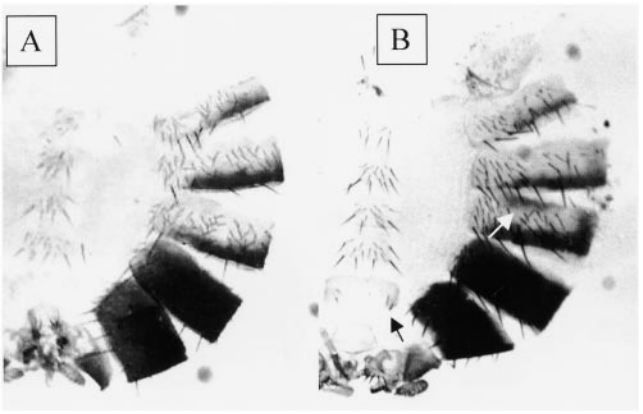

FIGURE 2.—Adult male phenotypes of hemizygous *dsp1*^{1} mutation. Whole mounts of abdominal male cuticles of wild type (A) or *dsp1*^{1} (B) are shown. Male abdomens were cut along the dorsal midline and flattened on a slide. The dorsal surface of each abdominal segment has a plate of hard cuticle called tergites. The ventral surface of abdominal segments is composed of pleura on the central midline of hard cuticle called sternites. In wild-type male (A), only the fifth and sixth tergites are pigmented. The sixth sternite is easily distinguished from the more anterior sternites by its different shape and by the absence of bristles. In *dsp1*^{1} male (B), the fourth tergite shows patches of pigmentation, suggesting ectopic activation of *iab-5* in A4 (white arrow). This activation probably does not take place in all cells. On the ventral side, bristles are found on the sixth sternite, indicating that *iab-6* is inactive, at least in some cells, in segment A6 (black arrow). The number of bristles observed on the sixth sternite can vary from one to more than six.

TABLE 2

Interactions of *dsp1*^{1} allele with trx-G and *Pc* mutations

Males	Females		
	Oregon-R	$\frac{dsp1^1}{dsp1^1}$	$\frac{dsp1^1}{dsp1^+}$
$dsp1^+/Y;\ trx^1/+$	878/0	822/25	—
$dsp1^+/Y;\ trx^{E2}/+$	508/0	380/60	538/24
$dsp1^+/Y;\ brm^2/+$	127/0	256/3	—
$dsp1^+/Y;\ ash1^{vv183}/+$	134/0	169/35	215/11
$dsp1^+/Y;\ ash2^1/+$	553/0	377/0	—
$dsp1^+/Y;\ ash2^{x2}/+$	124/0	184/1	—
$dsp1^+/Y;\ osa^2/+$	109/0	313/8	—
$dsp1^+/Y;\ Pc^{11}/+$	395/213	688/254	324/152

Homozygous female genotypes were crossed to male genotypes. Resulting male progeny were examined for homeotic transformations such as haltere to wing (trx-G) and second legs transformed to first leg (*Pc*). Data are presented as number of flies examined/number of flies showing homeotic transformations.

ble 2). Interaction with mutations in the *trx* gene was studied with two *trx* alleles: *trx*^{1} and *trx*^{E2}. In both cases, we observed an increase in the number of transformations of halteres into wings. This enhancement was more pronounced with the *trx*^{E2} allele (16%) than with the *trx*^{1} allele (3%). This can be explained by the hypomorph nature of the *trx*^{1} allele, which results from an insertion of 9 kb outside the coding sequences, and probably produces normal trx protein but at a reduced level (Ingham and Whittle 1980; Ingham 1985; Breen and Harte 1991). On the contrary, *trx*^{E2} is an amorph allele (Kennison and Tamkun 1988). A strong enhancement in transformation frequency of halteres into wings (21%) was also observed in *dsp1*^{1}*/Y; ash1*^{vv183}*/+* males. In contrast, interaction with mutations in other trx-G genes (*ash2* and *brm*) did not affect significantly the rate of homeotic transformations. As with *Ubx* alleles, the enhancement of transformations was reduced when the mothers were heterozygous for *dsp1*^{1}.

