Genetic Analysis of the *Additional sex combs* Locus of *Drosophila melanogaster*

Donald A. R. Sinclair, Roderick B. Campbell, Felicity Nicholls, Eric Slade and Hugh W. Brock

Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4

Manuscript received September 4, 1991
Accepted for publication December 16, 1991

ABSTRACT

Additional sex combs (*Asx*) is a member of the *Polycomb* group of genes, which are thought to be required for maintenance of chromatin structure. To better understand the function of *Asx*, we have isolated nine new alleles, each of which acts like a gain of function mutation. *Asx* is required for normal determination of segment identity. Asx^{P1} shows an unusual phenotype in that anterior and posterior homeotic transformations are seen in the same individuals, suggesting that Asx^{P1} might upset chromatin structure in a way that makes both activation and repression of homeotic genes more difficult. Analysis of embryonic and adult phenotypes of *Asx* alleles suggests that *Asx* is required zygotically for determination of segment number and polarity. The expression pattern of *even-skipped* is altered in *Asx* mutant embryos, suggesting that *Asx* is required for normal expression of this gene. We have transposon-tagged the *Asx* gene, and can thus begin molecular analysis of its function.

MUTATIONS in the *Polycomb* (*Pc*) group (PcG) of genes cause homeotic transformations similar to gain-of-function (GOF) mutations of the BX-C and ANT-C (LEWIS 1978; STRUHL 1981; DUNCAN 1982; INGHAM 1984). Molecular analyses have shown that this is due to ectopic expression of homeotic genes (STRUHL and AKAM 1985; WEDEEN, HARDING and LEVINE 1986; CARROLL *et al.* 1986; RILEY, CARROLL and SCOTT 1987; GLICKSMAN and BROWER 1988; DURA and INGHAM 1988; SMOUSE *et al.* 1988; MCKEON and BROCK 1991), suggesting that PcG genes are negative regulators of homeotic genes. Analysis of *extra sex combs* shows that it is not required for the initiation of segment-specific expression of homeotic genes, but is instead required to repress homeotic gene expression in inappropriate segments once spatial regulation of homeotic genes has been established by the action of segmentation genes (STRUHL and AKAM 1985; AKAM 1987; INGHAM 1988).

PcG genes have more general roles in development than regulation of homeotic genes. Mutations in various loci show nervous system defects (SMOUSE *et al.* 1988), small disc phenotypes (SHEARN, HERSPERGER and HERSPERGER 1978), modification of *zeste-white* interactions (WU *et al.* 1989; JONES and GELBART 1990), maternal-effect segmentation defects (INGHAM 1984; BREEN and DUNCAN 1986) as well as zygotic effects on expression of segmentation genes (DURA and INGHAM 1988; SMOUSE *et al.* 1988; BUSTURIA and MORATA 1988). However, no PcG mutations have been reported whose zygotic loss causes segmentation defects.

Recently, it has been reported that there is sequence similarity between the *Pc* and *Su(var)205* genes (PARO and HOGNESS 1991). This has led to suggestions that PcG genes might function analogously to modifiers of position-effect-variegation in establishment or maintenance of chromatin structure (PARO 1990; GAUNT and SINGH 1990; REUTER *et al.* 1990). Using a dosage argument, LOCKE, KARTOSKI and TARTOF (1988) have suggested that PcG gene products may form a multimolecular complex. In addition, ZINK and PARO (1989) have shown that *Pc* protein binds to chromosomal sites containing other PcG genes, supporting the idea that the PcG may form a regulatory network. Thus, study of PcG genes and their interactions should allow dissection of their role in establishment or maintenance of chromatin structure. Detailed analysis of individual PcG genes may provide the basis for understanding their individual functions.

Asx is located in region 51AB on the polytene chromosome map and was first described and classified as a member of the PcG by JURGENS (1985). BREEN and DUNCAN (1986) described the *Asx* zygotic phenotype, and showed that *Asx* has a maternal effect. Zygotic loss of *Asx* causes ectopic expression of homeotic genes (MCKEON AND BROCK 1991). Here, we report the isolation of new alleles of *Asx* using γ-irradiation, and report that *Asx* mutant embryos and adults exhibit segmentation defects in addition to previously identified homeotic defects. Mutant embryos show abnormal expression of *even-skipped* (*eve*) at germ band extension. Finally, we have isolated a *P* element-induced allele of the *Asx* locus, and can thus begin molecular analysis.

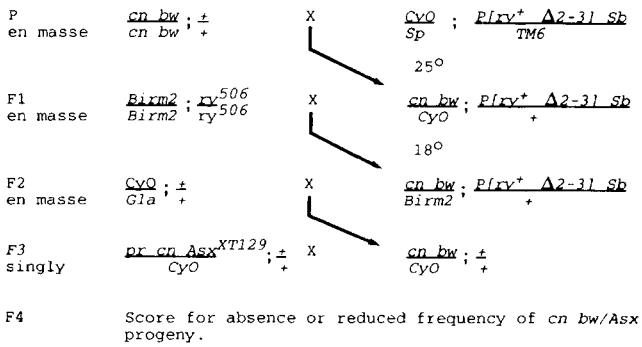

FIGURE 1.—*P* element mutagenesis of the *Asx* locus. Shown are the crosses used to generate *P* element-induced mutations at the *Asx* locus, together with the temperatures at which the crosses were made. Details can be found in ROBERTSON *et al.* (1988).

MATERIALS AND METHODS

Culture conditions and fly stocks: Flies were grown on standard cornmeal-sucrose medium, with Tegosept added as a mold inhibitor. Except where indicated, cultures were maintained and crosses performed at 22°. Descriptions of all visible mutations and balancers can be found in either LINDSLEY and GRELL (1968) or LINDSLEY and ZIMM (1985, 1986, 1987, 1990). *DTS91* is a dominant temperature sensitive mutation on the second chromosome supplied by T. GRIGLIATTI. *Df(2R)trix* deletes from 51A1-2 to 51B6, and was obtained from B. BAKER, and *Df(2R)L+48* deletes from 51A1-51B4, and was obtained from E. WIESCHAUS. The parental chromosome for the mutagenesis was derived from an isogenic *cn bw* line. The *pr cn Asx*^{XT129} strain was constructed by G. JURGENS and was obtained from P. SANTAMARIA, and the *cn Asx*^{IIF51} *bw sp* strain (JURGENS 1985), was obtained from the Bloomington stock center. The *al b pr cn Asx*^{P1} strain was obtained from I. DUNCAN, and is described in BREEN and DUNCAN (1986). Stocks for *P* element mutagenesis (*Birm2;ry*^{506}, *CyO/Sp;P[ry*$^{+}$ *2–3] Sb/TM6*) were obtained from W. ENGELS, and are described in ROBERTSON *et al.* (1988).

