# Mutations in *Drosophila* heat shock cognate 4 are enhancers of *Polycomb*

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**The homeotic genes controlling segment identity in *Drosophila* are repressed by the *Polycomb* group of genes (PcG) and are activated by genes of the *trithorax* group (trxG). An $F_1$ screen for dominant enhancers of *Polycomb* yielded a point mutation in the heat shock cognate gene, *hsc4,* along with mutations corresponding to several known PcG loci. The new mutation is a more potent enhancer of *Polycomb* phenotypes than an apparent null allele of *hsc4* is, although even the null allele occasionally displays homeotic phenotypes associated with the PcG. Previous biochemical results had suggested that HSC4 might interact with BRAHMA, a trxG member. Further analyses now show that there is no physical or genetic interaction between HSC4 and the Brahma complex. HSC4 might be needed for the proper folding of a component of the *Polycomb* repression complex, or it may be a functional member of that complex.**

The homeobox-containing clusters of genes are essential for proper body pattern formation in a variety of organisms (1). In *Drosophila melanogaster*, a correct body plan is laid out by differential expression of the homeotic transcription factors residing within the Antennapedia complex and the bithorax complex. Members of the *trithorax* group (trxG) genes and the *Polycomb* group (PcG) genes function to regulate this differential expression. The trxG members can be broadly categorized as activators of the homeotic transcription factors (2). Conversely, the PcG members collaborate to fix the repressed state of the homeotics within particular segments (3). Indirect lines of evidence had previously suggested that the PcG members accomplish at least part of their task through modulation of chromatin structure (4). The products of several genes, including *Polycomb* (*Pc*), *polyhomeotic* (*ph*), and *Posterior sex combs* (*Psc*), reside in a biochemical complex called PRC1 (5). Products of genes in the trxG, including *brahma* (*brm*) and *moira* (*mor*) are included in the BRM complex (6, 7), the *Drosophila* counterpart of the SWI/SNF activation complex of *Saccharomyces cerevisiae*. Recently, PRC1 has been shown to antagonize the chromatin remodeling function of a SWI/SNF complex *in vitro* (5), supporting the chromatin-effect hypothesis.

To date, at least 15 genes have been classified as PcG members (3, 8, 9). Several different genetic screens have been used to discover PcG member genes. *Polycomb* itself was recognized for its dominant (haplo-insufficient) phenotype of partial segmental transformations (10). Some PcG mutations were discovered as dominant enhancers of the *Pc*/+ phenotype, e.g., *Polycomblike* (*Pcl*) (11). Some were found as homozygous lethal mutations with homeotic defects, including *Additional sex combs* (*Asx*) (12) and *super sex combs* (*sxc*) (13). None of these screens was sufficiently large to claim saturation of all possible loci, and probably none of the screening criteria are sufficient to identify all PcG members. Zygotic phenotypes of many PcG mutations are masked by maternal contribution of the wild-type product, as seen most dramatically in the case of *extra sex combs* (*esc*) (14). Other PcG mutations show little or no dominant phenotype [such as *Su(z)2* (15)]. Virtually all, however, have been shown to enhance the dominant phenotype of *Pc*.

Jürgens (16) estimated that the PcG group may include as many as 40 members. This number now seems high, but there may be several PcG members yet to be discovered. We performed a new $F_1$ screen for dominant enhancers of a recessive viable allele of *Polycomb*, $Pc^{T3}$ (17), using 1-ethyl-1-nitrosourea for mutagenesis. Our screen recovered alleles of several known PcG loci but also an allele of the heat shock protein 70 (HSP70) cognate factor HSC4. We present here a genetic and molecular characterization of our *hsc4* allele, and of previously isolated alleles, to define the role of HSC4 in regulating the homeobox-containing complexes.

## Materials and Methods

**Mutagenesis.** Males from a *black, cinnabar; claret* stock were fed 1-ethyl-1-nitrosourea (18) and mated to $Pc^{T3}$ homozygous females. Progeny were examined for transformations of the abdominal tergites, wings, and legs. The mutations recovered were mapped to the second or third chromosome and then tested for complementation with alleles of known PcG genes. These included $Asx^{XT129}$, $esc^{2}$, $esc^{10}$, $Pcl^{1}$, $Pcl^{D5}$, $Psc^{h27}$, and $Su(z)2^{1.b8}$ (on the second chromosome), and $Scm^{28-16}$, $Scm^{D1}$, $Pc^{3}$, $Sce^{b1}$, and $Df(3L)lxd^{15}$ (on the third chromosome).