Interaction with *Pc* showed a decrease of the extra sex comb phenotype of *Pc* (Table 2): the number of T2 legs with sex comb was reduced (only 37% of T2 legs showed a sex comb in *dsp1*^{1}*/Y; Pc*^{11}*/+* flies *vs.* 54% in *dsp1*$^{+}$*/Y; Pc*^{11}*/+*). The number of T3 legs with a sex comb was also lower, 19% in *dsp1*^{1}*/Y; Pc*^{11}*/+* flies *vs.* 30% in *dsp1*$^{+}$*/Y; Pc*^{11}*/+*. It is worth noting that the size of the sex comb on T1 legs in the double mutant *dsp1*^{1}*/Y; Pc*^{11}*/+* was almost normal, as expected for two genes acting in an opposite manner in the same pathway.

Overexpression of *dsp1* enhances a *Polycomb* mutation: As loss of *dsp1* function led to a reduced expression of *Ubx* and *Scr*, we expected a perturbation of homeotic gene expression by an overexpression of *dsp1* and a subsequent ectopic expression of *Ubx* and *Scr*. To test it we used the Gal4/UAS system of induction to overexpress *dsp1*. As a driver we used *Gal4-HSP*, which is expressed after heat-shock treatments. Flies carrying the *Gal4-HSP* driver were crossed with those carrying the *UAS-dsp1* construct. Virgin females were recovered, submitted to heat shocks, and crossed with *Pc*^{11}*/TM3* males as described in materials and methods. The *Pc* offspring were analyzed for transformations of wings into halteres and for the extent of the extra sex comb phenotype of *Pc* in males. In control experiments, a majority of *Pc*^{11}*/+* flies showed normal wings and very few showed a mild transformation of the wing into haltere (Table 3). In contrast, when *UAS-dsp1* mothers were submitted to heat shock, the majority of the *Pc*^{11}*/+* progeny exhibited a mild transformation of wing into haltere and *Pc*^{11}*/+* male offspring showed a considerable increase in their number of T3 legs with a sex comb (Table 3). These results strengthen the hypothesis that *dsp1* is involved in the expression of different homeotic genes and could act as trx-G genes.

Absence of *dsp1* modified the chromatin structure at the *Mcp* locus: As already shown, *dsp1*^{1} flies exhibited a partial transformation of A4 into A5, which could be the result of an activation of *iab-5* in the A4 segment. The repression state of *iab-5* in the A4 segment is controlled by *Pc-G* genes and by a boundary region, the *Mcp* region, which ensures that *iab-4* and *iab-5* are functionally autonomous and that the activation state of *iab-4* does not spread into *iab-5*. The *Mcp* region is characterized by an unusual chromatin structure in embryos (Karch *et al.* 1994). One prominent nuclease hypersensitive region of ~300 bp has been identified. Deletion of this region leads to a transformation of A4 into A5. Thus it seemed to be of interest to determine whether the *Mcp* boundary region had the same chromatin structure in mutants lacking DSP1 protein. To examine the chromatin structure of the *Mcp* DNA segment, we prepared nuclei from 0–12-hr wild-type or *dsp1*^{1} embryos and digested them with DNase I. In the experiment shown in Figure 3, *Eco*RI-restricted chromatin digests were probed with a 2.5-kb DNA fragment spanning almost all the *Mcp* region (Figure 3A). As illustrated in the autoradiogram in Figure 3B, the wild-type 6.0-kb *Eco*RI *Mcp* fragment contained a prominent hypersensitive region, as revealed by the decrease of the amount of the full-length *Mcp* DNA fragment and the appearance of specific DNase cleavage products around 4.3 kb and 1.7 kb (Figure 3B, lanes 1–4). These DNase cleavage products are chromatin-specific as they are not detected in control digests of naked DNA (Figure 3B, lane 9). Such a result is in agreement with the location of the hypersensitive sites of the *Mcp* region described by Karch *et al.* (1994). When the *dsp1*^{1} 6.0-kb *Eco*RI *Mcp* fragment was DNase I digested, no specific DNase cleavage products appeared (Figure 3B, lanes 5–8). This result strongly suggests that the major DNase hypersensitive region of the *Mcp* boundary is absent in mutant *dsp1*^{1}

TABLE 3

Effect of DSP1 overexpression on the phenotype of polycomb

	Without heat shock		Heat shock	
Female genotypes	T3 to T1	Wing to haltere	T3 to T1	Wing to haltere
GAL4-HSP/+	159/55	255/7	134/60	230/34
UAS-dsp1/+ ; GAL4-HSP/+	216/75	167/7	308/197	416/232

