A Screen for New Trithorax Group Genes Identified *little imaginal discs*, the *Drosophila melanogaster* Homologue of Human Retinoblastoma Binding Protein 2

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ABSTRACT

The proteins encoded by two groups of conserved genes, the Polycomb and trithorax groups, have been proposed to maintain, at the level of chromatin structure, the expression pattern of homeotic genes during Drosophila development. To identify new members of the trithorax group, we screened a collection of deficiencies for intergenic noncomplementation with a mutation in *ash1*, a trithorax group gene. Five of the noncomplementing deletions uncover genes previously classified as members of the Polycomb group. This evidence suggests that there are actually three groups of genes that maintain the expression pattern of homeotic genes during Drosophila development. The products of the third group appear to be required to maintain chromatin in both transcriptionally inactive and active states. Six of the noncomplementing deficiencies uncover previously unidentified trithorax group genes. One of these deficiencies removes 25D2-3 to 26B2-5. Within this region, there are two, allelic, lethal *P*-insertion mutations that identify one of these new trithorax group genes. The gene has been called *little imaginal discs* based on the phenotype of mutant larvae. The protein encoded by the *little imaginal discs* gene is the Drosophila homologue of human retinoblastoma binding protein 2.

CELL determination can be defined as the process by which cells become committed to differentiate into the structures characteristic of specific tissues. In Drosophila the determination of imaginal disc cells is initiated during embryogenesis but terminal differentiation does not begin until the pupal stage (reviewed by Cohen 1993). The determined state must be maintained throughout the multiple rounds of cell proliferation that imaginal disc cells undergo during larval stages. At the molecular level, imaginal disc determination depends upon segment-specific expression of the homeotic genes of the bithorax and Antennapedia complexes. These genes encode homeobox containing transcription factors that are responsible for expression of specific target genes (*e.g.*, Gould and White 1992; reviewed by White *et al.* 1992). The initial pattern of expression of homeotic genes during early embryogenesis, *i.e.*, the initiation of determination, depends upon the products of the gap and pair rule genes (Akam 1987). However, maintenance of segment-specific expression of homeotic genes must depend on some other mechanisms since the gap and pair rule genes are not expressed late in embyrogenesis or during larval development (Akam 1987). This maintenance function has been ascribed to cross-regulation among homeotic genes themselves (Hafen *et al.* 1984; Carroll *et al.* 1986), to auto-regulation (Bergson and McGinnis 1990), and to two other groups of genes, the Polycomb group (reviewed in Simon 1995) and the trithorax group (reviewed in Kennison and Tamkun 1992). The proteins encoded by the Polycomb group are postulated to prevent transcription of homeotic genes outside of their normal expression domain and the proteins encoded by the trithorax group are postulated to allow transcription of homeotic genes within their normal expression domain. This paradigm is based primarily on the analysis of mutant phenotypes but also on some biochemical studies.

The *Polycomb* (*Pc*) gene was originally identified by P. Lewis (Lindsley and Zimm 1992) as a dominant mutation that causes sex comb teeth to form on the second and third legs of male *Drosophila melanogaster* (Puro and Nygren 1975). E. B. Lewis (1978) studied the phenotype of embryonic lethal *Pc* homozygotes and recognized that *Polycomb* encodes a negative *trans*-regulatory factor of the bithorax complex. Indeed, *Polycomb* mutations cause ectopic expression of genes of both the bithorax and Antennapedia complexes (Wedeen *et al.* 1986; Busturia and Morata 1988). E. B. Lewis (1968) identified a dominant enhancer of bithorax complex mutations and suggested that this gene encodes a positive *trans*-regulatory factor of the bithorax complex. It

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was subsequently discovered that this mutation originally called *Rg-bx* and now called trx^D is an allele of a gene now know as *trithorax* (Ingham and Whittle 1980). Breen and Harte (1991, 1993) showed that *trithorax* mutations cause reduced expression of genes of both the bithorax and Antennapedia complexes. The antagonistic action between the products of *Polycomb* and *trithorax* and their sensitivity to gene dosage was first reported by Capdevila and Garcia-Bellido (1981). Subsequently, mutations were recovered in other genes that cause phenotypes like mutations in *Polycomb* or *trithorax*.

When heterozygous, *Polycomb* null mutations cause transformations of the second and third legs of adult flies to the morphology of first legs. In males this includes the presence of sex combs, which gave rise to the name of the gene. When homozygous, *Polycomb* null mutations cause transformations of the thoracic and abdominal segments to the morphology of the eighth abdominal segment (Lewis 1978). The Polycomb group was defined by Jürgens (1985) as genes in which mutations cause phenotypes that "resemble weak *Polycomb* mutations in both their dominant adult and recessive embryonic phenotypes." He observed that homozygosis for mutations in pairs of Polycomb group genes caused an enhanced phenotype and used this observation as an assay to screen deficiencies that were then available for ones that enhance the phenotype of Polycomb group mutations. He estimated that there are ~40 Polycomb group genes in the genome. This estimate is based on the assumption that such enhancement indicates a Polycomb group gene uncovered by the deletion. Deficiencies that enhance the *Polycomb* phenotype but do not by themselves express a phenotype like *Polycomb* would inflate the estimate of Polycomb group genes. Another property shared by Polycomb group genes is that mutations in these genes show intergenic noncomplementation, *i.e.*, the phenotype caused by heterozygosis for a *Polycomb* mutation is enhanced by heterozygosis for a mutation in another Polycomb group gene (Campbell *et al.* 1995). The similar phenotypes of mutations in Polycomb group genes and their intergenic noncomplementation has suggested that the products of these genes act via a multimeric protein complex. Such a complex has been detected in embryos (Franke *et al.* 1992). It contains 10 to 15 proteins including the products of at least two Polycomb group genes, *Polycomb* and *Polyhomeotic*. As expected for components of a multimeric complex, the products of *Polycomb*, PC, and *Polyhomeotic*, PH, are localized at identical positions on polytene chromosomes (Franke *et al.* 1992). Neither PC nor PH demonstrates sequence-specific DNA binding; however, PHO, the product of the *pleiohomeotic* gene, may be responsible for sequence-specific DNA binding of the Polycomb multimeric complex (Brown *et al.* 1998). It has been postulated that the products of Polycomb group genes repress transcription at the level of chromatin structure. Indeed the *Polycomb* protein has been detected in inactive chromatin isolated from the bithorax complex (Orlando and Paro 1993; Strutt and Paro 1997).

The product of *posterior sex combs* (PSC) also binds to polytene chromosomes (Brunk *et al.* 1991). Rastelli *et al.* (1993) found that many of the PSC binding sites are similar to PC and PH sites. Moreover, they found that PSC binding to polytene chromosomes was normal in larvae homozygous for a temperature-sensitive allele of *Enhancer of zeste, [E(z)]*, when raised at a permissive temperature, but dramatically reduced when these larvae were raised at a nonpermissive temperature. This result indicates that *E(z)* function is required for normal PSC binding and is consistent with the hypothesis that PSC and E(Z) are also involved in the Polycomb multimeric protein complex. E(Z) is a nuclear protein that is bound to salivary gland polytene chromosomes (Carrington and Jones 1996). Direct protein:protein interactions of some Polycomb group gene products have been documented, as examples, PSC, PH, and PC (Kyba and Brock 1998a), PH and SCM, the product of *Sex combs on midleg* (Kyba and Brock 1998b), and ESC, the product of *extra sex combs*, and E(Z) (Jones *et al.* 1998; Tie *et al.* 1998). However, PSC, SCM, ESC, and E(Z) have *not* been shown to be components of the purified Polycomb multimeric protein complex. Kyba and Brock (1998a) have suggested that Polycomb group gene products may actually be components of several different multimeric complexes. If so, that would make them analogous to the trithorax group gene products that have now been shown to be components of several different multimeric complexes (Papoulas *et al.* 1998) as described below.

When heterozygous, *trithorax* mutations cause either no transformations or an extremely low frequency of transformations of the third thoracic segment to the second segment (Capdevila and Garcia-Bellido 1981). However, when homozygous, *trithorax* mutations cause transformations of the first and third thoracic segments to the second segment and anterior transformations of the abdominal segments (Ingham and Whittle 1980). Other genes in which mutations cause similar phenotypes have been classified as members of the trithorax group (Shearn 1989). Trithorax group genes have been identified by several approaches. Two of the trithorax group genes, *ash1* and *ash2*, were identified as pupal lethal mutations that disrupt imaginal disc development (Shearn *et al.* 1971). Most of the other trithorax group genes were identified in a genetic screen for dominant suppressors of the adult phenotypes of dominant *Polycomb* or *Antennapedia* mutations (Kennison and Tamkun 1988). Like mutations in Polycomb group genes, mutations in trithorax group genes show intergenic noncomplementation, *i.e.*, heterozygosis for recessive mutations in two different trithorax group genes can cause an adult mutant phenotype (Shearn 1989). The phenotype can

include partial transformations of the first and third thoracic segments to the second thoracic segment and partial anterior transformations of the abdominal segments. The similar phenotypes of mutations in trithorax group genes and their intergenic noncomplementation has suggested that the products of these genes also act via multimeric protein complexes. Indeed, a 2-MD complex has been detected in embryos that contains the products of the trithorax group genes, *brahma* (Dingwall *et al.* 1995), *snr1* (Dingwall *et al.* 1995), and *moira* (Papoulas *et al.* 1998; Crosby *et al.* 1998). However, this complex does not contain the products of the trithorax group gene *ash1*, which is in a different 2-MD complex (Papoulas *et al.* 1998) that also contains the product of the trithorax gene (Rozovskaia *et al.* 1999) nor does it contain the product of *ash2*, which is in a 0.5-MD complex (Papoulas *et al.* 1998).

Taking advantage of the phenomenon of intergenic noncomplementation, we have screened a large fraction of the Drosophila genome to look for new trithorax group genes. We crossed females heterozygous for an *ash1* mutation to males heterozygous for one of 133 deficiencies and examined the progeny doubly heterozygous for the *ash1* mutation and the deficiency for homeotic transformations. In this way we identified regions of the genome with candidate trithorax group genes.

MATERIALS AND METHODS

Fly culture: All crosses were performed at 20° in shell vials with yeast, cornmeal, molasses, and agar medium containing tegosept and proprionic acid as mold inhibitors.

Assay for *ash1* complementation: Five females heterozygous for an *ash1* (*brahma* or *trithorax*) mutation were mated to five males heterozygous for a deletion, insertion, or other mutation, incubated in vials containing a small piece of paper, and transferred daily. Special care was taken to prevent overcrowding since conditions that slow development can increase the penetrance and expressivity of the homeotic transformations being scored. Adult flies were examined within 24 hr of eclosion. Papering the vials and examining the flies as they eclose were essential for reproducible results since flies with transformations preferentially get stuck in the food. Individual flies of the correct genotype were examined under the dissecting scope for thoracic homeotic transformations including apical and preapical bristles on metathoracic legs, sternopleural bristles on the proximal lateral metathorax, bristles and wing blade on halteres, bristles on the metanotum, preapical and apical bristles on the prothoracic legs, and sternopleural bristles on the proximal lateral prothorax. The statistical significance of differences in penetrance were evaluated by the G-test (Sokal and Rohlf 1969). Only in cases with a high penetrance of third leg to second leg transformations was a low penetrance of haltere to wing and first leg to second leg transformations also observed. A similar result was previously reported for *ash1* and *trithorax* double heterozygotes (Shearn 1989) and *ash1* and *brahma* double heterozygotes (Tripoulas *et al.* 1994). Although differences in expressivity were not quantitated, the expressivity was more extreme in cases where the penetrance was higher and more extreme in triple heterozygotes than double heterozygotes.