***Drosophila* Crosses and Phenotypes.** Flies were raised on standard cornmeal, yeast, and sugar medium at 25°C. Mutations used are described by Lindsley and Zimm (19) or in Flybase. The initial *54.1* mutagenized chromosome was made heterozygous in females with the "*ru cu ca*" third chromosome (*ru h th st cu sr e ca*), and these were backcrossed to *ru cu ca* males. Single $F_1$ males carrying chromosomes recombined within each interval were crossed to $Pc^{3}/TM1$ females, to test for the presence of the *54.1* allele. Stocks were prepared for *54.1* mutant recombinant chromosomes with crossover points close to the *54.1* locus; these "cleaned up" chromosomes included *ru h th st cu 54.1* and *ru 54.1 sr e*. Heterozygotes between these chromosomes (*ru h th st cu 54.1/ru 54.1 sr e*) still showed larval lethality. For the scoring of larval cuticles, the cleaned up chromosomes were balanced with *TM6B(Hu)*, and progeny lacking the *Tubby* phenotype were chosen for inspection. The tested deficiencies that most closely

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Abbreviations: trxG, *trithorax* group; PcG, *Polycomb* group; HSC4, heat shock cognate 4; HSP, heat shock protein.

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**Table 1. *hsc4*$^{54.1}$ interactions with PcG mutations**

| Genotype | A4 → A5* | A6 → A7† | Extra sex combs‡ |
|---|---|---|---|
| *54.1*/+ | 4% | 1% | 0 |
| *54.1*/*Pc*$^{3}$ | 100% | 100% | 34 |
| *Pc*$^{3}$/+ | ND | 0% | 0.3 |
| *54.1*/*Asx*$^{xt129}$ | 26% | 7% | 0.8 |
| *Asx*$^{xt129}$/+ | 10% | 0% | 0.1 |
| *54.1*/*Pcl*$^{1}$ | 70% | 35% | 1.6 |
| *Pcl*$^{1}$/+ | 4% | 0% | 0.2 |
| *54.1*/*Scm*$^{D1}$ | 64% | 4% | 2.4 |
| *Scm*$^{D1}$/+ | 13% | 6% | 0 |

ND, not determined.

*Transformation of the fourth abdominal segment to the fifth, expressed as percentage of males with extra black pigmentation on the fourth abdominal tergite.

†Transformation of the sixth abdominal segment to the seventh, expressed as the percentage of males with a reduced sixth abdominal tergite.

‡Transformation of the second and third thoracic legs to the character of the forelegs, expressed as the average total number of extra sex comb teeth on the four posterior legs of a male.

flank the *hsc4* locus were *Df(3R)kar*$^{51}$ (87C7-D1 to 88E2–3) and *Df(3R)ea* (88E7–13 to 89A1).

Both *54.1* and *ru 54.1 sr e* chromosomes were tested for the PcG enhancement analysis, with each cross performed in both directions, except for the *ph*$^{410}$ allele. Table 1 presents averaged percentages of progeny with medium or strong transformations. Adult abdomens were mounted by the method of Duncan (11). ABDOMINAL-B protein was detected in embryos by using a mouse mAb as the primary antibody (20) and a horseradish peroxidase-coupled goat anti-mouse antibody (Bio-Rad) as the secondary antibody.

**Molecular Analysis.** The coding region was recovered from *hsc4*$^{54.1}$/*TM6B* heterozygotes by PCR amplification, by using the primer pairs as described (21). Multiple copies of each PCR product were cloned and sequenced. Only one difference with the published sequence (22) was found in two separate clones. This change was verified by direct sequencing of the mixed PCR product. The region of the *hsc4*$^{54.1}$ mutation was also sequenced from two stocks containing the background chromosome on which *hsc4*$^{54.1}$ was induced. These showed only the wild-type sequence.

The cloned PCR products covering the coding region were also used to probe the Southern blots of the various *hsc4* alleles; separate blots were done with two different enzymes. The alleles carrying P element insertions near *hsc4* (*l(3)03550* and *E(nd)*$^{195}$) gave shifted bands on the Southern blots with the enzymes chosen. Thus, changes in these bands in ''revertant'' lines could be scored in the presence of a wild-type copy of *hsc4* from a balancer chromosome.