Female *GAL4-HSP/+* or *UAS-dsp1/+ ; GAL4-HSP/+* were heat-shocked or not at 37° and crossed with $Pc^{11}/+$ males. Resulting male $Pc^{11}/+$ progeny were examined for homeotic transformations such as wing to haltere and third legs transformed to first legs. Data are presented as number of flies examined/number of flies showing homeotic transformations.

embryos and that DSP1 protein could act to remodel the chromatin structure at the *Mcp* locus.

DISCUSSION

***dsp1* is involved in homeotic gene expression:** Studies of the phenotype of a homozygous $dsp1^1$ mutant provide evidence that *dsp1* is involved in the determination of body segment identity. We show that $dsp1^1$ mutants exhibit homeotic transformations typical of loss-of-function mutants for the two homeotic genes *Ubx* and *Scr*, with halteres transformed into wings and a sex comb reduced in size on the T1 leg. In the case of *Scr*, we have shown that *Scr* expression is diminished in T1 imaginal discs in homozygous $dsp1^1$ mutants. Hemizygous $dsp1^1$ males also show a moderate transformation of A6 into A5, resembling mutants at the *iab-6* locus, and a mild transformation of A4 into A5, suggesting that *iab-5* is ectopically activated in A4. Furthermore, by studying genetic interaction between $dsp1^1$ and a gain-of-function mutation of *Antp*, we show that the absence of DSP1 alters the function of *Antp*. All these results argue that *dsp1* is implicated in the regulation of the function of homeotic genes.

***dsp1* could be a remodeling chromatin factor acting as a trithorax- or a Polycomb-group gene:** Two groups of genes are known to control the expression of homeotic genes: the trx-G genes and the Pc-G genes. In view of some phenotypic traits observed in the mutant lacking DSP1, it appears that *dsp1* could be classified as a trx-G