Assay for suppression of *Polycomb*: Five females with the genotype *Df(3L)Asc/TM3* were placed in shell vials with five males of a candidate deficiency or mutation balanced over *CyO* or *TM3* and transferred daily. Progeny heterozygous for *Df(3L)Asc*, which deletes *Polycomb*, and heterozygous for a candidate deficiency or mutation were scored for the presence of sex comb teeth on the mesothoracic and metathoracic legs. The control flies, *Df(3L)Asc/+*, were progeny of *Df(3L)Asc/TM3* females mated to Canton-S wild-type males.

Stage of lethality: To identify mutant larvae, stocks were constructed in which the X chromosomes are mutant for *yellow* and the mutant *l(2)10424*, *l(2)k06801*, or *Df(2L)cl-h3* chromosomes are heterozygous with a *CyO* balancer that carries the wild-type allele of *yellow* (Timmons *et al.* 1993). This allows mutant larvae to be identified by the *yellow* mutant phenotype. Five females heterozygous for *l(2)10424* or *l(2)k06801* were mated to five males heterozygous for *l(2)10424*, *l(2)k06801*, or *Df(2L)cl-h3*, incubated in vials containing a small piece of paper, and transferred daily. Homozygous or *trans*-heterozygous larvae were separated from nonmutant larvae, counted, and allowed to continue development. The stage of lethality is given as the stage when half of the mutant larvae ceased to develop.

***P*-element excision:** *l(2)10424* is a ry^+ lethal *P*-element insertion on the second chromosome with the genotype, *p[ry+]/CyO ; ry−/ry−*. Males from this stock were mass mated to females that have a source of transposase, *Sp/CyO ; Δ2-3 Sb ry−/TM6*. Male progeny of this cross with the genotype *p[ry+]/CyO ; Δ2-3 Sb ry−/ry−* were mated to female progeny with the genotype *Sp/CyO ; Δ2-3 Sb ry−/ry−*. Individual male *ry−* progeny with genotype $p[ry+]^{rev}/CyO$; *ry−/ry−* were mated to females of the original *P*-element stock, and males and females of the genotype $p[ry+]^{rev}/CyO$; *ry−/ry−* were mated to each other. The presence of *Cy+*, *ry−* progeny from this cross indicates that the lethal *P*-element insertion was precisely excised. Five males from each of these revertant stocks were mated to five *ash1/TM3* females, and the $p[ry+]^{rev}/+$; *+/ash1* progeny were examined for the presence of transformations.

Mounting and photography: Adults were dissected in PBS, transferred to a drop of Faure's medium on a glass slide, and covered with a coverslip. A small weight was placed on the coverslip for at least 24 hr to assure proper spreading. Third instar larvae were dissected in PBS, brains and imaginal disks were transferred to a drop of Permount on a glass slide, covered with a coverslip, and sealed. All photographs were taken with TMAX 100 film using a Zeiss Axioplan microscope.

Genomic DNA purification and plasmid rescue: Plasmid rescue of DNA flanking a *P*-element insertion was performed essentially by the method of Pirrotta (1986).

Plasmid DNA purification and sequencing: All plasmid DNA purifications were performed using a QIAGEN (Chatsworth, CA) plasmid purification kit as suggested in the supplied handbook. DNA sequencing was performed on a Perkin-Elmer (Norwalk, CT) 310 fluorescent sequencer using dye-terminator chemistries according to the manufacturer's instructions. Sequence assembly and comparison to genomic DNA from the Berkeley *Drosophila* Genome Project was performed using the AutoAssembler program from Perkin-Elmer. Amino acid motifs were determined using the Profilescan program and PsortII programs. Protein alignments were performed on the Blast server at the National Center for Biotechnology Information.

RESULTS

Deficiency screen: The transformations of the third thoracic segment to the second thoracic segment in *ash1*

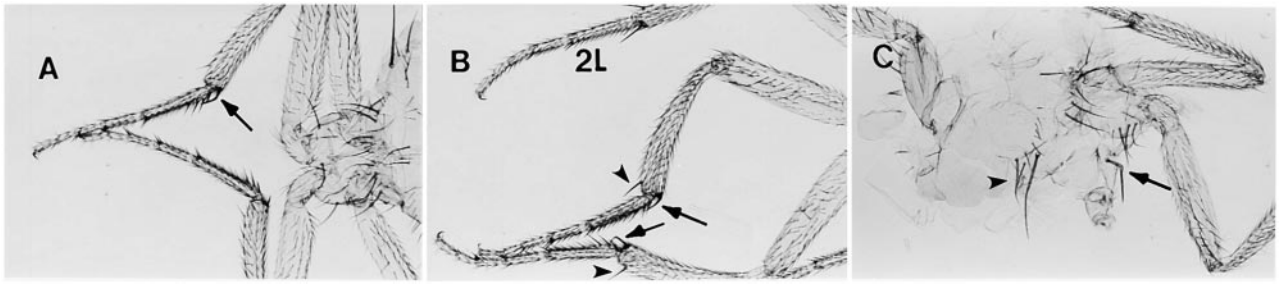

Figure 1.—Expressivity of third leg transformations. (A) Minimal transformation caused by intergenic noncomplementation. One of two third legs has an apical bristle (arrow) on the distal tibia, which is characteristic of second legs (genotype is $+/Sce^{D1}$; $brm^2/+$); the other third leg appears normal. Neither leg has sternopleural bristles that are characteristic of normal second legs. (B and C) More extreme transformations caused by enhancement of double mutant phenotype. (B) Both third legs have apical (arrows) and preapical (arrowheads) bristles on the distal tibia, which are characteristic of second legs. The second leg (2L) serves as a positive control showing both an apical and preapical bristle (genotype is $+/Sce^{D1}$; brm^2 $trx^{e2}/++$). (C) One of the third legs has sternopleural bristles (arrow), which are characteristic of second legs (arrowhead). The same leg has both apical and preapical bristles (not shown; genotype is $+/Sce^{D1}$; brm^2 $trx^{e2}/++$).

mutant homozygotes are caused by loss of *Ultrabithorax* expression and ectopic expression of *Antennapedia* in halteres and loss of *Ultrabithorax* expression and increased expression of *Antennapedia* in third legs (LaJeunesse and Shearn 1995). Recessive mutations in other genes of the trithorax group show intergenic noncomplementation with recessive mutations in *ash1* (Shearn 1989). The most common feature of the mutant phenotype is a partial transformation of the third leg to the second leg, as illustrated in Figure 1. The presence of an ectopic apical bristle on the third leg is an example of lesser expressivity (Figure 1A). The presence of ectopic apical, preapical, and sternopleural bristles is an example of greater expressivity (Figure 1, B and C). The penetrance of this transformation depends upon the alleles examined. To identify additional members of the trithorax group, we screened Drosophila deficiencies for intergenic noncomplementation with $ash1^{RE418}$, also know as $ash1^4$, an antimorphic mutation in *ash1* (Tripoulas *et al.* 1996). The 133 deficiencies tested represent 70% of the *D. melanogaster* genome. We found that 107 of the deficiencies fully complemented $ash1^{RE418}$. The data for just 4 of them, *Df(2R)eve1.27*, *Df(2R)en30*, *Df(2R)JP1*, and *Df(3R)Kx1*, are presented in Table 1. The 26 other deficiencies, representing 21 different cytogenetic regions, showed intergenic noncomplementation.

Two of the noncomplementing deficiencies were expected not to complement *ash1* mutations because they uncover the homeotic selector genes (Figure 2). *Df(3R)p115* (89B7-8;89E7-8) uncovers the bithorax complex and the trithorax group gene *moira*, and *Df(3R)Scr* (84A1-2;84B1-2) uncovers the Antennapedia complex. Three of the noncomplementing deficiencies uncover known trithorax group genes (Figure 2). $Df(3L)brm^{11}$ (71F1-4;72D1-10) uncovers *brahma*; *brahma* loss-of-function mutations have previously been shown to not complement *ash1* mutations (Tripoulas *et al.* 1994). $Df(3R)red^1$ (88B1;88D3-4) uncovers *trithorax; trithorax* loss-of-function mutations have previously been shown to not complement *ash1* mutations (Shearn 1989). *Df(3R)e-n19* (93B;94) uncovers *modifier of mdg4* also known as *E(var)3-93D* (Dorn *et al.* 1993); loss-of-function mutations in this gene have been shown to not complement *ash1* mutations (Gerasimova and Corces 1998). Finding intergenic noncomplementation of *ash1* mutations among deficiencies that uncover known homeotic selector genes and trithorax group genes suggested that the screen was working as expected and that some of the other noncomplementing deficiencies might uncover new trithorax group genes.

Five of the noncomplementing deficiencies *Df(1)C52* (8E-9C-D), *Df(2R)m41A4* (41A), *Df(2R)X58-7* (58A1-2; 58E4-10), *Df(2R)M60E* (60E2-3;60E11-12), and *Df(3R)XTA1* (96B;96D) uncover *Minute* genes (Figure 2). This was verified by crossing to smaller deficiencies of each of these regions and/or by crossing to the corresponding *Minute* mutations (data not shown). We had previously observed that some *Minute* mutations show intergenic noncomplementation with *ash1* mutations (A. Shearn, unpublished observation); however, the significance of these observations is not clear.