**Analysis of HSC4 Protein.** Superose 6 gel filtration chromatography, SDS/PAGE, and immunoblotting were performed as described by Papoulas *et al.* (6) with the following changes. For determination of the native molecular mass of HSC4, 4.6 mg of native protein extract from 0–12 h embryos was loaded on a Superose 6 gel filtration column. For immunoprecipitation of size fractionated BRM complexes, 5 mg of extract prepared from wild-type (Oregon R) or *w P[w*$^{+}$*, brm-HA6HIS]92C;brm*$^{2}$/*Df(3L)th102, h ri Sb ca*$^{2}$ embryos was passed over the column and eluted in IP buffer (10 mM Hepes, pH 8.0/1 mM EDTA/100 mM NaCl/0.05% Tween-20/10% glycerol). In each case, 1.3 ml of pooled fractions 17 to 19 (BRM peak) was incubated with 100 μl of anti-hemagglutinin (HA) immunoaffinity resin for 4.5 h at 4°C. Bound proteins were washed with 40 volumes of IP buffer and eluted with 100 μl of 0.5 mg/ml HA dipeptide (GeneMed Synthesis) in IP buffer (30 min, room temperature). BRM was detected by Western blotting with affinity-purified rabbit anti-BRM polyclonal antibodies (23). For native molecular weight determination, HSC4 was detected by using mouse anti-Hsp70/Hsc70 mAb (clone BB70, StressGen Biotechnologies, Victoria, Canada). HSC4 was detected in immunoprecipitation samples with affinity-purified rabbit anti-Hsc4p polyclonal antibody (24).

## Results

**Enhancer Screen and Complementation Analyses.** Mutagenized males were crossed to *Pc*$^{T3}$ females, and about 30,000 $F_1$ progeny were screened for multiple enhancement phenotypes. Five mutants that showed significant enhancement were recovered. The mutations were assigned to chromosomes II or III and then tested for complementation with strong loss-of-function PcG alleles on the relevant chromosome. One mutation was a new *Pc* allele, and three others failed to complement with mutations in the *esc*, *Pcl*, and *Scm* loci. Unexpectedly, the *esc* allele appears to be antimorphic; it does not require the removal of the maternal contribution to give lethality against either *esc*$^{2}$ *or esc*$^{10}$. With apparent null *esc* alleles, the removal of the maternal contribution is required for lethality (14). The molecular nature of this allele is under investigation. The remaining mutation, on the third chromosome, complemented the mutations in known PcG loci. This mutation, designated as *54.1*, is the focus of the studies reported here.

**Phenotypic Characterization of the *54.1* Allele.** When *54.1* is tested over *Pc*$^{3}$, we observe dramatic transformations along the body axis (Table 1). These include anterior-to-posterior transformations in many abdominal segments (Fig. 1*B*), a partial wing-to-haltere transformation in the thorax (Fig. 1*C*), and the occurrence of multiple sex combs on both the second and third pairs of legs in males (Fig. 1 *E* and *F*). The ectopic sex combs represent a posterior-to-anterior transformation, caused by misexpression of the homeotic gene *Sex combs reduced* (*Scr*), a member of the Antennapedia complex. Nearly 50% of these flies also showed partial antenna-to-leg transformations. The spectrum and severity of the transformations vary from fly to fly among the progeny of a single cross, but the penetrance and expressivity are equivalent whether the *54.1* mutation is transmitted from the father or the mother. However, the progeny of *54.1*/*Pc*$^{3}$ females often die as first instar larvae, even when the fathers are wild type, but *54.1*/*Pc*$^{3}$ males give viable offspring when mated to wild-type females.

*54.1* also enhances the phenotypes of several loss-of-function PcG alleles to varying degrees (Table 1). The table presents observations of progeny from *54.1*/+ mothers. In *54.1*/+ progeny, there is no correlation between the strength of enhancement and maternal versus paternal transmission of the *54.1* allele. However, transformations in *Asx*, *Pcl,* or *Scm*/*54.1* progeny were more penetrant when the *54.1*/+ parent was the mother. Of particular note is that *54.1* gives synthetic lethality against the *polyhomeotic* mutation *ph*$^{410}$. The *ph* locus encodes two related products; *ph*$^{410}$ is an inversion that breaks the proximal *polyhomeotic* transcript (25) and it is null for the *ph-proximal* product (26). The *ph*$^{410}$; *54.1*/+ males die as pharate adults that carry complete ectopic sex combs on the second and third thoracic legs. These animals show malformed or missing abdominal tergites and sternites, a reduced number of thoracic bristles, and missing aristae.