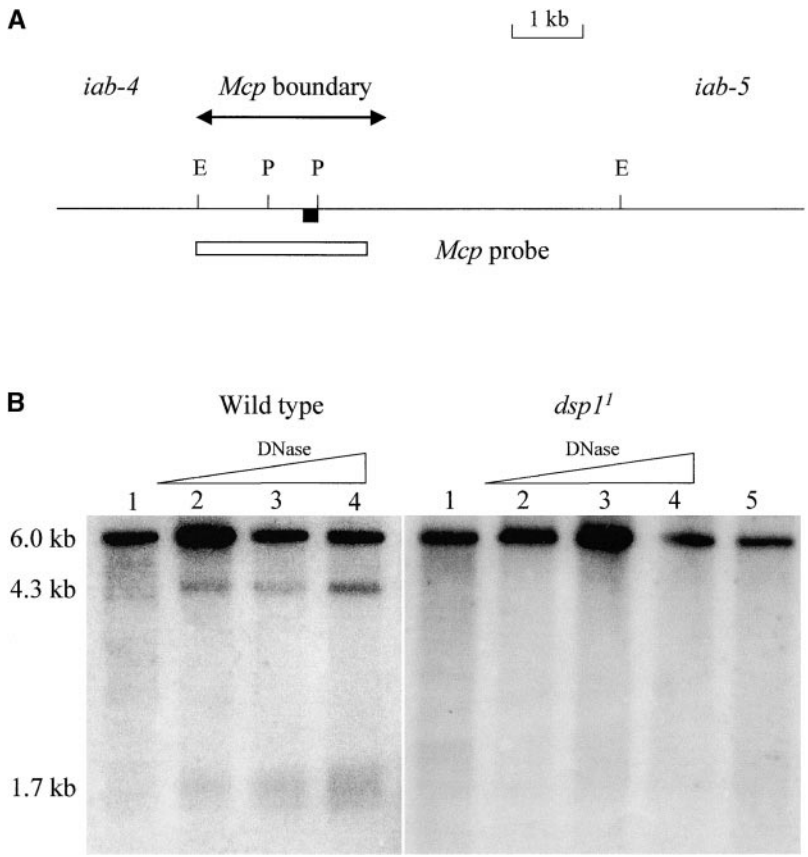

FIGURE 3.—Absence of the DNase hypersensitive region in the *Mcp* boundary in a $dsp1^1$ mutant. (A) Schematic representation of the 6.0-kb *Eco*RI *Mcp* fragment. The map is shown in the proximal to distal orientation, with *iab-4* to the left and *iab-5* to the right. The solid square indicates the strong hypersensitive DNase region as described by KARCH *et al.* (1994). The probe used in the experiment is indicated by an open rectangle below the map and the *Mcp* boundary is indicated by an arrow. E, *Eco*RI; P, *Pst*I. (B) Nuclei prepared from wild-type (lanes 1–4) or $dsp1^1$ (lanes 5–8) embryos were digested with DNase I. After isolating the DNase I-digested DNA, the DNA samples were restricted with *Eco*RI and electrophoresed onto an agarose gel. After blotting to nitrocellulose filters, the DNA was hybridized with a probe encompassing the DNase hypersensitive region. If the hypersensitive region is present, several fragments are revealed at ~4.3 and 1.7 kb; if it is absent, only one fragment is revealed at 6.0 kb. Lanes 1–4 and 5–8 correspond to different concentrations of DNase I (0, 1, 2, and 4 units/ml); lane 9 corresponds to naked DNA treated with DNase I (4 units/ml). Lanes containing a 1.0-kb M_r ladder were also included in the gels, but are not shown here.

gene. Studies of the genetic interaction between *dsp1*^{1} and mutations of various trx-G genes show a strong enhancement of the haltere into wing homeotic transformation. On the contrary, interaction between *dsp1*^{1} and a mutation in *Pc* reveals a partial suppression of the extra sex comb phenotype of *Pc*. Taken together, these findings suggest that DSP1 acts antagonistically to Pc to activate the transcription of *Ubx*, *Scr*, *Antp*, and *iab-6*. If this is the case, overexpression of *dsp1* is expected to induce ectopic expression of these homeotic genes. This has been confirmed by studying overexpression of *dsp1* in a *Pc* context. We observe an increase of transformations of wings toward halteres and an enhancement of the extra sex comb phenotype of *Pc*. Taken together, these results strongly support the idea that *dsp1* acts as a member of trx-G. Interestingly, *dsp1* function seems to be restricted to some particular loci. This is not unknown in flies since *kismet*, a suppressor of *Pc*, causes specific homeotic transformations when it is mutated (Daubresse *et al.* 1999): transformation of the fifth abdominal segment into the fourth, with the other abdominal segments being not affected. The Kis protein seems to interact specifically with the *iab-5 cis*-regulatory element of *AbdB* and not with the other *iab* regions.

Surprisingly, one phenotypic trait of *dsp1*^{1} seems to be characteristic of a mutation in Pc-G genes, the pigmentation of the A4 segment in adult males, corresponding to homeotic transformation of a segment into a more posterior one. However, analysis of the chromatin structure at the *Mcp* locus reveals that the DNase hypersensitive region is absent in *dsp1*^{1}. We propose that lack of DSP1 leads to remodeling of the chromatin structure at the *Mcp* locus, suppressing, at least in part, the boundary between *iab-4* and *iab-5*, and then allowing the extension of the activation state of *iab-4* to *iab-5* in the A4 segment.

These results demonstrate that *dsp1* could be a chromatin remodeling factor, acting as a trx-G or Pc-G gene depending on the considered function. These genes are involved in maintenance of an activation or repression state of homeotic genes. It has been proposed that they modify chromatin structure locally to maintain it in an "open" or "closed" configuration. DSP1 is an HMG1-like protein. It contains an HMG domain with two HMG boxes and a short acidic tail. HMG domains are known to interact with DNA, principally with bent DNA as four-way junctions or cisplatin-modified DNA. The interaction between an HMG box and DNA causes dramatic distortions on DNA structure and thus could participate with protein complexes in remodeling of the chromatin. In the case of the Brm complex, a protein, BAP111, containing an HMG domain has been identified. A counterpart for BAP111 has been identified in mammals as a member of a related SWI/SNF complex. This SWI/SNF complex is composed of BAF190, the human homologue of the Drosophila protein Brahma, and of BAF57, a high-mobility-group/kinesin-like protein (Wang *et al.* 1998). Considering the strong interaction between *dsp1*^{1} and *trx* or *ash1* mutations, DSP1 could be involved in a complex containing trx and ash1. Recently it has been shown that these two proteins interact with each other *in vivo* (Rozovskaia *et al.* 1999). Such HMG proteins would not be obligatory members of the activating complexes, but could participate in the recognition of a higher-order chromatin structure and allow the interaction between chromatin and the activating complexes.