Mutations in some Polycomb group genes fail to complement mutations in trithorax group genes: Six of the 26 noncomplementing deficiencies in 5 distinct regions, *Df(2R)en-A* (47D3;48B2-5), *Df(2R)CX1* (49C1-4;50C23-D2), *Df(2R)vg-B* (49B2-3;49E7-F1), *Df(2R)trix* (51A1-2; 51B6), *Df(3L)lxd6* (67E1-2;68C1-2), and *Df(3R)by62* (85D11-14;85F6) delete regions that contain genes of the Polycomb group (Figure 2; Table 1). This result was surprising because loss of Polycomb group gene function is expected to suppress, not enhance, the phenotype of a loss-of-function or antimorphic mutation in a trithorax group gene. *Df(3L)lxd6* (67E1-2;68C1-2) uncovers the *Enhancer of zeste* (also known as *polycombeotic*) gene. We have already reported that amorphic muta-

TABLE 1

Some deficiencies that fail to complement mutations in trithorax group genes uncover Polycomb group genes

Name of mutation	Cytogenetic location	Penetrance of T3 to T2 transformations[a]					
		ash1^{RE418}	*ash1*^{VV183}	*brm*^{2}	*trx*^{b11}	*ash1*^{VF101} *trx*^{b11}	*brm*^{2} *trx*^{e2}
Df(2R)eve1.27	46C3-4 ; 46C9-11	0 $n = 68$					
Df(2R)en-A	47D3 ; 48C5-6	34.7* $n = 49$					
E(Pc)^{1}	48A2	43.7 $n = 183$	29.0*** $n = 214$	13.1*** $n = 236$	9.2*** $n = 284$	96.0*** $n = 248$	66.8*** $n = 232$
E(Pc)^{2}	48A2		9.4*** $n = 224$	0 $n = 231$	1.5 $n = 132$	51.9 $n = 54$	33.3 $n = 135$
Df(2R)en30	48A3-4 ; 48C6-8	0 $n = 58$					
Df(2R)vg-c	49B2-3 ; 49E7-F1	30.1* $n = 156$					
Df(2R)CX1	49C1-4 ; 50C2-D2	53.5* $n = 43$					
Su(z)2^{1}	49E5	18.5 $n = 108$	8.8*** $n = 216$	2.9** $n = 272$	0 $n = 212$	77.4*** $n = 221$	48.5*** $n = 227$
Su(z)2^{arp}	49E5		3.7 $n = 107$	0 $n = 103$	0 $n = 32$	52.9 $n = 50$	55.3** $n = 47$
Psc^{1}	49E1	36.7 $n = 147$	27.8*** $n = 237$	3.9** $n = 229$	4.2*** $n = 212$	91.3*** $n = 218$	61.4*** $n = 220$
Psc^{arp}	49E1		0 $n = 101$	0.8 $n = 126$	0 $n = 32$	37.3** $n = 75$	35.3 $n = 102$
Df(2R)JP1	51C3 ; 52F5-9	0 $n = 74$					
Df(2R)trix	51A1-2 ; 51B6	18.6* $n = 86$					
Asx^{XF23}	51A2	39.1 $n = 128$	13.6*** $n = 235$	6.3*** $n = 239$	15.9*** $n = 226$	100*** $n = 208$	65.9*** $n = 205$
Asx^{3}	51A2		66.6*** $n = 50$	42.4*** $n = 99$	37.3*** $n = 75$	98.1*** $n = 52$	79.5*** $n = 39$
Asx^{13}	51A2		29.3*** $n = 140$	24.5*** $n = 184$	16.6*** $n = 83$	41.7 $n = 24$	74.4*** $n = 39$
Df(3R)Kx1	86C1 ; 87B1-5	0 $n = 88$					
Df(3R)by62	85D11-14 ; 85F6	60.0* $n = 40$					
Scm^{D1}	85E1-10	22.1 $n = 163$	12.6*** $n = 199$	0 $n = 176$	0 $n = 192$	68.7*** $n = 227$	40.0 $n = 164$
Scm^{m56}	85E1-10		5.1** $n = 117$	0.8 $n = 125$	0 $n = 63$	81.4*** $n = 97$	45.8 $n = 48$
Scm^{302}	85E1-10		0 $n = 73$	0 $n = 62$	0 $n = 61$	28.0** $n = 50$	28.6 $n = 42$
Canton-S			1.0 $n = 290$	0 $n = 178$	0 $n = 342$	52.1 $n = 361$	35.3 $n = 218$

* Penetrance highly significantly different from adjacent control deficiency ($P < 0.01$); ** Penetrance significantly different from *Canton-S* controls ($P < 0.05$); *** Penetrance highly significantly different from *Canton-S* controls ($P < 0.01$).

[a] Penetrance is presented as a percentage of the number (n) of flies examined.

tions in *Enhancer of zeste* show intergenic noncomplementation with *ash1* mutations (LaJeunesse and Shearn 1996). We tested mutations in the Polycomb group genes uncovered by the others of these 5 deficiencies for intergenic noncomplementation with the antimorphic mutation, *ash1*^{RE418}, and with an amorphic mutation, *ash1*^{VV183} (also known as *ash1*^{22}; Table 1). *Df(2R)en-A* uncovers the *E(Pc)* gene (Sato *et al.* 1984). The penetrance of third leg to second leg transformations in *Df(2R)en-A*/+ ; +/*ash1*^{RE418} double heterozygotes (34.7%) is indis-

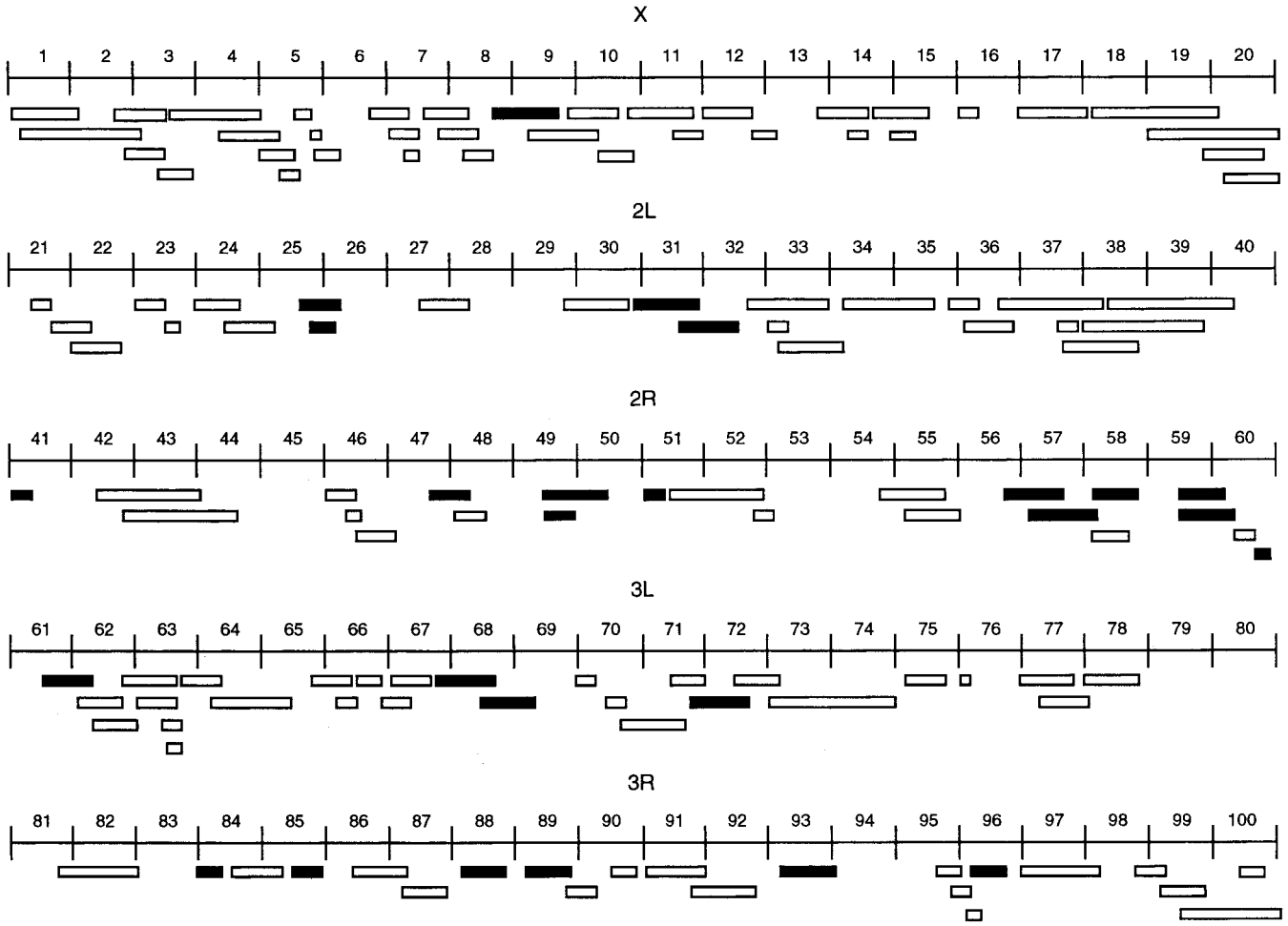

FIGURE 2.—A collection of 133 deficiencies were crossed to an antimorphic allele of $ash1^{RE418}$ (also known as $ash1^{4}$) and scored for the penetrance of homeotic transformations. Open bars indicate complementation, *i.e.*, no homeotic transformations. Solid bars indicate intergenic noncomplementation with $ash1^{RE418}$.

tinguishable from the penetrance in $E(Pc)^{1}/+$; $+/ash1^{RE418}$ double heterozygotes (43.7%). The penetrance of third leg to second leg transformations in both $E(Pc)^{1}/+$; $+/ash1^{VV183}$ double heterozygotes (29.0%) and $E(Pc)^{2}/+$; $+/ash1^{VV183}$ double heterozygotes (9.4%) is highly significantly greater than that of $ash1^{VV183}$ single heterozygotes (1.0%). *Df(2R)CX1* (and *Df(2R)vg-B*) uncovers both the *Su(z)2* and *Psc* genes (ADLER *et al.* 1989). The penetrance of third leg to second leg transformations in *Df(2R)CX1*/+ ; $+/ash1^{RE418}$ double heterozygotes (53.5%) is indistinguishable from the sum of the penetrances of $Su(z)2^{1}/+$; $+/ash1^{RE418}$ double heterozygotes (18.5%) and $Psc^{1}/+$; $+/ash1^{RE418}$ double heterozygotes (36.7%). The penetrance of third leg to second leg transformations in both $Su(z)2^{1}/+$; $+/ash1^{VV183}$ double heterozygotes (8.8%) and $Psc^{1}/+$; $+/ash1^{VV183}$ double heterozygotes (27.8%) is highly significantly greater than that of $ash1^{VV183}$ single heterozygotes (1.0%). *Df(2R)trix* uncovers the *Additional sex combs*, *Asx*, gene (JÜRGENS 1985; SINCLAIR *et al.* 1992). The penetrance of third leg to second leg transformations in *Df(2R)trix*/+ ; $+/ash1^{RE418}$ double heterozygotes (18.6%) is actually lower than the penetrance in $Asx^{XF23}/+$; $+/ash1^{RE418}$ double heterozygotes (39.1%). The penetrance of third leg to second leg transformations in $Asx^{XF23}/+$; $+/ash1^{VV183}$ double heterozygotes (13.6%), $Asx^{3}/+$; $+/ash1^{VV183}$ double heterozygotes (66.6%), and $Asx^{13}/+$; $+/ash1^{VV183}$ double heterozygotes (29.3%) is each highly significantly greater than that of $ash1^{VV183}$ single heterozygotes (1.0%). *Df(3R)by62* uncovers the *Scm* gene (BREEN and DUNCAN 1986). The penetrance of third leg to second leg transformations in *Df(3R)by62*/ $ash1^{RE418}$ double heterozygotes (60.0%) is significantly greater than the penetrance in $Scm^{D1}/ash1^{RE418}$ double heterozygotes (22.1%). This deficiency uncovers the *hyperplastic discs* gene (MANSFIELD *et al.* 1994), which also shows intergenic noncomplementation with *ash1* mutations (K. AMANAI and A. SHEARN, unpublished data). So, in this case as with *Df(2R)CX1*, intergenic noncomplementation with the deficiency is likely to be the consequence of the loss of two different genes. The penetrance of third leg to second leg transformations in $Scm^{D1}/ash1^{VV183}$

double heterozygotes (12.6%) is highly significantly greater, the penetrance of $Scm^{m56}/ash1^{VV183}$ double heterozygotes (5.1%) is significantly greater, but the penetrance of $Scm^{302}/ash1^{VV183}$ double heterozygotes (0%) is not significantly different than that of $ash1^{VV183}$ single heterozygotes (1.0%).