Heterozygous *54.1* males do not display the extra sex combs trait seen in many *Polycomb* group mutations (Table 1). However, they occasionally show a partial posterior transformation of the fourth abdominal tergite, seen as spots of black pigmented cuticle (Table 1). These anterior-to-posterior conversions are characteristic of loss-of-function PcG alleles, and they are pre-

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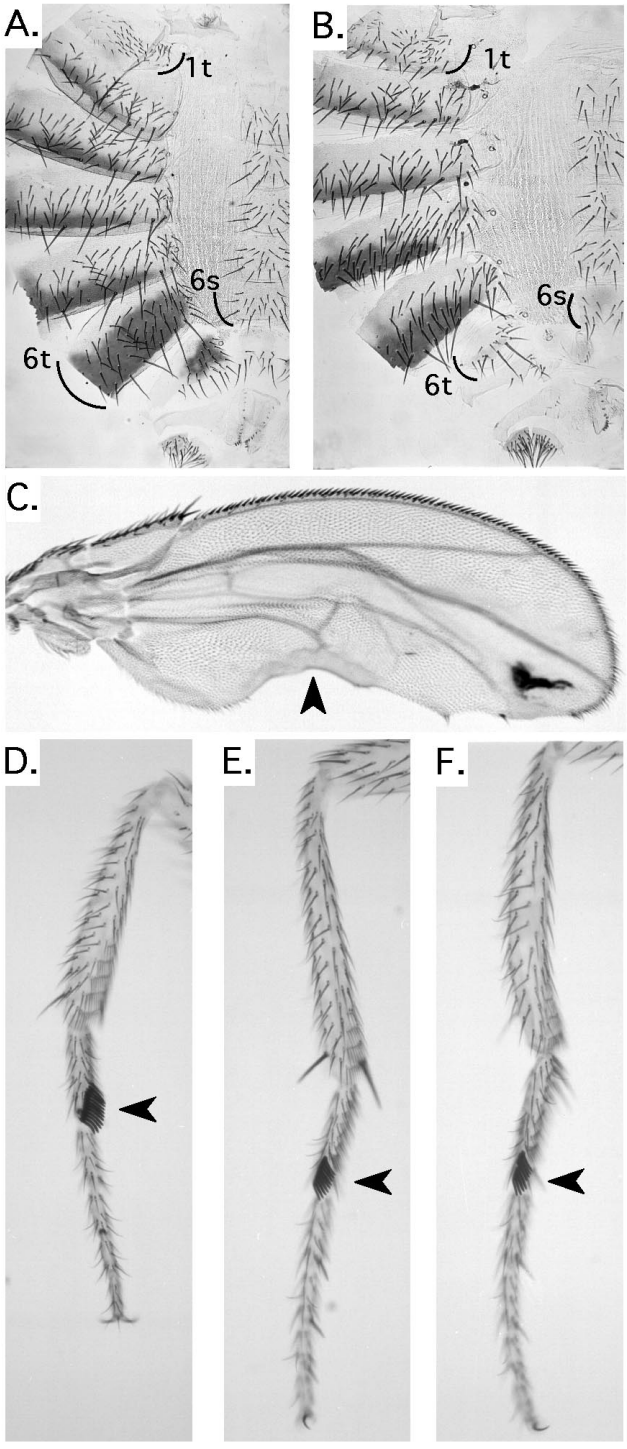


**Fig. 1.** *Polycomb* enhancement phenotypes of *hsc4*$^{54.1}$. (*A*) The abdominal cuticle of a wild-type female. (*B*) The abdominal cuticle of a *54.1*/*Pc*$^{3}$ female. Note that the first abdominal tergite (marked 1t) is transformed to the character of the second abdominal segment. Likewise, the sixth abdominal tergite (6t) and sternite (6s) are transformed to the character of the seventh abdominal segment. The seventh tergite and sternite are almost completely removed, partially transformed to the eighth abdominal segment. (*C*) The wing from a *54.1*/*Pc*$^{3}$ adult. Patches of wing tissue are transformed to the character of haltere, especially along the posterior margin (arrowhead). (*D–F*) Legs from a *54.1*/*Pc*$^{3}$ male. (*D*) The first thoracic leg, which shows a row of sex comb teeth (arrowhead) characteristic of the wild-type male foreleg. (*E*) The second thoracic leg. Note the complete row of ectopic sex comb teeth (arrowhead). (*F*) The third thoracic leg. There is a partial row of ectopic sex comb teeth (arrowhead).