We are grateful to M. J. Giraud-Panis for helpful discussions and useful suggestions and to A. Soulas and M. Martineau for excellent technical assistance. We are indebted to Dr. B. Limbourg-Bouchon for help in *P*-element-mediated transformation. We thank Bloomington Stock Center and Umeå Stock Center for supplying mutant strains used in this analysis. We especially thank A. Shearn for providing *ash* mutant strains. This work is supported in part by la Ligue contre le Cancer, la Fondation pour la Recherche Médicale, l'Association pour la Recherche contre le Cancer, and the E.U. (project ERB4061 PL97028).

LITERATURE CITED

Bianchi, M. E., M. Beltrame and L. Falciola, 1992 The HMG box motif, pp. 112–128 in *Nucleic Acids and Molecular Biology*, Vol. 6, edited by F. Eckstein and D. M. J. Lilley. Springer-Verlag, Berlin.

Bonne, C., P. Sautiere, M. Duguet and A. M. de Recondo, 1982 Identification of a single-stranded DNA binding protein from rat liver with high mobility group protein 1. J. Biol. Chem. **257:** 2722–2725.

Bonne-Andrea, C., F. Harper, J. Sobczak and A. M. de Recondo, 1984 Rat liver HMG1: a physiological nucleosome assembly factor. EMBO J. **5:** 1193–1199.

Breen, T. R., and P. J. Harte, 1991 Molecular characterization of the trithorax gene, a positive regulator of homeotic gene expression in Drosophila. Mech. Dev. **35:** 113–127.

Bryant, P. J., 1978 Pattern formation in imaginal discs, pp. 229–335 in *The Genetics of Drosophila*, Vol. 2c, edited by M. Ashburner and T. R. F. Wright. Academic Press, London.

Cairns, B. R., Y. Lorch, Y. Li, M. Zhang, L. Lacomis *et al.*, 1996 RSC, an essential, abundant chromatin-remodeling complex. Cell **87:** 1249–1260.

Calogero, S., F. Grassi, A. Aguzzi, T. Voigtländer, P. Ferrier *et al.*, 1999 The lack of chromosomal protein HMG1 does not disrupt cell growth, but causes lethal hypoglycaemia in newborn mice. Nat. Genet. **22:** 276–280.

Daubresse, G., R. Deuring, L. Moore, O. Papoulas, I. Zakrajsek *et al.*, 1999 The *Drosophila kismet* gene is related to chromatin-remodeling factors and is required for both segmentation and segment identity. Development **126:** 1175–1187.

Dingwall, A. K., S. J. Beek, C. M. McCallum, J. W. Tamkun, G. V. Kalpana *et al.*, 1995 The *Drosophila* snr1 and brm proteins are related to yeast SWI/SNF proteins and are components of a large protein complex. Mol. Biol. Cell **6:** 777–791.

Dorn, R., J. Szidonya, G. Korge, M. Sehnert, H. Taubert *et al.*, 1993 P transposon-induced dominant enhancer mutations of position-effect variegation in *Drosophila melanogaster*. Genetics **133:** 279–290.

Duncan, I., 1987 The bithorax complex. Annu. Rev. Genet. **21:** 285–319.

Eissenberg, J. C., T. C. James, D. M. Foster-Hartnett, T. Hartnett, V. Ngan *et al.*, 1990 Mutation in a heterochromatin specific chromosomal protein is associated with suppression of position-effect variegation in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA **87:** 9923–9927.

Eissenberg, J. C., G. D. Morris, G. Reuter and T. Hartnett, 1992 The heterochromatin associated protein HP-1 is an essential pro-

tein in Drosophila with dosage-dependent effects on position-effect variegation. Genetics **131:** 345–352.

Farkas, G., J. Gausz, M. Galloni, G. Reuter, H. Gyurkovics *et al.*, 1994 The *Trithorax-like* gene encodes the *Drosophila* GAGA factor. Nature **371:** 806–808.