In each case, we found that intergenic noncomplementation of the deficiency could be accounted for, at least in part, by deletion of the uncovered Polycomb group gene. To analyze whether this intergenic noncomplementation was specific for *ash1* mutations or was general for mutations in trithorax group genes, we also tested these mutations in Polycomb group genes for intergenic noncomplementation with mutant alleles of the trithorax group genes, *trithorax* (trx^{b11}) and *Brahma* (brm^{2}), and for increased penetrance of the phenotype of two different double mutants, $ash1^{VF101}$ trx^{b11} ($ash1^{VF101}$ is also known as $ash1^{17}$) and brm^{2} trx^{e2}. Mutations in four of the five genes [*E(Pc), Psc, Su(z)2*, and *Asx*] showed significant intergenic noncomplementation with one or the other or both of *trithorax* or *brahma* mutations and significant enhancement of the penetrance of both double mutants (Table 1). However, the *Scm* mutations only showed intergenic noncomplementation with *ash1* mutations and only increased the penetrance of the double mutant that included an *ash1* mutation, $ash1^{VF101}$ trx^{b11} (Table 1), suggesting a specific interaction between *Scm* and *ash1*.

Complementation with mutations in other Polycomb group genes: Finding intergenic noncomplementation between mutations in trithorax and Polycomb group genes was unexpected. So we set out to find how general a phenomenon these results represented. Loss-of-function mutations in nine other previously identified Polycomb group genes were analyzed for intergenic noncomplementation with amorphic mutations in *ash1*, *trithorax*, and *brahma* and for enhancement or suppression of the double mutant phenotypes. *Polycomb* is the archetypal Polycomb group gene (Puro and Nygren 1975). An amorphic *Polycomb* mutation (Pc^{3}) showed no intergenic complementation with $ash1^{VV183}$, trx^{b11}, or brm^{2} mutations and significantly suppressed the penetrance of both double mutants (Table 2). This is the result expected for a loss-of-function mutation in a Polycomb group gene. Such mutations are expected to antagonize the phenotype caused by mutations in trithorax group genes. Similar results were obtained for most of the mutations tested in six of the other eight genes *polyhomeotic* (Ph^{d503}; Dura *et al.* 1987), *Polycomb-like* (Pcl^{7}; Duncan 1982), *pleiohomeotic* (pho^{h}; Girton and Jeon 1994), *multi sex combs* (mxc^{m1} and mxc^{mbn}; Santamaria and Randsholt 1995; Docquier *et al.* 1996), *extra sex combs* (esc^{5}, esc^{9}, esc^{10}, and esc^{21}; Struhl 1981, 1983), and *super sex combs* (sxc^{4} and sxc^{5}; Ingham 1984). Some mutations in these genes behave anomalously. As examples, mxc^{G48} significantly enhances the penetrance of $ash1^{VF101}$ trx^{b11} but neither suppressed nor enhanced the penetrance of brm^{2} trx^{e2}; esc^{6} did not suppress the penetrance of $ash1^{VF101}$ trx^{b11}; and sxc^{1} significantly enhanced the penetrance of both $ash1^{VF101}$ trx^{b11} and brm^{2} trx^{e2}. These specific mutations may be causing partial gain-of-function phenotypes or there may be additional unknown mutations on the chromosomes that contain the mutations tested.

Mutations in the two other genes tested, *Sex combs extra* (Sce^{D1}; Breen and Duncan 1986) and *Enhancer of zeste* ($E(z)^{5}$; Phillips and Shearn 1990; Jones and Gelbart 1990), showed intergenic noncomplementation with mutations in one or more of the three single trithorax group genes and enhanced the penetrance of both double mutants. These are the results expected for mutations in trithorax group genes. For *Sex combs extra* no deficiencies and no other alleles are available, so it is unclear whether the results with this allele represent a loss-of-function phenotype. However, for *Enhancer of zeste*, these data extend previously reported results (LaJeunesse and Shearn 1996). So, we have identified at least six genes, *Enhancer of zeste*, *Enhancer of Polycomb*, *Posterior sex combs*, *Suppressor of zeste-2*, *Additional sex combs*, and *Sex comb on midleg*, that behave as if they are both Polycomb and trithorax group genes. We also tested a null mutation in *Trithorax-like* for intergenic noncomplementation. Mutations in *Trithorax-like* give a phenotype like *trithorax* (Farkas *et al.* 1994), hence the name, and enhance the phenotype of *Ultrabithorax* mutations as do mutations in other trithorax group genes (Shearn 1989). However, the same *Trithorax-like* mutations enhance the extra sex combs phenotype of *Polycomb* mutations as if *Trithorax-like* were a Polycomb group gene (Strutt *et al.* 1997). We observed that Trl^{R85} showed intergenic noncomplementation with all three single mutations and enhanced the phenotype of both double mutants (Table 2). So *Trithorax-like* also behaves as if it is both a Polycomb and trithorax group gene.

Complementation with *Suppressors of zeste*: Specific mutations in the *zeste* gene cause reduced expression of the *white* gene leading to yellow eye color (Gans 1953). Mutations in four of the six genes that behave as if they are both Polycomb and trithorax group genes have also been recovered as dominant suppressors of this *zeste-white* interaction: *Psc* (Kalisch and Rasmuson 1974; Wu *et al.* 1989), *Scm* (Kalisch and Rasmuson 1974; Wu *et al.* 1989), and *E(z)* (Kalisch and Rasmuson 1974; Wu *et al.* 1989; Phillips and Shearn 1990; Jones and Gelbart 1990). We have examined mutations in six other genes identified as dominant suppressors of the *zeste-white* interaction for intergenic noncomplementation with mutations in trithorax group genes. Three of these mutations, $Su(z)3^{1}$, $Su(z)5^{1}$, and $Su(z)12^{1}$ did not show intergenic noncomplementation with any of the three single mutations; one of these three, $Su(z)5^{1}$, suppressed the phenotype of brm^{2} trx^{e2} (Table 2). However, we found that two of these mutations, $Su(z)6^{1}$ and $Su(z)7^{1}$, show intergenic noncomplementation with all

TABLE 2

Mutations in some genes classified as members of the Polycomb group or classified as *Suppressors of zeste* fail to complement mutations in trithorax group genes

	Penetrance of T3 to T2 transformations				
Name of mutation	*ash1*^{VV183}	*brm*^{2}	*trx*^{b11}	*ash1*^{VF101} *trx*^{b11}	*brm*^{2} *trx*^{e2}
Canton-S	1.0	0	0	52.1	35.3
	$n = 290$	$n = 178$	$n = 342$	$n = 361$	$n = 218$
Pc^{3}	0	0	0	6.5**	9.3**
	$n = 223$	$n = 216$	$n = 316$	$n = 321$	$n = 216$
ph^{d503}	0	0	0	28.0**	1.4**
	$n = 242$	$n = 255$	$n = 264$	$n = 218$	$n = 208$
Pcl^{7}	0	0	0	1.2**	2.2**
	$n = 218$	$n = 249$	$n = 263$	$n = 243$	$n = 229$
pho^{b}	0	0	0	33.2**	1.9**
	$n = 232$	$n = 239$	$n = 235$	$n = 229$	$n = 209$
mxc^{G48}	2.2	0.9	0	79.1**	36.9
	$n = 226$	$n = 213$	$n = 224$	$n = 225$	$n = 222$
mxc^{m1}	0	0	0	28.9**	15.9*
	$n = 92$	$n = 88$	$n = 73$	$n = 45$	$n = 44$
mxc^{mbn}	0	0	0	22.9**	1.0**
	$n = 69$	$n = 99$	$n = 72$	$n = 74$	$n = 94$
esc^{5}	0	0	0	19.2**	22.8
	$n = 72$	$n = 104$	$n = 38$	$n = 52$	$n = 79$
esc^{6}	0	0	0	52.8	0
	$n = 100$	$n = 90$	$n = 45$	$n = 36$	$n = 33$
esc^{9}	0	0	0	5.6**	3.8**
	$n = 92$	$n = 32$	$n = 40$	$n = 89$	$n = 106$
esc^{10}	0	0	0	4.0**	12.3**
	$n = 80$	$n = 32$	$n = 59$	$n = 25$	$n = 81$
esc^{21}	0	0	0	22.9*	17.1*
	$n = 86$	$n = 61$	$n = 56$	$n = 70$	$n = 35$
Sxc^{1}	1.8	0	3.1**	89.0**	58.6**
	$n = 204$	$n = 248$	$n = 262$	$n = 219$	$n = 181$
Sxc^{4}	0	0	0	8.3**	7.1**
	$n = 61$	$n = 78$	$n = 78$	$n = 72$	$n = 70$
Sxc^{5}	0.9	1.1	0.9	26.1**	26.2
	$n = 113$	$n = 88$	$n = 110$	$n = 111$	$n = 65$
Sce^{D1}	54.0**	41.2**	24.0**	95.5**	85.0**
	$n = 335$	$n = 243$	$n = 254$	$n = 222$	$n = 233$
E(z)^{5} also known as *pco*^{1902}	14.7**	0	1.7	84.4**	46.5*
	$n = 211$	$n = 338$	$n = 239$	$n = 243$	$n = 241$
Trl^{R85}	25.6**	4.6**	10.6**	100**	69.7**
	$n = 234$	$n = 239$	$n = 293$	$n = 229$	$n = 244$
Su(z)3^{1}	0.4	0	0	55.9	32.1
	$n = 225$	$n = 229$	$n = 252$	$n = 213$	$n = 243$
Su(z)4^{1}	1.0	0	4.8**	86.1**	57.6**
	$n = 290$	$n = 232$	$n = 209$	$n = 266$	$n = 217$
Su(z)5^{1}	0	0	0	ND	11.1**
	$n = 81$	$n = 89$	$n = 112$		$n = 72$
Su(z)7^{1}	6.0**	2.6*	5.4**	83.1**	44.6*
	$n = 402$	$n = 310$	$n = 370$	$n = 278$	$n = 323$
Su(z)6^{1}	18.0**	22.9**	27.2**	84.8**	52.5**
	$n = 456$	$n = 314$	$n = 254$	$n = 224$	$n = 221$
Su(z)12^{1}	0	0	0	ND	ND
	$n = 94$	$n = 110$	$n = 101$		

ND, not done. * Penetrance significantly different from *Canton-S* control according to G-test ($P < 0.05$); ** Penetrance highly significantly different from *Canton-S* control according to G-test ($P < 0.01$).