Isolation of *Asx* mutants. New mutations of *Asx* were isolated by crossing males of an isogenized *cn bw* stock that had been irradiated with 4000 R of γ-rays from a ^{60}Co source to *DTS91/CyO* females *en masse*. The bottles were cleared every 2 days for a total of 6 days, and then parents were discarded. F_1 males that were phenotypically *Curly*, were crossed individually to several *pr cn Asx*^{XT129}*/CyO* females and F_2 progeny from each cross were examined for the absence of straight-winged flies. Stocks of each putative mutant were established from the balanced siblings. Chromosomes containing each confirmed *Asx* allele were examined cytologically when heterozygous with wild-type chromosomes, using standard lacto-acetic-orcein staining.

Hybrid dysgenesis screen for *Asx* alleles: Figure 1 shows the screen used to isolate *P* element-induced mutations in the *Asx* locus. Homozygous *cn bw* females were crossed to *CyO/Sp;P[ry*$^{+}$ *2–3] Sb/TM6* males, and their male *Cy;Sb* progeny crossed to the *Birm* females, which were allowed to lay at 18°. Male *Sb* progeny were crossed en masse to *CyO/Gla* females, and single male *cn bw/CyO* progeny were crossed individually to *Asx*^{XT129} females, and their progeny scored for lethality or reduced viability. A single putative *P* element-induced *Asx* mutation designated *Asx*^{P1} was recovered. Revertants were isolated by mating males from the *Asx*^{P1}*/CyO* stock to *CyO/Sp;P[ry*$^{+}$ *2-3]/TM6* females, and then crossing their straight-winged, *Sb*$^{+}$ progeny to *Asx*^{XT129}*/CyO*, and examining the F_2 progeny for the presence of straight-winged flies.

Mapping and complementation analysis: Each newly induced mutation that failed to complement the *Asx*^{XT129} chromosome was recombinationally mapped by crossing males of the stock to *S Sp Tft nw*^{D} *Pin*^{YT}*/CyO* females, and then backcrossing *S Sp Tft nw*^{D} *Pin*^{YT}*/cn Asx*^{n} *bw* female progeny to *cn Asx*^{n} *bw* males (*n* = newly induced *Asx* allele). Approximately 800–1000 progeny were scored for each mapping cross. These studies revealed that mutations at two other loci were recovered, and control experiments established that this was because of the presence of two additional lethals on the *Asx*^{XT129} screening chromosome. Only mutations mapping to *Asx* were examined here. Complementation analysis was performed in conventional fashion, and at least 100 progeny were scored for lethality in each complementation test.

Cuticle analysis: To observe cuticular patterns in late embryos, overnight egg collections were aged for a further 24 hr at 25°. Unhatched embryos were dechorionated in 50% commercial bleach, fixed on the interface of a heptane/3.7% formaldehyde solution, devitellinized by removal of the aqueous fix and addition of methanol, postfixed in glycerol:acetic acid 1:4, and mounted in Hoyer's medium for observation under phase and dark field illumination. Where necessary, abdomens or appendages were mounted in a 1:1 mixture of Canada balsam and methyl salicylate, and cleared at 60° on a hot plate for several days. Sometimes abdomens were cut middorsally and spread to show dorsal and ventral surfaces.

Analysis of interactions with even-skipped: A transformed line containing an *even-skipped* (*eve*) β-galactosidase fusion on the *X* chromosome, described in LAWRENCE *et al.* (1987) and obtained from J.-M. DURA, was crossed to *Asx* mutant females. Their doubly heterozygous female progeny were crossed to *Df(2R)trix* or other *Asx* alleles. β-galactosidase activity was detected according to BIER *et al.* (1989).

***In situ* hybridization:** *In situ* hybridization using biotinylated probes was performed essentially as described by MCDONALD and GOLDSTEIN (1990).

RESULTS

Generation of new *Asx* alleles: To better understand the *Asx* phenotype, and to provide breakpoints that could be useful for identifying DNA sequences corresponding to *Asx*, we used γ-irradiation to induce new alleles of this gene on isogenic *cn bw* chromosomes (see MATERIALS AND METHODS). Nine new *Asx* alleles were recovered from a total of 6720 chromosomes screened. All mutations were mapped by recombination as described. The new *Asx* mutants plus *Asx*^{IIF51} mapped to between 69 and 72 on chromosome 2R, in agreement with the published map position of 2–72 (JURGENS 1985). The lethality associated with *Asx*^{XT129} could not be localized in chromosome 2R because of a small inversion that spans from 48C to 51C on the polytene chromosome map. However, neither of the inversion breakpoints is included in the smallest deficiency that defines *Asx* cytologically. The nine new *Asx* alleles will hereafter be referred to as *Asx*$^{8-16}$, since JURGENS (1985) and BREEN and DUNCAN (1986) have described previously 6 and 1 *Asx* alleles respectively. Cytological examination of these strains

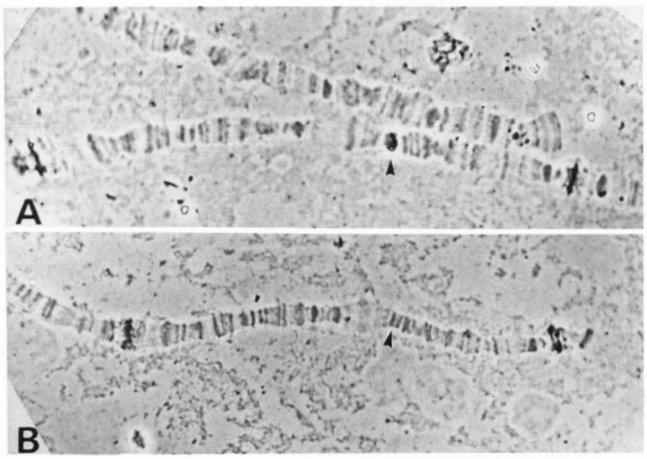

FIGURE 2.—*In situ* hybridization to putative *P* element-induced *Asx* mutations and a revertant. A, *In situ* hybridization to polytene chromosomes of homozygous Asx^{P1} mutants. Proximal is on the left, and distal on the right. Sites of hybridization are visible at regions 47B, 51A and 53D. The arrowhead marks the site of 51A. B, *In situ* hybridization to polytene chromosomes of an Asx^{P1} revertant. Proximal is left, and distal right as before, and hybridization is still visible at 47B and 53D, but is no longer visible at 51A (marked with an arrowhead).

did not reveal chromosomal abnormalities associated with the 51A-B region.