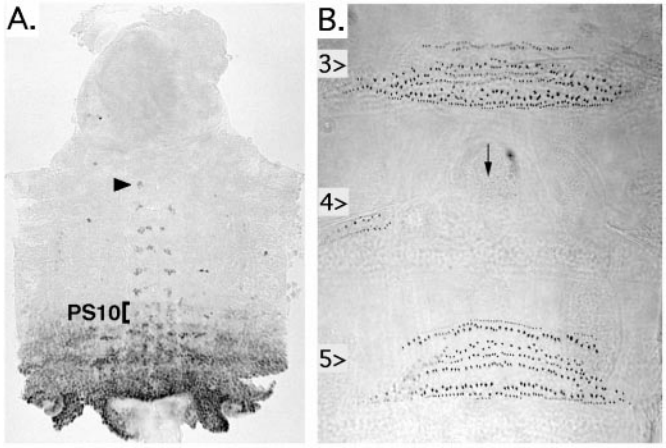


**Fig. 2.** Embryonic and larval phenotypes of *hsc4*$^{54.1}$. (*A*) ABD-B staining of a stage 14 embryo obtained from crossing the *ru hsc4*$^{54.1}$ *sr e* and *ru h th st cu hsc4*$^{54.1}$ stocks. The embryo was cut open along the dorsal midline and flattened. Note the ectopic expression of ABD-B, especially in the central nervous system, up through the second thoracic segment, or parasegment 4 (arrowhead). A bracket marks parasegment 10, the anterior limit of ABD-B expression in wild-type embryos. (*B*) The ventral cuticle of a *ru h th st cu hsc4*$^{54.1}$/+ second instar larva. The denticle belts of the third, fourth, and fifth abdominal segments are marked. Most of the denticles of the fourth abdominal segment are missing (arrow).

sumably caused by anterior misexpression of the *Abdominal-B* (*Abd-B*) gene. A small fraction of embryos carrying *54.1* (<5%) show spotty anterior expression of the *Abd-B* gene product (Fig. 2*A*).

*54.1* homozygous individuals survive embryogenesis but die as larvae before the third instar. (The mutagenized chromosome was first "cleaned" by recombination; see *Materials and Methods*.) We examined both *54.1* heterozygous and homozygous larvae for signs of homeotic transformations. Neither class displays any obvious homeotic phenotypes. However, we noticed that 7% of the larvae carrying one copy of *54.1* from their mothers show either partial or complete loss of one or more ventral denticle belts, predominantly in the A4 segment (Fig. 2*B*). This percentage is higher for *54.1* homozygous larvae (13%).

**Recombination and Deletion Mapping Analyses.** Salivary polytene chromosomes from *54.1*/+ heterozygotes were examined for potential rearrangements on the third chromosome; none were found. We proceeded to map the *54.1* mutation by meiotic recombination with a multiply marked third chromosome (*ru h th st cu sr e ca*). *54.1* was assigned to a region between the *curled* (*cu*) and *stripe* (*sr*) loci. The *54.1* allele was then mapped relative to the dominant *Stubble* marker (at 89B9–10 on the cytological map); it lies approximately 1 map unit proximal to *Stubble*. We also tested a series of overlapping deletions spanning the interval 86C1–88E2–3 and another series spanning 88E13–89E. None of them uncovered our *54.1* allele. This analysis suggested that *54.1* resides within the 88E2–3 to 88E13 region, for which we had no deficiencies, or that the *54.1* allele might be viable over a deficiency for its site.

**Allelism with HSC4.** We first checked candidate genes in the 88D-E region. *54.1* fully complemented a mutation in *E(Pc)88D* (27). It also failed to modify the position-effect variegation of the *w*$^{m4h}$ inversion, and so it seemed unlikely to be allelic to *E(var)88D* (28) or *Su(var)3–9* (29). We then tested a collection of lethal P-element insertions within the 88E segment. The original *54.1* stock and the derivative recombinants *ru 54.1 sr e* and *ru h th st*

**Table 2. Interaction phenotypes of *hsc4* alleles**

| *hsc4* allele (from father) | *Pc*$^3$ | | | *ph*$^{410}$ | | *trx*$^{B11}$ | |
|---|---|---|---|---|---|---|---|
| | T2,3 → T1* | A4 → A5† | A6 → A7‡ | Lethal?§ | T2,3 → T1 | T1 → T2¶ | A5 → A4‖ |
| *54.1* | +++ | + | ++ | l. | ++ | 0 | 0/− |
| *l(3)03550* | ++ | 0 | 0 | v. | + | 0 | 0 |
| *II-35-R2* | + | +/0 | 0 | l. | + | 0 | + |
| *II-35-R7* | + | +/0 | 0 | l. | + | 0 | 0/+ |
| *I-35-R7* | 0 | +/0 | 0 | l. | + | ++ | ++ |
| *E(nd)*$^{195}$ | − | + | 0 | v. | − | 0 | − |
| *195rvPB2* | − | + | 0 | v. | −/0 | 0 | − |
| *Dp-P[hsc4]* | 0 | 0 | 0 | v. | 0 | 0 | − |

*Transformation of the second and third thoracic legs toward that of the first, seen as extra sex combs on the second and third legs of the males. Enhancements are categorized as complete (+++), strong (++), weak (+), or not more than seen with wild-type fathers (0). −, Denotes suppression of the indicated transformation.