TABLE 3

Some deficiencies that fail to complement mutations in trithorax group genes do not suppress the extra sex comb phenotype of a deficiency of *Polycomb*

Name of deficiency	Cytogenetic location	Penetrance[a] T3 to T2 transformations				Penetrance[a] extra sex comb teeth	
		ash1^{RE418}	*ash1*^{VV183}	*brm*^{2}	*trx*^{b11}	L2 Df Asc	L3 Df Asc
Df(2L)MdhA	30D1-F6;31F1-5	90.6*	86.6*	50.8*	85.2*	24.4	3.3
		$n = 75$	$n = 164$	$n = 134$	$n = 197$	$n = 90$	$n = 90$
Df(2R)or-Br6	59D5-10;60B3-8	49.2*	34.4*	18.9*	10.5*	24.2	6.0
		$n = 63$	$n = 154$	$n = 122$	$n = 153$	$n = 33$	$n = 33$
Df(3L)Ar14.8	61C5-8;62A8	90.4*	71.4*	6.6*	8.1*	19.6	2.8
		$n = 42$	$n = 42$	$n = 106$	$n = 124$	$n = 107$	$n = 107$
Df(3L)vin6	68C8-10;69A4-5		31.0*	3.2	6.2*	22.3	5.1
			$n = 126$	$n = 124$	$n = 128$	$n = 54$	$n = 54$
Df(2L)cl-h3	25D2-3;26B2-5	75.4*	64.3*	9.6*	24.0*	1.0*	0.0
		$n = 57$	$n = 157$	$n = 230$	$n = 196$	$n = 91$	$n = 91$
Df(2R)Pu^{D17}	57B5;58B1-2	93.5*	85.5*	57.2*	74.4*	4.3*	0.0
		$n = 31$	$n = 131$	$n = 173$	$n = 129$	$n = 90$	$n = 90$
Canton-S		0	1.0	0	0	19.3	4.1
		$n = 149$	$n = 290$	$n = 178$	$n = 342$	$n = 145$	$n = 145$

* Penetrance highly significantly different than *Canton-S* controls ($P < 0.01$).
[a] Penetrance is presented as a percentage of the number (n) of flies examined.

three single mutations and enhanced the phenotype of both double mutants; one of these mutations, *Su(z)4*^{1}, enhanced the phenotype of both double mutants. No other alleles of these genes were available and no deficiencies are known to uncover these genes. So, at this point it is not possible to confirm that the observed intergenic noncomplementation is due to these *Su(z)* mutations rather than to other mutations on the chromosomes.

Assay for suppression of *zeste*: Finding that mutations in some of the genes identified as *Suppressors of zeste* behave as if they are both Polycomb and trithorax group genes led us to examine mutations in genes identified as Polycomb group genes for their ability to suppress the *zeste-white* interaction. We found that mutations in none of six genes (*Polycomb*, *polyhomeotic*, *Polycomb-like*, *pleiohomeotic*, *extra sex combs*, and *super sex combs*) that suppress the penetrance of the two different double mutants, *ash1*^{VF101} *trx*^{b11} and *brm*^{2} trx^{e2}, affect the *zeste-white* interaction (data not shown). Mutations in *Su(z)2* (Kalisch and Rasmuson 1974), *Scm* (Wu *et al.* 1989), *Psc* (Wu *et al.* 1989), and *mxc* (Santamaria and Randsholt 1995) have already been reported to suppress the *zeste-white* interaction. We confirmed those results and observed in addition that mutations in *E(Pc)* suppress the *zeste-white* interaction and the *Sce*^{D1} mutation enhances the *zeste-white* interaction (data not shown).

***lid* is a new trithorax group gene:** The 10 other noncomplementing deficiencies are located in six different cytogenetic regions that do not contain homeotic selector genes or known Polycomb or trithorax group genes (Figure 2). Two of these deficiencies uncover *Minute* genes, but noncomplementing regions were separated from the *Minute* genes by using smaller deficiencies. The original screening of the deficiencies utilized the *ash1*^{RE418} (also known as *ash1*^{4}) allele because it causes the most extreme phenotype and was therefore believed to be an amorphic allele. However, a substantial amount of synthetic lethality occurs among flies doubly heterozygous for *ash1*^{RE418} and these 10 noncomplementing deletions, making it difficult to obtain adequate numbers of progeny. Subsequently, we discovered that *ash1*^{RE418} is actually an antimorphic allele (Tripoulas *et al.* 1996; J. J. Gildea, unpublished observation), so all further work was done with *ash1*^{VV183} (also known as *ash1*^{22}), which we believe to be an amorphic allele because it is predicted to stop translation after the 47th of 2144 amino acids (Tripoulas *et al.* 1996). Each of these deficiencies was also crossed to amorphic alleles of two other trithorax group genes, *brahma*, *brm*^{2} (Kennison and Tamkun 1988), and *trithorax*, *trx*^{B11} (Mazo *et al.* 1990), and to a deficiency of Polycomb, *Df(3L)Asc*, to determine if these deficiencies fail to complement mutations in trithorax group genes and suppress loss of *Polycomb* function as expected for loss of function of trithorax group genes.

Four of the six noncomplementing deficiencies, *Df(2L)MdhA* (30D1-F6;31F1-5), *Df(2R)vw* (59D6-E1; 60C1-8), *Df(3L)Ar14.8* (61C5-8;62A8), and *Df(3L)vin7* (68C8;69B4-5), fail to complement mutations in all three of the trithorax group genes tested, *ash1*, *brahma*, and *trithorax*, as expected for deficiencies that uncover trithorax group genes (Figure 2; Table 3). However, none of these four deficiencies suppress loss of *Polycomb*

TABLE 4

Penetrance of T3 to T2 transformations in flies doubly heterozygous for *ash1*^{vv183}, an amorphic mutation, and deficiencies that overlap *Df(2L)cI-h3*

Name of deficiency	Cytogenetic location	Penetrance[a] T3 to T2 transformations		
Df(2L)cI-h3	25D2-3;26B2-5	64.3 *n* = 157	*	
Df(2L)GpdhA	25E1;26A8-9	26.9 *n* = 119	*	**
Df(2L)cI-h4	25E1;25E5	14.3 *n* = 91	*	**
Df(2L)cI-h1	25D4;25F1-2	47.7 *n* = 218	*	**
Df(2L)2802	25F2-3;25F4-5	0.0 *n* = 124		**
Df(2L)E110	25F3-26A1;26D3-11	37.8 *n* = 119	*	**
Canton-S		1.0 *n* = 290		**

* Penetrance highly significantly different from *Canton-S* ($P < 0.01$); ** Penetrance highly significantly different from *Df(2L)cI-h3* ($P < 0.01$).

[a] Penetrance is percentage of the number (*n*) of flies examined.

function as expected for deficiencies that uncover trithorax group genes (Table 3). These deficiencies may uncover genes that represent a group undefined until now. Further work will be necessary to investigate this issue.

Two of the six noncomplementing deficiencies, *Df(2L)cl-h3* (25D2-3;26B2-5) and *Df(2R)Pu*^{D17} (57B5; 58B1-2), fail to complement mutations in all three of the trithorax group genes tested, *ash1*, *brahma*, and *trithorax*, and suppress loss of *Polycomb* function as expected for deficiencies that uncover trithorax group genes (Figure 2; Table 3). As a first step toward identifying the trithorax group gene uncovered by *Df(2L)cl-h3*, we more precisely determined its cytogenetic location by assaying the ability of deficiencies that overlap *Df(2L)cl-h3* to complement the *ash1* mutant phenotype. We found that *Df(2L)GpdhA* (25E1;26A8-9), *DF(2L)cl-h4* (25E1;25E5), *DF(2L)cl-h1* (25D4;25F1-2), and *Df(2L)E110* (25F3-26A1; 26D3-11) all significantly fail to complement *ash1*^{VV183}, but *Df(2L)2802* (25F2-3;25F4-5) does complement (Table 4). The complementation of *Df(2L)2802* and failure of complementation both by deficiencies distal to *Df(2L)2802*, such as *Df(2L)cl-h4* and *Df(2L)cl-h1*, and proximal, such as *Df(2L)E110*, suggest that there are two different genes uncovered by *Df(2L)cl-h3* that are responsible for the noncomplementation originally observed. This interpretation is strongly supported by the fact that for both distal deficiencies and for the proximal deficiency, the penetrance is significantly less than the penetrance of *Df(2L)cl-h3* (Table 4). Based on the breakpoints of these deletions it appears that the distal gene uncovered by *Df(2L)cl-h3* is at least partially within 25E1-5 because it is uncovered by *Df(2L)cl-h4* (Figure 3). However, the penetrance of *Df(2L)cl-h1* is significantly greater than that of *Df(2L)cl-h4* ($P < 0.01$), suggesting that *Df(2L)cl-h4* causes only a partial loss of function of the distal gene. So, based on this data, the distal gene is within 25D4;25F1-2. The proximal gene uncovered by *Df(2L)cl-h3* must be within 25F4-5;26B2-5 because it is not uncovered by *Df(2L)2802* (Figure 3).

As the next step toward identifying the two trithorax group genes uncovered by *Df(2L)cl-h3*, we assayed five *P*-element insertion lethal mutations that had been localized to the interval of 25D4 to 26B2-5 for failure to complement *ash1*^{VV183}. Two of the five, *l(2)10424* and *l(2)k06801*, failed to complement (Table 5). We found that these mutations are allelic to each other and are lethal in combination with *Df(2L)cl-h3*, *Df(2L)GpdhA*, and *Df(2L)E110* (data not shown). As might be expected for allelic mutations, the insertion sites of the *P* elements in *l(2)10424* and *l(2)k06801* are essentially identical, 26A8-9 and 26B1-2, respectively (Berkeley *Drosophila* Genome Project; http://www.fruitfly.org). The *l(2)k06801* allele exhibits intergenic noncomplementation with *brahma* and *trithorax* mutations, enhances the phenotype of *ash1*^{VF101} *trx*^{h11} and *brm*^{2} *trx*^{e2} double mutations, and suppresses the phenotype of a *Polycomb* deletion (Table 5). These data suggest that *l(2)10424* and *l(2)k06801* identify the proximal trithorax group gene uncovered by *Df(2L)cl-h3*. This interpretation is supported by the fact that the penetrance of either *l(2)10424 ; ash1* or *l(2)k06801 ; ash1* double heterozygotes is not significantly different from *Df(2L)E110*; *ash1*^{VV183} or *Df(2L)GpdhA ; ash1*^{VV183} double heterozygotes. Since the lack of complementation caused by *Df(2L)GpdhA* can be fully accounted for by uncovering this proximal gene (Table 4), the distal gene uncovered by *Df(2L)cl-h3* must be within 25D4;25E1, *i.e.*, distal of the distal breakpoint of *Df(2L)GpdhA* as indicated in Figure 3.

To examine whether the mutation in the proximal

TABLE 5

Effect of mutations in the 25D-26B region on the T3 to T2 phenotype of mutations in trithorax group genes and on the extra sex comb phenotype of a deficiency of *Polycomb*

Name of mutation	Cytogenetic location	Penetrance[a] T3 to T2 transformations					Penetrance[a] sex comb teeth	
		ash1^{VV183}	*brm*^{2}	trx^{b11}	*brm*^{2} *trx*^{E2}	*ash1*^{VF101} *trx*^{b11}	Leg 2 *Df Asc*	Leg 3 *Df Asc*
l(2)04884	25D4-5	0 $n = 67$						
l(2)03771	25D4-6	0 $n = 102$						
l(2)04643	25E4-6	0 $n = 84$						
l(2)10424	26A8-9	17.8** $n = 118$						
l(2)k06801	26B1-2	21.6** $n = 217$	4.8* $n = 103$	9.2** $n = 131$	81.5** $n = 108$	100** $n = 106$	5.4** $n = 111$	0 $n = 111$
Canton-S		1.0 $n = 290$	0 $n = 178$	0 $n = 342$	35.3 $n = 218$	52.1 $n = 361$	19.3 $n = 145$	4.1 $n = 145$

* Penetrance significantly different from *Canton-S* controls ($P < 0.05$); ** Penetrance highly significantly different than *Canton-S* controls ($P < 0.01$).