Transposon tagging the *Asx* locus: A screen for *P* element-induced alleles of *Asx* on isogenic *cn bw* chromosomes was carried out as described (see MATERIALS AND METHODS). Five mutations were recovered from 7224 chromosomes scored. Only one of these exhibited semilethality in combination with *Df(2R)trix* and other *Asx* alleles. This strain has been designated Asx^{P1}. We isolated eleven revertant lines of the Asx^{P1} mutation (see MATERIALS AND METHODS). Asx^{P1} is homozygous viable, but semi-lethal with *Df(2R)trix* and other *Asx* alleles. It is likely a weak hypomorph. All eleven revertants were completely wild type in combination with *Df(2R)trix* (data not shown).

To determine if the Asx^{P1} strain contained a *P* element in region 51AB, *P* element DNA was hybridized to polytene chromosomes of this stock after replacement of the *X* and third chromosomes. As shown in Figure 2A, there is a *P* element present in region 51A5-6. Two other *P* elements are also visible on chromosome 2R, at positions 47B and 53E, respectively. Subsequently, polytene chromosomes from four of the eleven Asx^{P1} revertants were examined by *in situ* hybridization. All four strains lost the *P* element at 51AB but retained the *P* elements in 47B and 53E (Figure 2B, and results not shown).

Embryonic phenotype of *Asx* mutants: The embryonic phenotypes of strong *Asx* mutants described by JURGENS (1985), and by BREEN and DUNCAN (1986), are summarized here for comparison. *Df(2R)trix* embryos show head defects and do not complete head involution. The cephalopharyngeal apparatus shows some shortening of the lateral graten, and the mouthhooks are mildly splayed. All thoracic denticle belts show occasional medial abdominal denticles, and the Keilin's organs are occasionally missing in the third thoracic segment, but ventral pits are unaffected (Figure 3B). All abdominal segments show posteriorly directed transformations. The first abdominal segment (A1) is transformed toward A3; A2, A3 and A4 are transformed toward A6 or A7; and A6 and A7 are transformed toward A8 (Figure 4B). Although the ability of *Df(2R)trix* to cause segmentation defects has not been previously reported, in our hands 10% of the homozygous embryos scored exhibited such traits. These included missing denticle belts or half denticle belts, and occasional segment fusions or reversed polarity of denticle hairs, typical of mild phenotypes of segment polarity genes. Similar defects were observed in about 10% of embryos homozygous for $Df(2R)L^{+48}$, which uncovers 51A1-2;51B4 but was made in a different background (Figure 3E).

In general, the newly induced $Asx^{8\text{-}16}$ alleles have more severe head phenotypes than deficiencies that uncover *Asx*, although the severity varies among alleles, and different progeny from the same cross also show variations in the amount of head involution. The cephalopharyngeal apparatus is often reduced to small condensations of chitin, and individual components of the mouth parts are difficult to distinguish. Typically, the mouth hooks and cirri are displaced laterally and the H piece reaches only to the level of the first thoracic segment (T1) (Figure 3C). These alleles also exhibit more severe transformation of thoracic segments toward abdominal segments than *Asx* deficiencies, as evidenced by the presence of more abdominal denticles in thoracic denticle belts. Most of these transformed denticles point forward, suggesting transformation of thoracic segments toward at least A2. T2 and T3 are somewhat trapezoidal, also supporting the idea of partial transformation toward at least A2. Usually all three thoracic segments exhibit abdominal denticles. The Keilin's organs are usually present in the third thoracic segment. This is not the case with Asx^{10} mutant embryos, which have no Keilin's organs in the third thoracic segment. $Asx^{8\text{-}16}$ show less severe posterior transformation of abdominal segments than do *Asx* deficiencies (Figure 4C). All the newly induced alleles show some segmentation defects, including missing segments, denticles missing in the midline, and hemisegments, at frequencies varying from 1 to 10% of scorable embryos (Figure 4F). As shown in Figure 4H, most of the defects affect even-numbered segments. Homozygous *cn bw* embryos do not show segmentation defects (results not shown).

Because the head and thorax phenotypes of the new alleles are stronger than those of the deficiency, this suggests the possibility that most of the new *Asx* mu-