†Transformation of the fourth abdominal segment toward the fifth, seen as black pigmentation appearing on the fourth abdominal tergite of the male.

‡Transformation of the sixth abdominal segment to the seventh, seen as reduction of the sixth abdominal tergite in males and females.

§Pharate lethality of males with the *ph*$^{410}$ mutation, indicated as lethal (l.) or viable (v.).

¶Transformation of the first leg toward the second, seen as loss of sex comb teeth in the male.

‖Transformation of the fifth abdominal segment toward the fourth, seen as loss of black pigment on the male fifth abdominal tergite.

*cu 54.1 ca* all gave lethality against the insertion line *l(3)03550* in the 88E region. This P-insertion behaves as a strong hypomorphic allele of the locus that encodes for the HSC4 product (21). The P element is inserted 431 bp upstream of the likely RNA start site for the *hsc4* mRNA (Berkeley Fly Database; ref. 22). Schmucker *et al.* (30) named the locus *scattered* before its identity to *hsc4* was discovered (21). However, the *scattered* name had already been given to a locus on the second chromosome; we prefer to call the third chromosome locus *hsc4* to avoid confusion.

We tested a variety of *hsc4* alleles, including a likely null mutation, for complementation with *54.1*. Schmucker *et al.* (30) had treated the *l(3)03550* P insertion with x-rays to induce deletions for the locus. Among the recovered chromosomes lacking the P element selectable marker were several derivatives that gave more severe phenotypes. We obtained three of these revertant lines, examined them cytologically, and analyzed their DNAs by Southern blots using a probe for the *hsc4* coding region. The II-R35-R2 line showed a small deficiency in the 88E3–5 region and an altered restriction map according to the Southern blots. The II-R35-R7 line looked normal (like the parental P insertion) in both assays. The third line, I-R35-R7, lacked an *hsc4* band on Southern blots, although it was cytologically normal. This line is our best candidate for a complete null. Hing *et al.* (21) isolated the *hsc4*$^{195}$ allele as an enhancer of *notchoid*. In the same study, they reverted the enhanced phenotype with additional mutations in *hsc4.* We also obtained *hsc4*$^{195}$ and a revertant called *hsc4*$^{PB2}$, which had been induced by P element-mediated dysgenesis. All five of the *hsc4* alleles mentioned above were lethal when heterozygous to *54.1*.

*54.1* showed no abnormality on Southern blots in the *hsc4* coding region. The coding region was recovered from *54.1/TM6B* heterozygotes by PCR amplification and sequenced. A single sequence change was found relative to the published sequence (22), a T to C transition changing $Leu_{380}$ to Pro. This change was absent in the background chromosome on which *54.1* was induced. Thus, the *54.1* mutation is designated below as *hsc4*$^{54.1}$.

The $Leu_{380}$ residue is conserved among HSP70 homologs from bacteria to mammals (31). A crystal structure has been determined for the 44-kDa amino-terminal ATPase domain of bovine HSP70 (32). The equivalent leucine residue lies within an extended $\alpha$-helix, although it is very near the end of the peptide studied, and so it is not possible to describe its environment in the intact protein.

**PcG Interactions with Additional *hsc4* Alleles.** The *hsc4*$^{54.1}$ allele was compared with six other *hsc4* mutations described above for enhancement of the *Pc*$^3$ mutation in trans-heterozygotes (Table 2). Although three other alleles showed some enhancement of the segmental transformations, none were as pronounced as those of *hsc4*$^{54.1}$, and the likely null allele, I-R35-R7, showed no clear interaction. Because *hsc4*$^{54.1}$ gives synthetic lethality against the *ph*$^{410}$ allele, we checked the additional *hsc4* alleles for interaction with *ph*$^{410}$. All three revertants of the *l(3)03550* P insertion, including the likely null, also caused lethality of late pupae, although with less severe segmental transformations than in the *hsc4*$^{54.1}$ case (Table 2). Other *hsc4* alleles, not likely to be null, were viable in combination with *ph*$^{410}$ (Table 2). *ph*$^{410}$; *hsc4*$^{54.1}$/+ males carrying a duplication for *hsc4* (P[*hsc4*]) (21) occasionally survive, and they show less severe phenotypes than pharate adults without the duplication. Likewise, *hsc4*$^{54.1}$/*Pc*$^3$ trans-heterozygotes with a duplication for the *hsc4* gene still show some enhancement of the *Pc* transformations, although less pronounced than the enhancement without the duplication. These observations suggest that *hsc4*$^{54.1}$ is an antimorphic allele.