[a] Penetrance is percentage of the number (n) of flies examined.

trithorax group gene found on the chromosome that contains *l(2)10424* was indeed caused by a *P*-element insertion, excisions of the *l(2)10424* insertion were generated. Nine different, apparently precise, excisions were recovered. In each case both the homozygous lethality and noncomplementation with *ash1* was fully reverted. These data demonstrate that the insertion of the *P* element in *l(2)10424* is responsible for the mutant phenotype and that *l(2)10424* is a mutation in the proximal trithorax group gene uncovered by *Df(2L)cl-h3*.

Mutant homozygotes of *l(2)10424* and *trans*-heterozygotes of *l(2)10424/l(2)k06801* are lethal at a number of different stages of development. Some homozygotes and *trans*-heterozygotes appear to die before hatching although no obvious defects in the larval cuticle could be observed. Most of the homozygotes appear to die at the early pupal stage. Of 10 late third instar homozygous *l(2)10424* larvae, 7 displayed a small optic brain lobe phenotype (Figure 4, A and B) and small imaginal discs (Figure 4, D and E). So, we named this gene *little imaginal discs* (*lid*). A small percentage of mutant larvae complete metamorphosis and die either as pharate adults or newly eclosed adults. These adult escapers often have duplicated thoracic macrochaetae (Figure 4C). Most hemizygous mutants die as late embryos, with rare escapers showing only minor disk proliferation defects as late third instar larvae.

To clone the *little imaginal discs* gene, genomic DNA was prepared from both *lid*^{1} [*l(2)10424*] and *lid*^{2} [*l(2)k06801*] heterozygous flies, and DNA flanking the insertions was isolated by plasmid rescue. The sequence of the flanking DNA was used to search the Drosophila genomic DNA sequence database generated by the Berkeley *Drosophila* Genome Project using the BlastN program. DNA flanking both *P*-element insertions matched genomic sequence from the P1 clone DS05973. Expressed sequence tags from the 5′ end of eight different cDNAs (LD08387, LD14429, LD06125, LD17452, LD19310, LD12254, LD12410, and CK01604) were found to match genomic sequence from this region. The longest cDNA, LD19310, was sequenced on both strands by primer walking; it was found to be 5947 bp long with a single open reading frame of 5516 bp. Comparison of this cDNA sequence to that of the genomic sequence revealed four introns of 2767, 143, 127, and 65 bp. The exon assembly program Genie (http://www.fruitfly.org/) precisely predicted the exon structure and open reading frame of this gene. The sequence of the cDNA matched exactly the DNA sequenced by the Berkeley *Drosophila* Genome Project. Both *P*-element insertions map very close to each other within the large first intron of *lid* (Figure 5). The LD19310 cDNA detects a transcript of approximately 8 kb on blots of RNA from Canton-S third instar larvae. The amount of this transcript is dramatically decreased in RNA from mutant third instar larvae (data not shown). This indicates that LD19310 cDNA is derived from the *lid* transcript.

Multiple stop codons are found upstream of the first methionine codon in the sequence of LD19310, suggesting that this cDNA contains the entire open reading frame. This open reading frame codes for a conceptually translated protein of 1838 amino acids with a predicted molecular weight of 203 kD and pI of 6.2. The protein contains a number of amino acid motifs found in both trithorax and Polycomb group genes. It contains an N-terminal RING double zinc finger at amino acids 451–495, which also matches the consensus for a PHD double zinc finger (Schindler *et al.* 1993; Aasland *et al.* 1995),

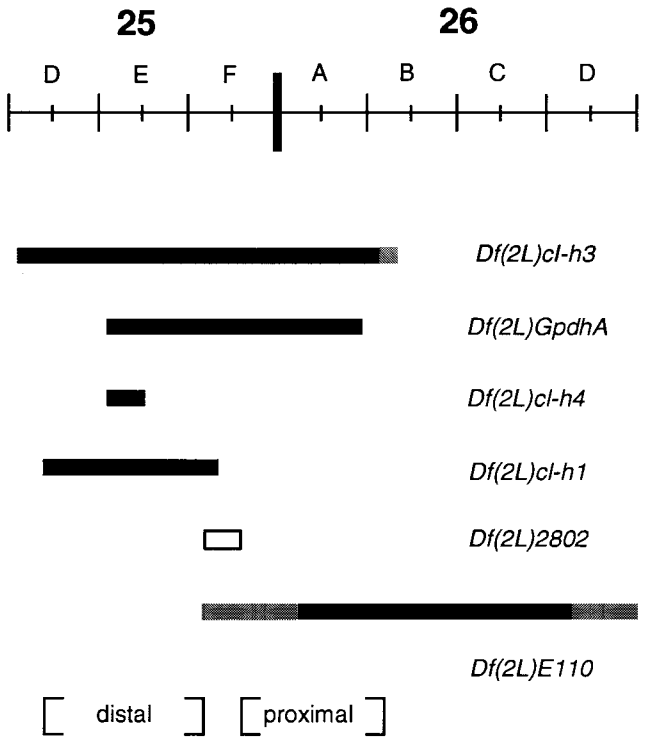

FIGURE 3.—Smaller deficiencies that overlap *Df(2L)cl-h3* were crossed to *ash1* and tested for intergenic noncomplementation. No shading indicates complementation. Dark shading indicates intergenic noncomplementation with *ash1*. Light shading indicates uncertainty as to the endpoint(s) of deficiencies. Areas labeled distal and proximal indicate deduced localization of two noncomplementing regions.

one centrally located PHD double zinc finger at amino acids 1293–1354, and a C-terminal PHD double zinc finger at 1753–1808. A predicted leucine zipper domain is found at amino acids 1033–1056; a bipartite nuclear localization signal is found at amino acids 1599–1616. Each of these amino acid motifs is found in human retinoblastoma binding protein 2 (RBP-2) in the same order (Figure 5), suggesting that LID may be the orthologue of human RBP-2. Overall, these two proteins share 47% identity; smaller regions contain substantially higher identity (Figure 5). It had been appreciated before that human RBP-2 has multiple novel zinc finger motifs that are very similar to those in *trx* and *Pcl* (STASSEN *et al.* 1995).

When alignments of these two proteins were performed, it became apparent that there is a domain N-terminal to the RING finger that also has a high degree of identity. This domain has a previously described amino acid motif called ARID (*A*T-*r*ich *i*nteraction *do*main; HERRSCHER *et al.* 1995). Among the proteins that contain this motif is SWI1/ADR6, a component of the yeast SWI/SNF multiprotein complex (CAIRNS *et al.* 1994); OSA, a component of the Drosophila BRM chromatin remodeling complex (COLLINS *et al.* 1999; VAZQUEZ *et al.* 1999); and another Drosophila protein, DEADRINGER (SHANDALA *et al.* 1999).

DISCUSSION

It has generally been observed that heterozygosis for recessive loss-of-function mutations in trithorax group genes can suppress the adult phenotype caused by heterozygosis for dominant mutations in *Polycomb*. Indeed, KENNISON and TAMKUN (1988) screened for suppressors of the dominant *Polycomb* mutant phenotype and recovered mutations in *trithorax* and 10 other genes considered to be members of the trithorax group including *brahma*. We used a different strategy to identify additional genes of the trithorax group. On the basis of the observation that mutations in trithorax group genes show intergenic noncomplementation (SHEARN 1989), we tested 133 large deficiencies and found 26 that showed intergenic noncomplementation with an antimorphic *ash1* allele, $ash1^{RE418}$. Each of the noncomplementing deficiencies was subsequently tested for complementation with mutations in two other trithorax group genes, *brahma* and *trithorax*, and for suppression of a *Polycomb* deletion. The implicit assumption of our approach was that mutations in trithorax group genes fail to complement mutations in other trithorax group genes and suppress the dominant phenotype of amorphic mutations in the *Polycomb* gene (SHEARN 1989). Five of the noncomplementing deficiencies uncovered homeotic selector genes and/or previously identified trithorax group genes. This result validated the rationale of our screen.

Five noncomplementing deficiencies identify *Minute* genes: Among the noncomplementing deficiencies, we recovered two groups that were not expected. Five of the deficiencies uncovered *Minute* genes. The *Minute* genes that have been analyzed to date encode ribosomal proteins, ribosomal RNAs, or are otherwise involved in the mechanism of protein synthesis, like aminoacyl-tRNA synthetases (LAMBERTSSON 1998). Therefore *Minute* mutations most likely cause a general decrease in translation rate or efficiency. The noncomplementation of the *ash1* mutant phenotype observed in this screen by *Minute* mutations is most likely due to the additive effects of decreased transcription of the *Ultrabithorax* gene caused by the *ash1* mutation (LAJEUNESSE and SHEARN 1995) and decreased translation of the *Ultrabithorax* transcript caused by the *Minute* mutations.

Six noncomplementing deficiencies identify genes previously classified as members of the Polycomb group: Six of the deficiencies uncovered genes that were previously classified in the Polycomb group. They were so classified, because they either enhanced the *Polycomb* mutant phenotype or caused a phenotype like *Polycomb* mutants. This result was quite unexpected because the antagonism between trithorax and Polycomb group genes suggested that loss of function of Polycomb group genes should suppress trithorax mutant phenotypes. Nevertheless, as shown in Table 1, it is likely that the Polycomb group genes uncovered by these deficiencies are responsible for the observed intergenic noncom-