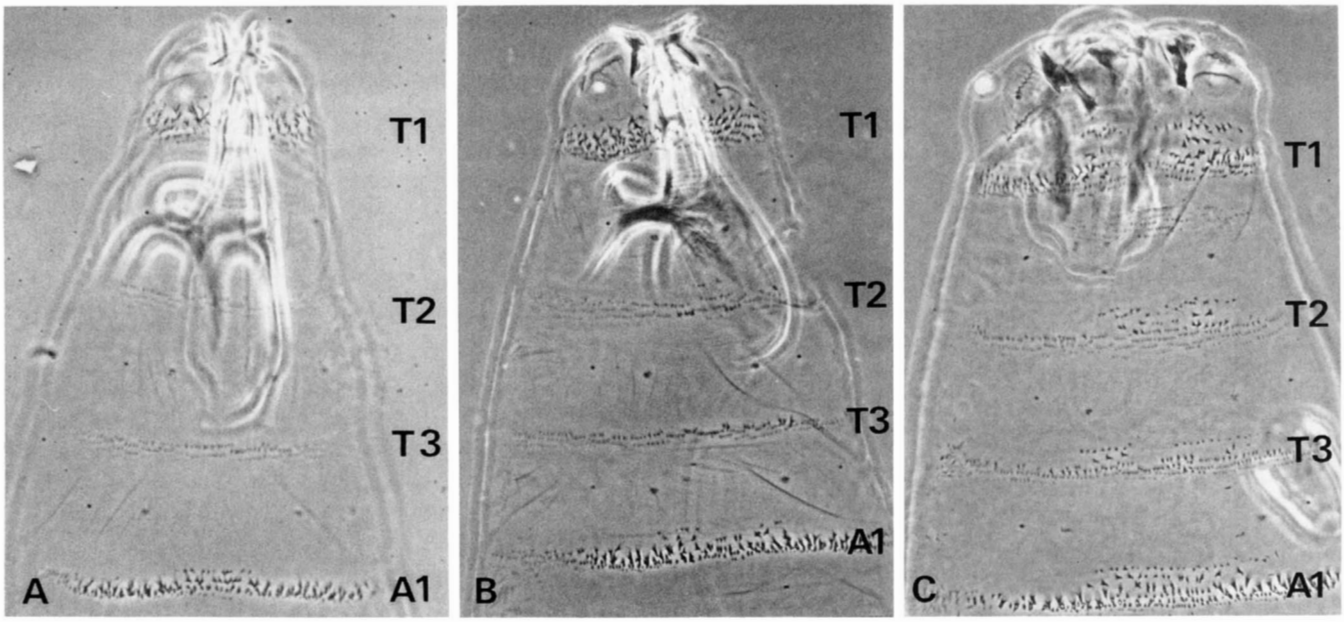

FIGURE 3.—Detail of head and thoracic segments from wild-type and *Asx* mutant embryos. T1–T3, first, second, and third thoracic segments, respectively. A1, first abdominal segment. A, Wild-type embryo. Note that the H-piece extends almost to T3, and that the mouthhooks are closely apposed in the midline. T1 lacks forward-pointing large denticles, and T2 and T3 lack large denticles of any kind. B, Homozygous *Df(2R)trix* embryo. The mouthhooks are displaced from the midline and the posterior end of the H-piece is more anterior than in wild type. Anteriorly directed denticles are visible in T1, and several large denticles are visible in T2 and T3. However, the shape of the thoracic denticles remains like that of wild-type embryos. C, Homozygous Asx^{12} embryo. The mouthhooks and cirri are displaced laterally, and the H-piece does not reach T2. Many forward-pointing denticles are visible in T1, and T2 and T3 have more large denticles, and the outline of the denticle belts appears more trapezoidal, showing that the extent of posterior transformation is greater in Asx^{12} than in *Df(2R)trix* embryos.

tations are GOF alleles. The phenotypes of embryos hemizygous for $Asx^{8,13,15,16}$ were examined. The majority of hemizygous embryos had weak head and thoracic defects that resemble the null phenotype, and weak abdominal homeotic transformations that resemble the homozygous phenotype of the new *Asx* alleles. However, between 5 and 10% of hemizygous embryos exhibit nearly complete posterior transformation of thoracic and abdominal segments (Figure 4F), and a few embryos show strong segmentation defects (Figure 4G). Figure 4H shows a histogram of the segmentation defects plotted against segment. It can be seen that abdominal segments are most affected, and that there are more defects in even-numbered segments.

Effect of *Asx* mutations on expression of segmentation genes in embryos: We examined the effects of *Asx* mutations on the expression of an *eve-lacZ* construct. The *eve-lacZ* construct contains 6.3 kb of the *eve* promotor (LAWRENCE *et al.* 1987), and in wild-type embryos, the β-galactosidase expression patterns at germ band exension are nearly normal compared to the distribution of *eve* protein, although stripes 2, 3 and 7 appear broader than normal (Figure 5A, and HARDING *et al.* 1989). However, this construction is not suitable for examining *eve* expression patterns at blastoderm. Males carrying the *X* chromosome bearing the *eve-lacZ* transformant were crossed separately to Asx^{8}, Asx^{13} and Asx^{16} balanced females. Their *eve-lacZ*/+;*Asx*/+ female progeny were then crossed to Asx^{15} or *Df(2R)trix* males. One quarter of the progeny from the F_2 crosses that stain for β-galactosidase will be either hemizygous for *Asx* if crossed to *Df(2R)trix* males, or transheterozygous for *Asx* if crossed to Asx^{15} males, and be heterozygous for the *eve-lacZ* transformant. About 10% of the stained embryos scored (25% should be mutant) had β-galactosidase expression patterns that had sharp anterior borders, but broader and more diffuse posterior borders than those of wild-type embryos, and these presumably correspond to Asx^{8}/Asx^{15}, $Asx^{13}/Df(2R)trix$ and $Asx^{16}/Df(2R)trix$ embryos (Figure 5B). This suggests that approximately 40% of the mutant *Asx* embryos exhibit some ectopic expression of *eve*.

Adult phenotypes of *Asx* alleles: Adult phenotypes of heterozygous *Asx* alleles have not been described previously. Homeotic transformations are observed most frequently in the abdomen, with mild transformation of A4 to A5 in males, manifested by darkly pigmented patches in the A4 tergite (see Figure 6B). Occasionally, long bristles can be seen in A1, showing transformation of A1 toward a more posterior segment. Extra sex combs are seen only occasionally, unless enhanced by the presence of another PcG gene. In less than 1% of flies, humeral bristles are missing, or rarely, the entire humerus is gone.