**HSC4 and the trxG.** A simple interpretation of the genetic data is that *hsc4* is required for the function of one or more PcG proteins. Alternatively, *hsc4* could be a negative regulator of a trxG protein. Consistent with this second interpretation, HSC4 was identified as a component of the purified BRM complex (6). The *brm* gene was first identified as a suppressor of *Pc* mutations (33), and it encodes the ATPase subunit of a protein complex related to the SWI/SNF chromatin-remodeling complex in yeast. However, the presence of HSC4 in purified BRM complexes could represent contamination by this very abundant protein. To resolve this issue, we examined the physical association of HSC4 with BRM by comparing their native molecular weights in whole embryo extracts. Western blotting of fractions from a gel-filtration column showed some overlap between the elution peaks of these two proteins (Fig. 3*A*), consistent with the prior copurification from similar extracts. However, it was not clear whether the small fraction of HSC4 in the BRM-containing fractions was physically associated with BRM. When BRM complexes were immunoprecipitated from these partially purified fractions, no specific association with HSC4 was detected (Fig. 3*B*). Because these immunoprecipitations were performed by using near-physiological salt and buffer conditions, even a relatively weak interaction should have been detected. We note

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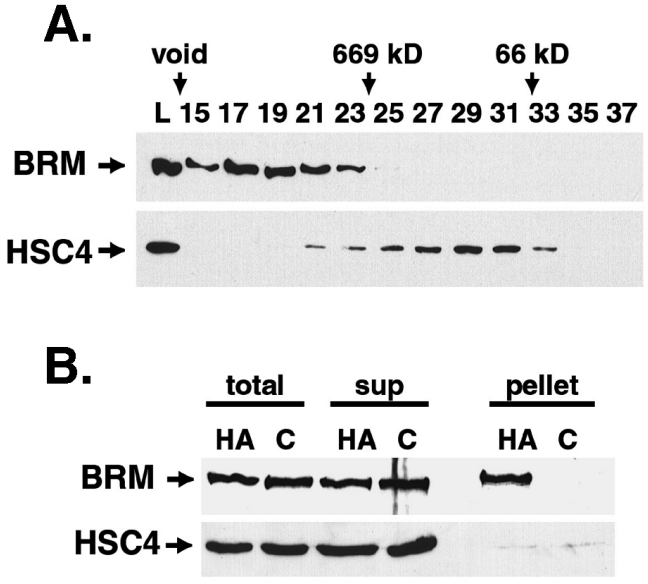


**Fig. 3.** HSC4 separation from the BRM complex in embryos. (*A*) Superose 6 gel filtration chromatography of embryo extracts, with fractions separated on Western blots and probed for BRM or HSC4. Lanes are designated by column fraction number or L, material loaded. Elution volumes of native molecular mass standards are indicated above. There is only a small amount of overlap between the BRM and HSC4 peaks. (*B*) BRM immunoprecipitates. An anti-HA mAb was used to immunoprecipitate extracts from wild-type control embryos (C) or embryos containing HA-epitope-tagged BRM protein (HA). Immunoprecipitation was from the pooled BRM peak fractions of a Superose 6 column. Western blotting of the starting material (total), supernatant (sup), and pellet samples revealed only trace amounts of HSC4 in both pellets, indicating no specific association with BRM.

also that the BRM complex purified by Kal *et al.* (7) did not include HSC4. It is therefore unlikely that HSC4 is a subunit of the BRM complex.

To determine whether we could detect a functional interaction between *hsc4* and *brm*, we used two genetic assays. First, we asked whether *hsc4* mutations could either enhance or suppress phenotypes created by a dominant-negative *brm* allele, $brm^{K804R}$. $brm^{K804R}$ induces a rough eye phenotype when expressed from a *UAS-*$brm^{K804R}$ transgene under control of an *eyeless-Gal4* driver (O.P., J.A., and J.T., unpublished observations). This eye phenotype is enhanced by alleles of *brm* or *mor*. The three tested *hsc4* alleles ($hsc4^{54.1}$, *l(3)03550*, and *II-R35-R7*) neither enhanced nor suppressed the eye phenotype. We then tested a second phenotype of the antimorphic $brm^{K804R}$ transgene. An insertion of this transgene on the X chromosome (*P[*$w^+$, $brm^{K804R}$*]22D*) reduces the amount of functional *brm* in males to levels barely sufficient for survival to adulthood. Mutations in *brm* or *mor* cause dominant lethality of males in this sensitized background (6). $hsc4^{54.1}$, *l(3)03550*, and *II-R35-R7* did not produce male lethality in this test.