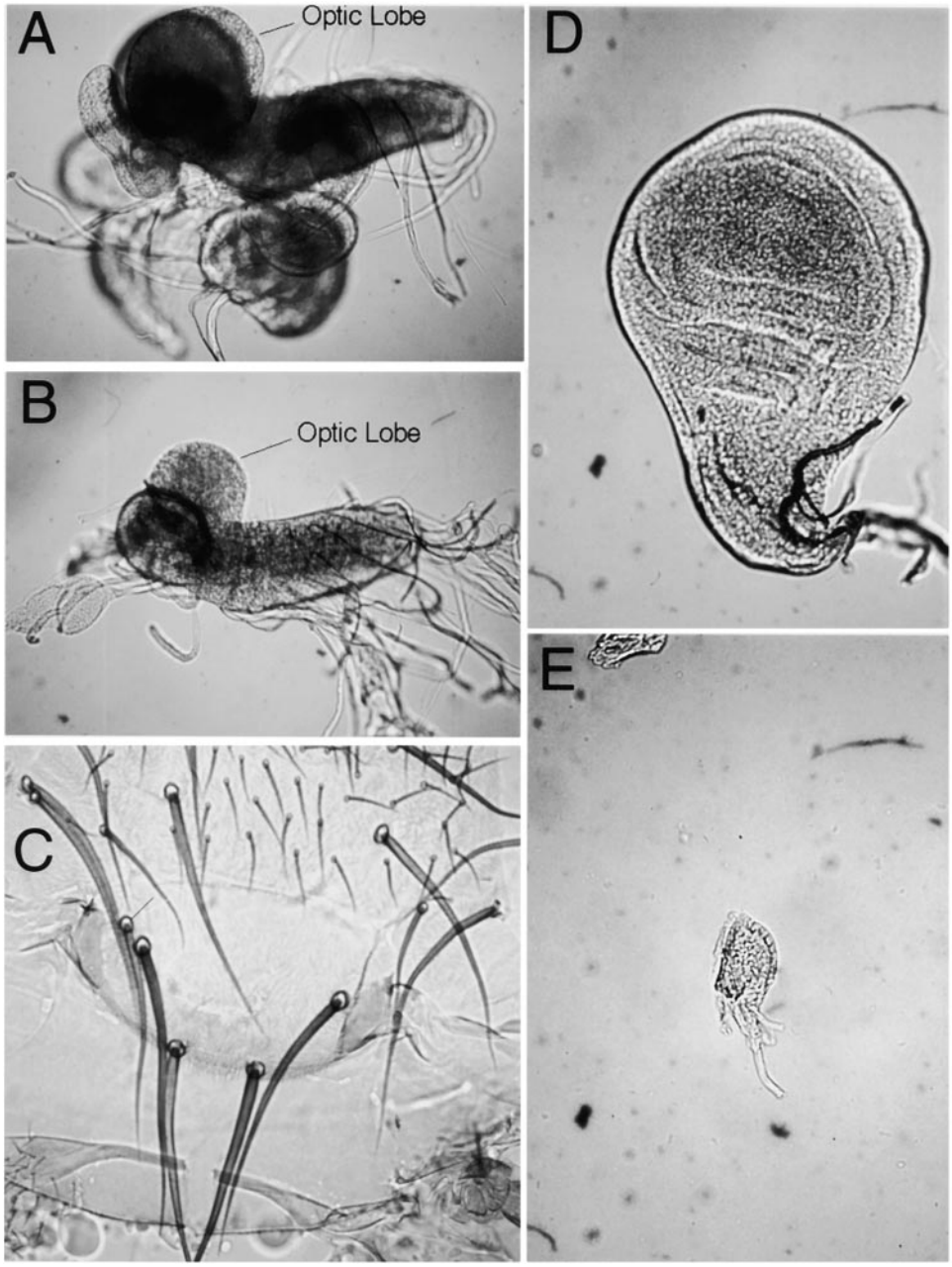

FIGURE 4.—The phenotype of homozygous *lid* mutants. (A) Brain dissected from wild-type, late third instar larva. (B) Brain from a homozygous *lid* mutant. Note the reduction in the size of the optic lobes and the absence of imaginal discs that are normally associated with the brain. (C) Two duplications of thoracic macrochete bristles in rare homozygous *lid* pharate adult escaper. (D) Wing imaginal disc dissected from wild-type, late third instar larva. (E) Wing imaginal disc dissected from homozygous *lid* mutant.

plementation with $ash1^{RE418}$. Another possibility is that each of the chromosomes with Polycomb group mutations we tested, $E(Pc)^1$, Psc^1, $Su(z)2^1$, Asx^{XF23}, and Scm^{D1}, also contains a mutation in some other gene that is responsible for the observed intergenic noncomplementation. This possibility is remote because it is unlikely that each of the deficiencies that uncover these Polycomb group genes also uncover mutations in the same other genes that fail to complement. Nevertheless, we have directly examined this possibility by testing other mutations in these five genes. We observed that $E(Pc)^2$, Asx^3, Asx^{13}, and Scm^{m56} all show intergenic noncomplementation with $ash1^{VV183}$ (Table 1). It was possible that the observed intergenic noncomplementation was specific for *ash1* mutations rather than general for mutations in trithorax group genes. This possibility was excluded for four of the five genes by showing that $E(Pc)^1$, Psc^1, $Su(z)2^1$, Asx^{XF23}, Asx^3, and Asx^{13} also show intergenic noncomplementation with trx^{b11} and/or brm^2 and increase the penetrance of two different double mutants, $ash1^{VF101}$ $^{trx\,b11}$ and brm^2 trx^{e2} (Table 1). Recently, another group has also reported that *Asx* mutations show intergenic noncomplementation with mutations in trithorax group genes (cited in SINCLAIR *et al.* 1998). In some of these cases, the different mutant alleles tested gave inconsistent results. For example, both Scm^{D1} and Scm^{m56} show intergenic noncomplementation with $ash1^{VV183}$ and enhance the phenotype of the $ash1^{VF101}$ trx^{b11} double mutant, whereas Scm^{302} does not enhance the phenotype of $ash1^{VV183}$ and suppresses the phenotype of $ash1^{VF101}$

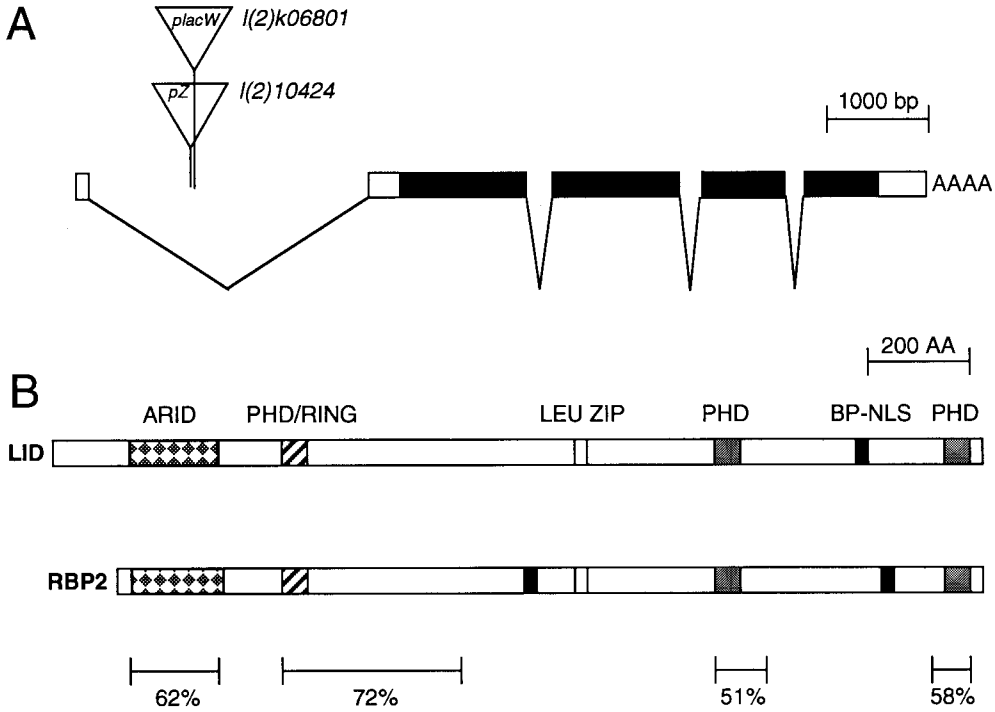

FIGURE 5.—(A) The intron-exon structure of *lid*. The *P* elements in *l(2)k06801* and *l(2) 10424* are located in the first large intron. The solid boxed areas indicate the open reading frame. (B) The motif structure of the conceptually translated LID protein along with that of human RBP-2. Note the overall similarity in size and arrangement of motifs. Four areas with a high degree of identity are indicated. LEU ZIP, leucine zipper motif; BP-NLS, bipartite nuclear localization motif.

trx^{b11}. We suppose that this difference is due to differences in the specific alterations of the SCM protein caused by these mutations.

Until now the antagonism of function between the products of Polycomb group genes and trithorax group genes has been demonstrated unidirectionally by the suppression of Polycomb group mutant phenotypes by mutations in trithorax group genes. We have taken advantage of the intergenic noncomplementation of mutations in trithorax group genes to assay suppression of trithorax group mutant phenotypes by mutations in genes previously classified as Polycomb group genes. Among $ash1^{VF101}$ trx^{b11} and brm^2 trx^{e2} heterozygotes, 52 and 35%, respectively, of adult flies express transformations of the third thoracic segment to the second thoracic segment. We observed that most mutations in seven of the genes that have been classified as members of the Polycomb group, *Polycomb*, *polyhomeotic*, *pleiohomeotic*, *Polycomb-like*, *multi sex combs*, *extra sex combs*, and *Super sex combs* suppress the penetrance of these transformations, in both of these double heterozygotes. Moreover, most mutations in these genes do not show intergenic noncomplementation with mutations in any of the three trithorax group genes that we have tested. We suggest that these genes represent the Polycomb group (Table 6) defined here as genes in which loss-of-function mutations enhance the dominant phenotype caused by *Polycomb* mutations and suppress the phenotype caused by heterozygosity for double mutations in trithorax group genes such as $ash1^{VF101}$ trx^{b11} and brm^2 trx^{e2}.

The *zeste* (*z*) gene encodes a transcription factor that binds DNA in a sequence-specific manner (BIGGIN *et al.* 1988). The z^1 mutation causes reduced *white* gene transcription (JACK and JUDD 1979). It was first recognized by WU *et al.* (1989) that mutations in three genes identified as dominant modifiers of the *zeste-white* interaction, *Enhancer of zeste*, *Suppressor of zeste-2*, and *Sex comb on midleg*, can also cause phenotypes like mutations in Polycomb group genes. We have shown that mutations in these three genes also behave as mutations in trithorax group genes: they show intergenic noncomplementation with mutations in trithorax group genes and/or increase the penetrance of $ash1^{VF101}$ trx^{b11} and/or brm^2 trx^{e2} heterozygotes. Moreover, we have shown that mutations in three other genes identified as suppressors of the *zeste-white* interaction, *Suppressor of zeste-4*, *Suppressor of zeste-6*, and *Suppressor of zeste-7*, may show intergenic

TABLE 6

Genes formerly classified as Polycomb group genes and/or *Suppressors of zeste*, reclassified according to the data presented herein

Polycomb group: enhancers of Polycomb group mutations and suppressors of trithorax group mutations	ETP group: enhancers of Polycomb group mutations and enhancers of trithorax group mutations
Polycomb (*Pc*)	*Enhancer of zeste [E(z)]*
polyhomeotic (*ph*)	*Enhancer of Polycomb [E(Pc)]*
Polycomb-like (*Pcl*)	*Additional sex combs* (*Asx*)
pleiohomeotic (*pho*)	*Sex combs on midleg* (*Scm*)
multi sex combs (*mxc*)	*Posterior sex combs* (*Psc*)
extra sex combs (*esc*)	*Suppressor of zeste 2 [su(z)2]*
super sex combs (*sxc*)	

noncomplementation with mutations in trithorax group genes and/or increase the penetrance of *ash1*^{VF101} *trx*^{b11} heterozygotes. The biochemical mechanism by which mutations in these genes modify the *zeste-white* interaction is not known. However, we think it is significant that many of the genes identified as *Suppressors of zeste* behave as if they are both trithorax and Polycomb group genes, that *Enhancer of Polycomb* is a suppressor of *zeste*, and that *sex combs extra* is an enhancer of *zeste*.