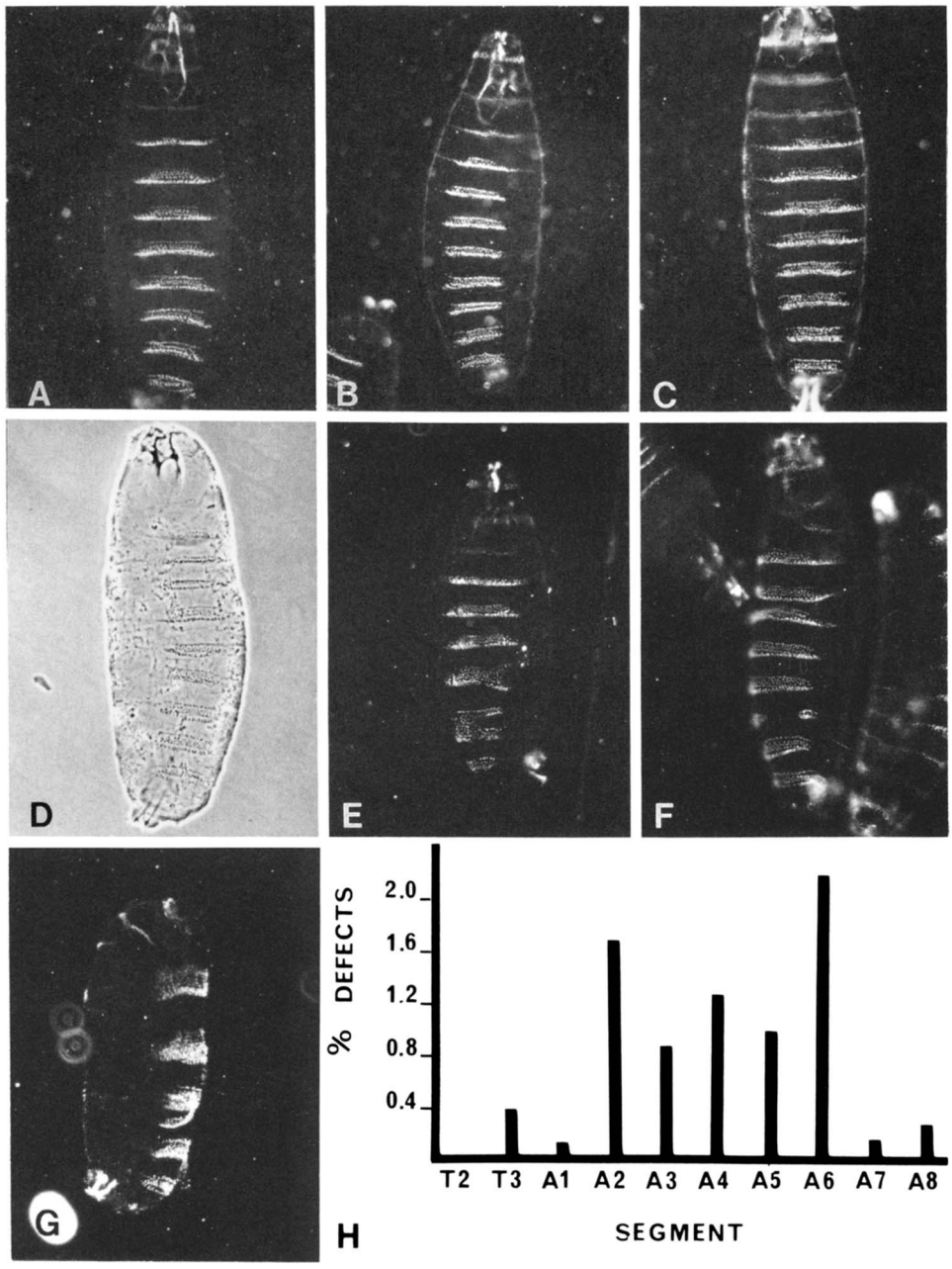

FIGURE 4.—Cuticle preparation of wild-type and mutant *Asx* embryos, and plot of segmentation-defect frequencies. Embryos are oriented with the anterior up, and posterior down, and are in ventral view, except for G, which is in lateral view. All embryos are shown in dark field illumination, except panel D, which is phase contrast. A, Wild-type embryo. Only the eighth abdominal segment (A8) is rectangular, and the remainder are trapezoidal and increase in width as one moves anteriorly. B, Homozygous *Df(2R)trix* embryo. All abdominal segments are posteriorly transformed, and A5 resembles A8. Most abdominal segments have polarity defects, seen as an increase in the number of larger denticles, and decreased width. C, Homozygous Asx^{16} embryo. Only A7 is clearly transformed toward A8, and most other abdominal segments appear normal, except A1 which is transformed toward A2. D, Hemizygous Asx^{16} embryo. Head defects are very severe, but T1 and T2 are relatively unaffected. All segments posterior to T2 appear to be transformed toward A8. E, Homozygous *Df(2R)L+48* embryo. A4–5, and A6–7 are fused. F, Homozygous Asx^{15} embryo. T3 is partially missing, and A6 is absent in the midline. G, Hemizygous Asx^{16} embryo. A7 and A8 are present, but the rest of the embryo shows a very strong pair-rule phenotype. H, Plot of segment-defect frequencies in homozygous *Asx* embryos. The percentage of defects (fusions, unfused, partial, or missing denticle belts) found in each segment is indicated on the *Y* axis, and the segment affected shown on the *X* axis.

Asx^{P1} shows a different phenotype. Asx^{P1} homozygotes and hemizygotes frequently exhibit extra sex comb teeth on their second thoracic leg, and occasional pigmented patches on their fourth tergites, consistent with this mutation permitting ectopic expression of homeotic genes. Surprisingly, homozygous flies also show anteriorly directed homeotic transformations. Unpigmented cuticle is frequently seen in the fifth abdominal tergites of males, and halteres are transformed toward wings in about 10% of flies (not shown). These transformed halteres usually have triple row bristles on their anterior margins, and are swollen. The anteriorly directed mutations are consistent with loss of function homeotic gene expression in the appropriate segments.

Some strong *Asx* alleles, notably Asx^{11} and Asx^{14} show odd-numbered tergite defects, including missing tergites, unfused tergites, or hemitergites. The sternites are not affected (Figure 5B). Curiously, these defects are found most often in odd-numbered segments, in contrast to the embryonic segmentation defects which normally affect even-numbered segments.

DISCUSSION

To better understand the function of the *Asx* locus, and to facilitate molecular analysis, new *Asx* alleles were induced using γ-irradiation and hybrid dysgenesis. The *Asx* mutation rate of 0.13% in the γ-irradiation screen is higher than that normally observed in similar screens in other genes. For example,

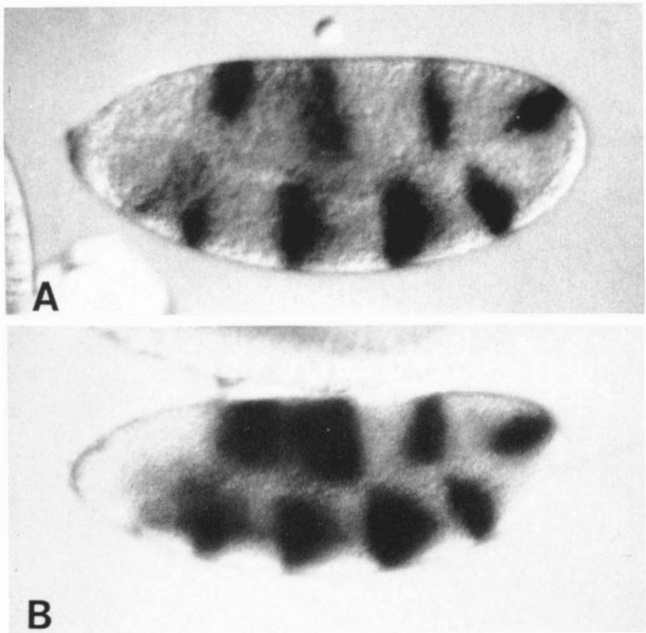

FIGURE 5.—Effect of *Asx* mutations on *even-skipped* expression. Germ band extended embryos are oriented with anterior to the left and dorsal to the top. A, Expression of β-galactosidase under the control of an *eve* promotor in a wild-type embryo. Stripes 2, 3 and 7 are somewhat wider than the other stripes, but there is not detectable β-galactosidase between the stripes. B, Expression of β-galactosidase in a putative $Asx^{16}/Df(2R)trix$ embryo. Most stripes are wider than in wild-type embryos, and appear more diffuse.