We also tested the *hsc4* alleles for interactions with a strong allele of *trithorax* (*trx*). *trx* is the prototypic activator gene of the trxG, but its biochemical function is not understood. For some alleles of *hsc4* (especially *I-R35-R7*), the *hsc4,* $trx^{B11}$ double heterozygous adult males showed enhancement of the mild anterior transformations of the fifth, sixth, and seventh abdominal segments seen in $trx^{B11}/+$, and/or reduced numbers of sex comb teeth on the first thoracic legs (Table 2). Conversely, an extra copy of *hsc4* suppressed the transformations of $trx^{B11}/+$ adults. The simultaneous effects on repression (*Polycomb*) and activation (*trithorax*) functions might explain why *hsc4* mutations alone have only subtle and variable effects on segmental specification.

## Discussion

The *hsc4* gene encodes the major heat shock cognate protein, which is expressed at all times of development, but is particularly abundant in ovaries and embryos (34). A variety of phenotypes have been associated with *hsc4* mutations. Removal of both the zygotic and maternal contributions of *hsc4* causes embryonic lethality accompanied by variable segmental deletions (35). Loss of zygotic activity alone leads to larval lethality (21). These dying larvae develop melanotic tumors (36) and are defective in the development and projection of the larval optic nerve, referred to as Bolwig's nerve (30). We have observed that heterozygotes for all alleles show malformations of the fourth abdominal tergite in a small fraction of adults. A dominant negative allele of *hsc4*, $hsc4^{195}$, was found to modulate Notch signaling during development (21). The same allele enhances the dominant adult phenotypes of mutations in the ecdysone receptor (37). We described above the interaction with mutations in the PcG, which constitutes another distinctive class of phenotypes. We also noted a seemingly contradictory enhancement of *trithorax* mutation, itself an antagonist of *Polycomb*. One mechanism that might connect all of these phenotypes is the one suggested by the protein sequence of HSC4, namely, the protein folding process. Protein targets of the HSC4 chaperone function might be involved in many diverse developmental pathways.

The interaction of the $hsc4^{54.1}$ allele with *Polycomb* is distinctive. The HSC4 protein is not particularly limiting, because heterozygotes for a null allele appear nearly wild type. Heterozygotes for $hsc4^{54.1}$ do show a phenotype, suggesting that the $hsc4^{54.1}$ protein product might act as a poison. Perhaps the $hsc4^{54.1}$ protein product sequesters a target protein or misfolds it into a nonfunctional product. If POLYCOMB were the target, this could inactivate 50% of the POLYCOMB protein, assuming half of the POLYCOMB nascent chains interact with the mutant form of HSC4. A 50% reduction in functional POLYCOMB would explain the weak phenotypes of $hsc4^{54.1}/+$, and the lethality of *hsc4* alleles with $ph^{410}$ (Table 2), because another *ph-proximal* null, $ph^{409}$, is lethal in a $Pc^-/+$ background (38).

An alternative explanation for the $hsc4^{54.1}$ phenotype is that HSC4 is more directly involved in the process of *Polycomb* group repression. Recently, HSC4 has been identified among the components of the *Polycomb* complex purified from *Drosophila* embryos (A. Saurin, Z. Shao, H. Erdjument-Bromage, P. Tempst, and R. Kingston, personal communication). Because HSC4 is so abundant, it could be merely a contaminant, but the purification included immunoprecipitation of an epitope-tagged complex, and it was demonstrated that some HSC4 protein coelutes with the complex on a sizing column. It is possible that HSC4 remains bound to one of its targets and becomes incorporated into the complex with that protein. It is also possible that HSC4 has a catalytic role. HSC4 is related to a variety of ATPases, including actin (39). A bacterial homolog of HSC4, DnaK, is thought to rearrange the protein complex involved in phage lambda DNA replication (40). Other actin-related proteins have been found in the SWI/SNF and RSC chromatin remodeling complexes (41), and it has been suggested that they catalyze conformational changes in those complexes or their substrates (42). HSC4 could have a similar function in PRC1 repression.

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