Grosschedl, R., K. Giese and J. Pagel, 1994 HMG domain proteins: architectural elements in the assembly of nucleoprotein structures. Trends Genet. **10:** 94–100.

Ingham, P. W., 1985 A clonal analysis of the requirement for the trithorax gene in the diversification of segments in Drosophila. J. Embryol. Exp. Morphol. **89:** 349–365.

Ingham, P. W., and R. Whittle, 1980 Trithorax: a new homeotic mutation of Drosophila melanogaster causing transformations of abdominal and thoracic imaginal segments. Mol. Gen. Genet. **179:** 607–614.

Jowett, T., 1986 Preparation of nucleic acids, pp. 275–286 in *Drosophila a Practical Approach*, edited by D. B. Roberts. IRL Press, Oxford.

Karch, F., M. Galloni, L. Sipos, J. Gausz, H. Gyurkovics *et al.*, 1994 *Mcp* and *Fab-7*: molecular analysis of putative boundaries of *cis*-regulatory domains in the bithorax complex of *Drosophila melanogaster*. Nucleic Acids Res. **22:** 3138–3146.

Kaufman, T. C., M. A. Seeger and G. Olsen, 1990 Molecular and genetic organization of the *Antennapedia* gene complex of *Drosophila melanogaster*. Adv. Genet. **27:** 309–362.

Kennison, J. A., 1995 The Polycomb and trithorax group proteins of Drosophila: trans-regulators of homeotic gene function. Annu. Rev. Genet. **29:** 289–303.

Kennison, J. A., and J. W. Tamkun, 1988 Dosage-dependent modifiers of *Polycomb* and *Antennapedia* mutations in *Drosophila*. Proc. Natl. Acad. Sci. USA **85:** 8136–8140.

Lehming, N., D. Thanos, J. M. Brickman, J. Ma, T. Maniatis *et al.*, 1994 An HMG-like protein that can switch a transcriptional activator to a repressor. Nature **371:** 175–179.

Lehming, N., A. Le Saux, J. Schüller and M. Ptashne, 1998 Chromatin components as part of a putative transcriptional repressing complex. Proc. Natl. Acad. Sci. USA **95:** 7322–7326.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

Locke, J., M. A. Kotarski and K. D. Tartof, 1988 Dosage-dependent modifiers of position effect variegation in Drosophila and a mass action model that explains their effect. Genetics **120:** 181–198.

Masucci, J. D., R. J. Miltenberger and F. M. Hoffmann, 1990 Pattern-specific expression of the Drosophila decapentaplegic gene in imaginal disks is regulated by 3′ cis-regulatory elements. Genes Dev. **4:** 2011–2023.

Mosrin-Huaman, C., L. Canaple, D. Locker and M. Decoville, 1998 *DSP1* gene of *Drosophila melanogaster* encodes an HMG-domain protein that plays multiple roles in development. Dev. Genet. **23:** 324–334.

Papoulas, O., S. J. Beek, S. L. Moseley, C. M. McCallum, M. Sarte *et al.*, 1998 The *Drosophila* trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. Development **125:** 3955–3966.

Paro, R., and D. S. Hogness, 1991 The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of *Drosophila*. Proc. Natl. Acad. Sci. USA **88:** 263–267.

Pattatucci, A. M., D. C. Otteson and T. C. Kaufman, 1991 A functional and structural analysis of the *sex comb reduced* locus of *Drosophila melanogaster*. Genetics **129:** 423–441.

Pirotta, V., 1997 Pc-G complexes and chromatin silencing. Curr. Opin. Genet. Dev. **7:** 249–258.

Read, C. M., P. D. Cary, S. Crane-Robinson, P. C. Driscoll, M. O. M. Carillo *et al.*, 1995 The structure of the HMG box and its interaction with DNA, pp. 222–250 in *Nucleic Acids and Molecular Biology*, Vol. 9, edited by F. Eckstein and D. M. J. Lilley. Springer-Verlag, Berlin.

Reynolds, E., and M. Tanouye, 1998 Cloning and characterization of bangsensitive mutants. A. Dros. Res. Conf. **39:** 588C.