rates varying between 0.02% and 0.048% have been reported for *chapotin* and *torpedo* at the same dose of γ-irradiation (VAN VACTOR *et al.* 1988; PRICE, CLIFFORD and SCHUPBACH 1989). However, rates as high as 0.24% have been reported for *Antennapedia*, which is an unusually large gene (HAZELRIGG and KAUFMAN 1983). Our data suggest that either *Asx* is a hot spot for damage by γ-irradiation, or that it is a large gene. The new *Asx* alleles are not associated with visible cytological rearrangements. As reviewed in ASHBURNER (1989), about half of γ-ray induced mutations are expected to be cytologically visible, and of these, most will be deletions. However, all nine of the *Asx* mutations lack cytologically detectable lesions. Because of the absence of suitable chromosome rearrangements, the previous best cytological location of *Asx* was 51A1-2;51B4. *In situ* hybridization to Asx^{P1} places the cytological location of *Asx* in 51A5-6

The phenotypes of deficiencies that include *Asx* differ from those of the newly induced *Asx* alleles. It appears that these alleles are not null or hypomorphic mutations because they have stronger head and thoracic phenotypes than do *Asx* deficiencies. However, the data do not exclude the possibility that *Df(2R)trix* uncovers a closely linked modifier of *Asx*, because a molecularly characterized null mutation is not available. Conversely, it is possible that the genetic background common to $Asx^{8\text{-}16}$ contains an enhancing modifier. However, we note that the *Asx* mutations described by JURGENS (1985) which were made in a different background, more closely resemble the new *Asx* mutations described here than the deletion phenotype described by BREEN and DUNCAN (1986), making this hypothesis unlikely.

These data support the hypothesis that the new *Asx* alleles are GOF mutations. However, the abdominal phenotypes of $Asx^{8\text{-}16}$ are weaker than the deficiency phenotype, so for this phenotype, the new *Asx* mutations act like loss of function mutations. Most embryos hemizygous for new *Asx* mutations showed weaker head and thoracic phenotypes than homozygotes for the same mutation, suggesting that the new alleles are antimorphic with respect to head and thoracic defects. It appears likely that the new mutations are complex, and that domains of the *Asx* protein with different functions are affected differentially by the mutations. A similar argument has been made for *Psc* mutations by ADLER, CHARLTON and BRUNK (1989). It is formally possible that some of the γ-ray-induced *Asx* mutations are regulatory mutations.

All *Asx* mutations known to us except Asx^{P1}, appear to be GOF mutations. Asx^{D1} was thought to be a hypomorph, but this mutation and all other *Asx* alleles tested show GOF interactions with *super sex combs* that are not exhibited by *Df(2R)trix* (D. SINCLAIR and H. BROCK, unpublished). It is surprising that most *Asx* mutations are GOF considering that they were generated by either ethylmethane sulfonate (Asx^{D1}) and X- or γ-irradiation. In principle, most should be point or deletion mutations. Perhaps the *Asx* protein retains some functional properties after most mutagenic events. If *Asx* protein functions as part of a multimeric complex as suggested by LOCKE, KOTARSKI and TARTOF (1988), then perhaps complete absence or reduction of the *Asx* protein might have a different effect on the complex than does the presence of abnormal *Asx* protein. Alternatively, the *Asx* mutations recovered might all be regulatory mutations. Distinguishing among these alternatives awaits molecular analysis of *Asx* mutations.

The zygotic embryonic phenotype of an *Asx* deletion is not as strong as the zygotic phenotype observed for *Pc* (DUNCAN and LEWIS 1982); *esc* (STRUHL 1983), or *ph* (DURA *et al.* 1987), and is similar to that observed for *Psc* and *Scm* (JURGENS 1985) and *Sce* (BREEN and DUNCAN 1986). Homeotic transformations are seen in all abdominal and thoracic segments, and head involution is abnormal, but the continued presence of Keilin's organs in most thoracic segments, and absence of the wartlike sensory organs in abdominal segments other than the eighth abdominal segment shows that these transformations are incomplete.

The adult homeotic phenotypes of *Asx* are relatively mild compared to those seen in *Pc* or *ph* flies, as only occasional transformations of T2 to T1 legs are seen.

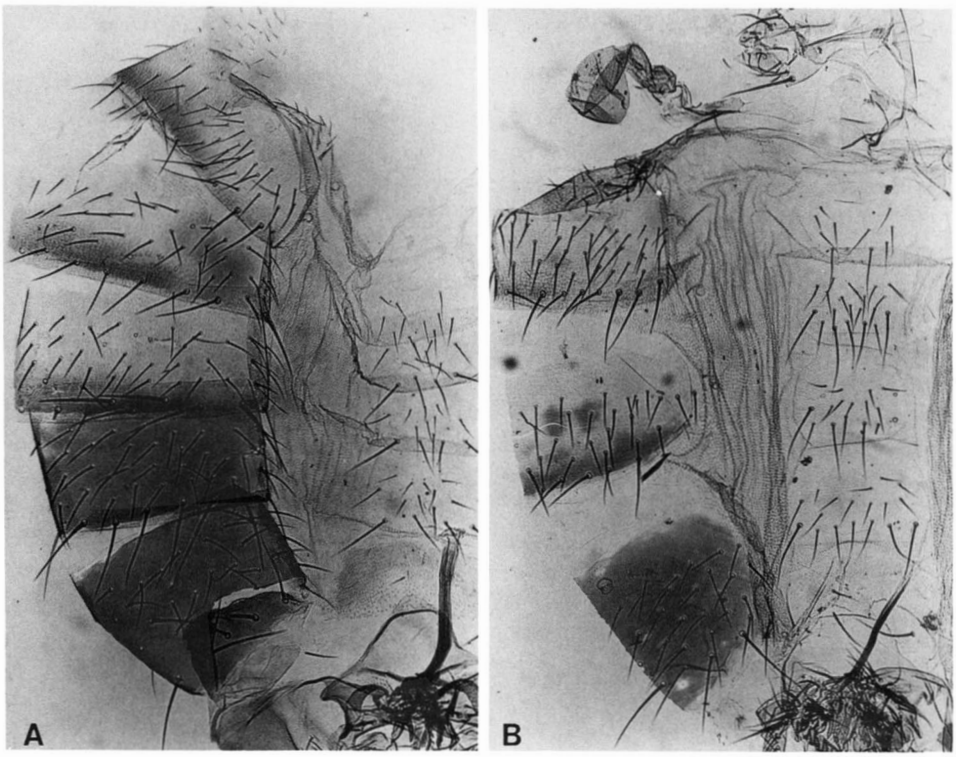

FIGURE 6.—Abdominal phenotype of wild-type and *Asx* male adults. Abdomens were split in the dorsal midline, and spread flat, and mounted in a 1:1 mixture of methyl salicylate and Canada balsam. Anterior is up, and posterior is down. A, Wild-type adult. A5 and A6 tergites are darkly pigmented, and A2–A4 are unpigmented except at the posterior border. B, Heterozygous Asx^{XT129}/+ adult. The A3 and A5 tergites are missing, but that the sternites are unaffected. The A4 tergite has pigmented spots, suggesting a posterior transformation of A4 to A5.