We propose that the six genes previously classified as Polycomb group genes in which loss-of-function or antimorphic mutations show intergenic noncomplementation with mutations in trithorax group genes and increase the penetrance caused by double heterozygosis of mutations in trithorax group genes belong in a distinct group (Table 6). We propose that this group be called the ETP (*E*nhancers of *t*rithorax and *P*olycomb mutations) group. Loss-of-function mutations in this group of genes enhance the dominant phenotype caused by *Polycomb* mutations like mutations in Polycomb group genes but also enhance the phenotype caused by heterozygosity for double mutations in trithorax group genes such as *ash1*^{VF101} *trx*^{b11} and *brm*^{2} *trx*^{e2} like mutations in trithorax group genes. Jürgens (1985) estimated that there were ~40 genes in the Polycomb group based on the enhancement of the Polycomb mutant phenotype by a sample of deficiencies. We suggest that this number may be an overestimate. Many of the genes in which mutations enhance the Polycomb mutant phenotype, according to our data, would also be expected to enhance the trithorax group mutant phenotype and hence should not be classified as Polycomb group genes.

Several studies have documented that mutations in many of the genes we have classified in the ETP group lead to ectopic expression of homeotic genes in embryos (*e.g.*, Simon *et al.* 1992; reviewed in Simon 1995). It has been inferred from such results that the normal function of the products of these genes is to repress transcription. However, a recent study of the consequences of mutations in one of these genes, *Enhancer of zeste*, demonstrated both ectopic expression and loss of expression of the same homeotic genes (LaJeunesse and Shearn 1995). That study was made possible by the availability of a strong temperature-sensitive allele. Without such alleles it would be very difficult to directly assay other members of the group for loss of homeotic gene expression. Nevertheless, we interpret the enhancement of the phenotype of mutations in both Polycomb and trithorax group genes by loss-of-function mutations in genes of the ETP group as an indication that the products of these genes are required for both activation and repression of transcription. It has recently been proposed that the product of the *zeste* gene itself is also involved in both activation and repression of transcription (Rosen *et al.* 1998). We have little information on the biochemical mechanism of action of any of these genes. There is evidence of a multimeric protein complex containing the products of the Polycomb group genes, *Polycomb* and *Polyhomeotic*, and of three different complexes containing the products of the trithorax group genes, *brahma*, *ash1*, and *ash2*. One way of rationalizing how mutations in the ETP group of genes could behave as both Polycomb and trithorax group mutations would be to suggest that the products of the ETP genes are components of complexes required for both repression and activation. Perhaps they are responsible for the structure of these complexes or different protein variants encoded by these genes are components of different complexes. Although Polycomb and trithorax group genes were first identified in Drosophila, homologous genes exist in mammals (reviewed in Schumacher and Magnuson 1997), *Caenorhabditis elegans* (Garvin *et al.* 1998), and plants (Goodrich *et al.* 1997). Until now, most interpretations of the functions of the products of such genes have been based on the idea that the products of Polycomb group genes repress gene transcription and the products of trithorax group genes activate gene transcription. The data presented here together with earlier data (LaJeunesse and Shearn 1995) suggest that some of the genes previously classified as Polycomb group genes and at least some of the genes identified as suppressors or enhancers of *zeste* belong to a group of genes whose products play a role in both the repression and activation of gene transcription. These data will require new interpretations of the functions of such genes.

Six noncomplementing deficiencies may identify new trithorax group genes: The 133 deficiencies examined collectively uncover ~70% of the genome. Of these, only 6 exhibited intergenic noncomplementation with mutations in all 3 of the trithorax group genes tested and do not uncover previously identified trithorax group genes. Either there must be only a small number (*i.e.*, closer to 10 than to 100) of genes in the entire genome in which mutations fail to complement mutations in the trithorax group genes tested or only deficiencies that uncover 2 or more such genes are detected in our assay. Four of the deficiencies failed to complement mutations in all 3 trithorax group genes but did not suppress the *Polycomb* mutant phenotype. Perhaps these deficiencies uncover genes whose products act downstream of the homeotic selector genes, for example, as cofactors necessary for the activity or stability of homeotic selector gene products.

Two of these six deficiencies suppressed the *Polycomb* mutant phenotype and did not uncover a known trithorax group gene. We have provided evidence that one of these six deficiencies, *Df(2L)cl-h3* (25D2-3;26B2-5), uncovers two different trithorax group genes. The distal gene is within 25D4 ; 25E1. It may be identical to *E(var)2-25E*, which was recovered in a screen for enhancers of position-effect variegation (Dorn *et al.* 1993). Several of the mutations recovered in that screen proved

to be allelic to trithorax group genes. The proximal gene is within 25F4-4;26B2-5. We have presented three lines of evidence that the allelic mutations *l(2)10424* (now known as *lid*^{1}) and *l(2)k06801* (now known as *lid*^{2}) represent *P*-element insertion mutations within this proximal gene that we have named *little imaginal discs*. First, both alleles are lethal in combination with deficiencies that remove 25F4-4;26B2-5. Second, *lid*^{2} enhances the phenotype of *ash1*, *brahma*, and *trithorax* mutations and suppresses the phenotype of a *Polycomb* deletion. Third, precise revertants of *lid*^{1} are homozygous viable and fail to enhance the phenotype of *ash1*, *brahma*, or *trithorax* mutations and fail to suppress the phenotype of a *Polycomb* deficiency.

Despite the fact that *lid* mutations satisfy the criteria we used for mutations in trithorax group genes, we did not observe homeotic transformations in homozygous or *trans*-heterozygous mutant embryos or larvae. Instead, we observed a small disc phenotype (Shearn *et al.* 1971). Certain allelic combinations of *ash1* mutations also cause a small disc phenotype (Shearn *et al.* 1987). The few *lid* mutants that survived the pupal stage expressed bristle phenotypes like mutations in the trithorax group genes *ash2* (Adamson and Shearn 1996) and *brahma* (Elfring *et al.* 1998). So, *lid* mutations do cause phenotypes like those caused by mutations in other trithorax group genes. We interpret the failure to detect a high frequency of homeotic transformations in the two *lid* mutants as a consequence of the nature of the mutations caused by the *P*-element insertions in these alleles.

The predicted *lid* gene product is extremely similar to the human *retinoblastoma binding protein 2* gene product (RBP-2). RBP-2 was discovered in a screen for proteins that interact with the pocket domain of the retinoblastoma protein (pRB; Defeo-Jones *et al.* 1991). The full-length sequence of RBP-2 was later determined and found to contain nuclear localization motifs as well as sequence motifs characteristic of transcriptional regulators (Fattaey *et al.* 1993). RBP-2 has been shown to physically interact with mammalian TATA-binding protein as well as with p107 and Rb (also known as p110; Kim *et al.* 1994). We have no information about the molecular mechanism of LID function. However, given the similarity of LID to RBP-2 and the binding of RBP-2 to pRB there are several intriguing possibilities.

The role of pRB in cell cycle regulation and proliferation is mediated, at least in part, by its interaction with the transcription factor E2F. It interacts physically with E2F to repress transcription and cell cycle progression. Overexpression of RBP-2 in cultured cells was shown to overcome the pRB-mediated suppression of E2F activity (Kim *et al.* 1994). A Drosophila mutant of E2F, *E(var)3-95E*, was discovered as a dominant enhancer of variegation (Seum *et al.* 1996). E2F is necessary for proliferation and differentiation in the Drosophila eye (Brook *et al.* 1996; Du *et al.* 1996a) and interacts genetically with a Drosophila homologue of Rb, RBF (Du *et al.* 1996b). Finding that *lid* mutations cause defects in imaginal disc cell proliferation may be due to the loss of negative regulation of RBF leading to increased E2F repression of *cyclin E*.

Histone acetylation has profound effects on transcriptional regulation and both global and local chromatin structure (Luger and Richmond 1998). The Rb protein has recently been found to physically associate with a histone deacetylase, HDAC1, and to repress transcription (Brehm *et al.* 1998; Luo *et al.* 1998; Magnaghi-Jaulin *et al.* 1998). The function of LID could be to counteract the repressive activity that histone deacetylation has on chromatin. Two multiprotein complexes from yeast, ADA and SAGA, function as nucleosome acetyltransferases, with GCN5 as the catalytic subunit (Grant *et al.* 1997); GCN5 mutations display synthetic lethality with SWI/SNF mutations. This is especially interesting in that *brahma* is a Drosophila homologue of yeast SWI2/SNF2 (Dingwall *et al.* 1995), and *lid* interacts genetically with *brahma*. Further evidence for an association of trithorax group gene products and pRB is that by both two-hybrid and coimmunoprecipitation studies, Hbrm and Brg1, two human homologues of *brahma*, are associated with pRB family members (Dunaief *et al.* 1994; Singh *et al.* 1995). The balance between acetylation and deacetylation is clearly implicated in the function of trithorax group genes. Though the role RBP-2 plays in chromatin regulation is not known, the fact that it could be involved in the inactivation or relocation of a histone deacetylase fits well with how we think trithorax group genes help to maintain an open chromatin conformation.

In addition to the connections of pRB with E2F, cyclin E, and the cell cycle and to the connections of pRB with histone deacetylation and repression of transcription, there is a connection of pRB with the nuclear matrix and nuclear matrix-associated proteins. p110^{Rb} is associated with the nuclear matrix in a cell cycle-dependent manner (Mancini *et al.* 1994). Many p110^{Rb}-associated factors have been previously found to be associated with the nuclear matrix, including SV40 large T antigen, adenovirus E1a, human papilloma E7 protein, lamin A, p84, and NRP/B (Durfee *et al.* 1994). One model is that functions within the nucleus occur at specific sites, and this functional compartmentalization of the nucleus is accomplished by localizing the machinery for each task to a specific site. For example, a hypothetical scenario consistent with this model would be that once activated, a homeotic selector gene may be bound by one or more trithorax group protein complexes that maintain the activated state by creating a site on the nuclear matrix for the transcription machinery itself and for proteins involved in acetylation and/or nucleosome remodeling and/or phosphorylation that are necessary for optimal expression. In this context, the change in subnuclear localization of the *modifier of mdg-4* gene

product may be relevant. *Modifier of mdg4*, also known as *E(var)3-93D*, is a trithorax group gene. Loss-of-function mutations enhance the phenotype of *ash1 trithorax* and *brahma trithorax* double mutations and suppress the phenotype of *Polycomb* mutations (Gerasimova and Corces 1998). The product of this gene, MOD, is normally associated with the nuclear matrix. However, the subnuclear localization of MOD is dramatically altered in both trithorax group and Polycomb group mutant backgrounds. In trithorax group mutants MOD is primarily cytoplasmic; in Polycomb group mutants MOD is present in the central region of the nucleus rather than the nuclear matrix (Gerasimova and Corces 1998). Many of the models for the organization of higher order chromatin structures are based on associations with nuclear matrix components. It will be interesting to determine the subnuclear localization of LID and observe whether there are changes in this localization during the cell cycle and/or in trithorax group and Polycomb group mutant backgrounds.

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Note added in proof: The lid gene corresponds to CG9088 of the annotated Drosophila genome.

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