Rozovskaia, T., S. Tillib, S. Smith, Y. Sedkov, O. Rozenblatt-Rosen *et al.*, 1999 Trithorax and ASH1 interact directly and associate with the trithorax group-responsive bxd region of the Ultrabithorax promoter. Mol. Cell. Biol. **19:** 6441–6447.

Simon, J., 1995 Locking in stable states of gene expression: transcriptional control during Drosophila development. Curr. Opin. Cell Biol. **7:** 376–385.

Sinclair, A. H., P. Berta, M. S. Palmer, J. R. Hawkins, B. L. Griffiths *et al.*, 1990 A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature **346:** 240–244.

Sinclair, D. A., V. K. Lloyd and T. A. Grigliatti, 1989 Characterization of mutations that enhance position-effect variegation in *Drosophila melanogaster*. Mol. Gen. Genet. **191:** 326–333.

Sinclair, D. A., A. A. Ruddell, J. K. Brock, N. J. Clegg, V. K. Lloyd *et al.*, 1992 A cytogenetic and genetic characterization of a group of closely linked second chromosome mutations that suppress position-effect variegation in *Drosophila melanogaster*. Genetics **130:** 333–344.

Sinclair, D. A., N. J. Clegg, J. Antonchuk, T. A. Milne, K. Stankunas *et al.*, 1998a *Enhancer of polycomb* is a suppressor of position-effect variegation in *Drosophila melanogaster*. Genetics **148:** 211–220.

Sinclair, D. A. R., T. A. Milne, J. W. Hodgson, J. Shellard, C. A. Salinas *et al.*, 1998b The *Additional sex combs* gene of *Drosophila* encodes a chromatin protein that binds to shared and unique polycomb group sites on polytene chromosomes. Development **125:** 1207–1216.

Singh, J., and G. H. Dixon, 1990 High mobility group proteins 1 and 2 function as general class II transcription factors. Biochemistry **29:** 6295–6302.

Spradling, A. C., and G. M. Rubin, 1982a Transposition of cloned P-element into *Drosophila* germ lines chromosomes. Science **218:** 341–347.

Spradling, A. C., and G. M. Rubin, 1982b Genetic transformation of *Drosophila* with transposable element vectors. Science **218:** 348–353.

Tautz, D., and C. Pfeifle, 1989 A non-radioactive in situ hybridization method for the localization of specific mRNAs in Drosophila embryos reveals translational control of the segmentation gene hunchback. Chromosoma **98:** 81–85.

Travis, A., A. Amsterdam, C. Belanger and R. Grosschedl, 1991 *LEF-1*, a gene encoding a lymphoid-specific protein with an HMG domain, regulates T-cell receptor alpha enhancer function. Genes Dev. **5:** 880–894.

Tremethick, D. J., and P. L. Molloy, 1988 Effects of high mobility group proteins 1 and 2 on initiation and elongation of specific transcription by RNA polymerase II in vitro. Nucleic Acids Res. **16:** 11105–11122.

Tsiersch, B., A. Hofmann, V. Krauss, R. Dorn, G. Korge *et al.*, 1994 The protein encoded by the *Drosophila* position-effect variegation suppressor gene *Su(var)3-9* combines domains of antagonistic regulators of homeotic gene complexes. EMBO J. **13:** 3822–3831.

Wang, W., T. Chi, Y. Xue, S. Zhou, A. Kuo *et al.*, 1998 Architectural DNA binding by a high-mobility-group/kinesin-like subunit in mammalian SWI/SNF-related complexes. Proc. Natl. Acad. Sci. USA **95:** 492–498.

Waterman, M. L., W. H. Fisher and K. A. Jones, 1991 A thymus-specific member of the HMG protein family regulates the human T cell receptor alpha enhancer. Genes Dev. **5:** 656–669.

Wustmann, G., J. Szidonya, H. Taubert and G. Reuter, 1989 The genetics of position-effect variegation modifying loci in *Drosophila melanogaster*. Mol. Gen. Genet. **217:** 520–527.

Yamamoto, Y., F. Girard, B. Bello, M. Affolter and W. J. Gehring, 1997 The *cramped* gene of *Drosophila* is a member of the *Polycomb*-group, and interacts with *mus209*, the gene encoding proliferating cell nuclear antigen. Development **124:** 3385–3394.

Communicating editor: T. C. Kaufman