Like the embryonic transformations, the adult phenotypes can be explained by *Asx* acting as a negative regulator of homeotic genes, so that in the absence of the wild-type product, the homeotic genes are expressed ectopically. Indeed, ectopic expression of *Antp*, *Scr* and *Ubx* has already been demonstrated in *Df(2R)trix* embryos (MCKEON and BROCK 1991).

Asx^{P1} is an interesting exception. Hemizygous flies show gain of function homoeotic transformations because most males have sex comb teeth on the second thoracic leg. However, these flies also exhibit loss of function homeotic transformations because halteres and the fifth abdominal segment are transformed anteriorly. This combination of phenotypes is difficult to explain in terms of *Asx* being solely a repressor of homeotic gene expression, unless the Asx^{P1} mutation is assumed to be simultaneously interfering with activation and repression of homeotic genes. One way this could occur would be if the mutation upset chromatin structure in such a way that made both normal activation and repression of homeotic gene expression more difficult. Alternatively, since most *P* elements insert into 5′-regulatory regions (FASANO *et al.* 1991), Asx^{P1} could be a regulatory mutation that allows inappropriate expression of *Asx*. Another possibility is that *Asx* is primarily a repressor, but the activation of some homeotic genes in some parasegments may require positive regulators that themselves interact with *Asx* protein.

One of the most striking phenotypes of *Asx* mutations is the segmentation defects observed at low penetrance and with variable expressivity in all *Asx* alleles except Asx^{D1}. Even in the presence of the maternal Asx^{+} product, zygotic loss of *Asx* results in segment defects and upset patterns of *eve* expression, suggesting that *Asx* is required very early in development. These segmentation defects are not owing to background, because they are observed in individuals homozygous or heterozygous for *Asx* alleles made in different backgrounds. Moreover, the siblings generated from crosses to make *Asx* heterozygotes do not exhibit segmentation defects. More embryos show upset *eve* expression than exhibit cuticle defects, suggesting that the embryo can rectify disturbances segmentation gene expression. Segmentation defects are also seen in adults, supporting the hypothesis that *Asx* is required for the normal regulation of segmentation genes.

No other PcG gene mutations are reported to cause zygotic segmentation defects visible in embryonic cuticle preparations. However, a number of observations support the hypothesis that PcG genes are required for regulation of segmentation genes. *ph* is required for the normal expression of *en* in early ectoderm (DURA and INGHAM 1988; SMOUSE *et al.* 1988). Three PcG genes *sxc*, *Pcl* and *l(4)29* show similar segmentation defects at low penetrance when the homozygous mutant embryos arise from mothers with homozygous mutant germ lines (INGHAM 1984; BREEN and DUNCAN 1986). SIMON, CHIANG and BENDER (1991) have shown that expression of *abdominal-A* and *abdominal-B* are pair-rule modulated in PcG mutations. How-

ever, the significance of these results remains to be determined. It may be that segmentation genes are less sensitive to the loss of any one PcG product than are homeotic genes, or that PcG genes have an indirect effect on segmentation.

Because the *Asx* locus is transposon-tagged, it should be possible to investigate the molecular basis of *Asx* genetics. In particular, one can test if *Asx* is a protein required for the maintenance or establishment of chromatin structure. These studies should contribute to our understanding of the function of the PcG.

We thank B. BAKER, P. INGHAM, E. LEWIS and P. SANTAMARIA for stocks. This work was supported by grants from the National Cancer Institute of Canada, and British Columbia Health Care Resarch Foundation to H.W.B.

LITERATURE CITED

ADLER, P. N., J. CHARLTON and B. BRUNK, 1989 Genetic interactions of the *Suppressor 2 of zeste* region genes. Dev. Genet. **10:** 249–260.

AKAM, M. E., 1987 The molecular basis for metameric pattern in the *Drosophila* embryo. Development **101:** 1–22.

ASHBURNER, M., 1989 *Drosophila, a Laboratory Handbook.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

BIER, E., H. VAESSIN, S. SHEPHERD, K. LEE, K. MCCALL, S. BARBEL, L. ACKERMAN, R. CARRETTO, T. VEMURA, E. GRELL, L. Y. JAN and Y. N. JAN, 1989 Searching for pattern mutations in the *Drosophila* genome with a P lacZ vector. Genes Dev. **3:** 1273–1287.

BREEN, T. R., and I. M. DUNCAN, 1986 Maternal expression of genes that regulate the bithorax complex of *Drosophila melanogaster*. Dev. Biol **118:** 442–456.

BUSTURIA, A., and G. MORATA, 1988 Ectopic expression of homeotic genes caused by the elimination of the *Polycomb* gene in *Drosophila* imaginal epidermis. Development **104:** 713–720.

CARROLL, S. B., R. A. LAYMON, M. A. MCCUTCHEON, P. D. RILEY and M. P. SCOTT, 1986 The localization and regulation of *Antennapedia* protein expression in *Drosophila* embryos. Cell **47:** 113–122.

DUNCAN, I. M., 1982 *Polycomblike*: a gene that appears to be required for the normal expression of the bithorax and Antennapedia complexes of *Drosophila melanogaster*. Genetics **102:** 49–70.

DUNCAN, I. M., and E. B. LEWIS, 1982 Genetic control of body segment differentiation in *Drosophila*, pp. 533–554 in *Developmental Order: Its Origins and Regulation*, edited by S. SUBTELNY and P. B. GREEN. Alan R. Liss, New York.

DURA, J.-M., and P. W. INGHAM, 1988 Tissue- and stage-specific control of homeotic and segmentation gene expression in *Drosophila* embryos by the *polyhomeotic* gene. Development **103:** 733–741.

DURA, J.-M., N. RANDSHOLT, J. DEATRICK, I. ERK, P. SANTAMARIA, J. D. FREEMAN, S. F. FREEMAN, D. WEDDELL and H. W. BROCK, 1987 Maternal and zygotic requirement for *polyhomeotic*, a genetically complex locus required for normal expression of segmental identity and cuticular development. Cell **51:** 829–839.

FASANO, L., L. RODER, N. CORE, E. ALEXANDRE, C. VOLA, B. JACQ and S. KERRIDGE, 1991 The gene *teashirt* is required for the development of Drosphila embryonic, trunk segments and encodes a protein with widely spaced zinc finger motifs. Cell **64:** 63–79.

GAUNT, S. J., and P. B. SINGH, 1990 Homeogene expression patterns and chromosomal imprinting. Trends Genet. **6:** 208–212.

GLICKSMAN, M. A., and D. L. BROWER, 1988 Misregulation of homeotic gene expression in *Drosophila* larvae resulting from mutations at the *extra sex combs* locus. Dev. Biol. **126:** 219–227.

HARDING, K., T. HOEY, R. WARRIOR and M. LEVINE, 1989 Autoregulatory and gap gene response elements of the *even-skipped* promotor of *Drosophila*. EMBO J. **8:** 1205–1212.

HAZELRIGG, K. J., and T. C. KAUFMAN, 1983 Revertants of dominant mutations associated with the *Antennapedia* gene complex of *Drosophila melanogaster*: cytology and genetics. Genetics **105:** 581–600.

INGHAM, P. W., 1984 A gene that regulates the bithorax complex differently in larval and adult cells of *Drosophila*. Cell **37:** 815–823.

INGHAM, P. W., 1988 The molecular genetics of embryonic pattern formation in *Drosophila*. Nature **335:** 25–31.

JONES, R. S., and W. M. GELBART, 1990 Genetic analysis of the *Enhancer of zeste* locus and its role in gene regulation in *Drosophila melanogaster*. Genetics **126:** 185–199.

JURGENS, G., 1985 A group of genes controlling expression of the bithorax complex in *Drosophila*. Nature **316:** 153–155.

LAWRENCE, P. A., P. JOHNSTON, P. MCDONALD and G. STRUHL, 1987 Borders of parasegments in *Drosophila* embryos are delimited by the *fushi tarazu* and *even-skipped* genes. Nature **329:** 440–442.

LEWIS, E. B., 1978 A gene complex controlling segmentation in *Drosophila*. Nature **276:** 565–570.

LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melangaster*. Carnegie Inst. Wash. Publ. 627.

LINDSLEY, D. L., and G. ZIMM, 1985 The genome of *Drosophila melanogaster*. Part 1: Genes A-K. Drosophila Inform. Serv. **64:** 1–227.

LINDSLEY, D. L., and G. ZIMM, 1986 The genome of *Drosophila melanogaster*. Part 2: lethals; maps. Drosophila Inform. Serv. **64:** 1–158.

LINDSLEY, D. L., and G. ZIMM, 1987 The genome of *Drosophila melanogaster*. Part 3: rearrangements. Drosophila Inform. Serv. **65:** 1–224.

LINDSLEY, D. L., and G. ZIMM, 1990 The genome of *Drosophila melanogaster*. Part 4: Genes L-Z, balancer, transposable elements. Drosophila Inform. Serv. **68:** 1–382.

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

MCDONALD, H. B., and L. S. B. GOLDSTEIN, 1990 Identification and characterization of a gene encoding a kinesin-like protein in *Drosophila*. Cell **61:** 991–1000.

MCKEON, J., and H. W. BROCK, 1991 Interactions of the *Polycomb* group of genes with homeotic loci of *Drosophila*. Roux's Arch. Dev. Biol. **199:** 387–396.

PARO, R., 1990 Imprinting a determined state into the chromatin of *Drosophila*. Trends Genet. **6:** 416–421.

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.

PRICE, J. V., R. J. CLIFFORD and T. SCHUPBACH, 1989 The ventralizing locus *torpedo* is allelic to *faint little ball*, an embryonic lethal that encodes the *Drosophila* EGF receptor homolog. Cell **56:** 1085–1092.

REUTER, G., M. GIARRE, J. FARAH, J. GAUSZ, A. SPIERER and P. SPIERER, 1990 Dependence of position-effect variegation in *Drosophila* on dose of a gene encoding an unusual zinc-finger protein. Nature **344:** 219–223.

RILEY, P. D., S. B. CARROLL and M. P. SCOTT, 1987 The expression and regulation of *Sex combs reduced* protein in *Drosophila* embryos. Genes Dev. **1:** 716–730.

ROBERTSON, H. M., C. R. PRESTON, R. W. PHILLIS, D. M. JOHNSON-SCHLITZ, W. K. BENZ and W. R. ENGELS, 1988 A stable genomic source of *P* element transposase in *Drosophila melanogaster*. Genetics **118:** 461–470.

SHEARN, A., G. HERSPERGER and E. HERSPERGER, 1978 Genetic analysis of two allelic temperature sensitive mutants of *Drosophila melanogaster* both of which are zygotic and maternal-effect lethals. Genetics **89:** 341–353.

SIMON, J., A. CHIANG and W. BENDER, 1991 Ten different *Polycomb* group genes are required for spatial control of the *abdA* and *AbdB* homeotic products. Development (in press).

SMOUSE, D., C. S. GOODMAN, A. MAHOWALD and N. PERRIMON, 1988 *Polyhomeotic*: a gene required for the embryonic development of axon pathways in the central nervous system of *Drosophila*. Genes Dev. **2:** 830–842.

STRUHL, G., 1981 A gene product required for the correct initiation of segment determination in *Drosophila*. Nature **293:** 36–41.

STRUHL, G., 1983 Role of *esc*$^{+}$ gene product in ensuring the selective expression of segment-specific homeotic genes in *Drosophila*. J. Embryol. Exp. Morphol. **76:** 297–331.

STRUHL, G., and M. E. AKAM, 1985 Altered distributions of *Ultrabithorax* transcripts in *extra sex combs* mutant embryos of *Drosophila*. EMBO J. **4:** 3259–3264.

VAN VACTOR, D., D. E. KRANTZ, R. REINKE and S. L. ZIPURSKY, 1988 Analysis of mutants in chaoptin, a photoreceptor cell-specific glycoprotein in *Drosophila* reveals its role in cellular morphogenesis. Cell **52:** 281–290.

WEDEEN, C., K. HARDING and M. LEVINE, 1986 Spatial regulation of *Antennapedia* and bithorax gene expression by the *Polycomb* locus. Cell **44:** 739–748.

WU, C.-T., R. S. JONES, P. F. LASKO and W. M. GELBART, 1989 Homeosis and the interaction of *zeste* and *white* in Drosophila. Mol. Gen. Genet. **218:** 559–564.

ZINK, B., and R. PARO, 1989 *In vivo* binding pattern of a *trans* regulator of homeotic genes in *Drosophila melanogaster*. Nature **337:** 468–471.

Communicating editor: R. E. DENELL