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Genetics

Dosage-dependent modifiers of Polycomb and Antennapedia mutations in *Drosophila*

(homoeotic)

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ABSTRACT **Two genes known to control the determination of segmental identity in *Drosophila melanogaster* are Polycomb and Antennapedia. To identify additional genes involved in the determination of segmental identity, we have isolated dominant modifers (both suppressors and enhancers) of Polycomb and/or Antennapedia mutations. Sixty-four such modifier mutations have been recovered and mapped to 18 complementation groups. All of the mutations identify genes necessary for viability of the zygote. Six of the 18 genes that were identified by mutations that interact with Polycomb and/or Antennapedia have been previously characterized as homoeotic genes [i.e., Sex combs reduced (*Scr*), Brista (*Ba*), trithorax (*trx*), Polycomb (*Pc*), Polycomblike (*Pcl*), and Sex comb on midleg (*Scm*)]. Mutations in several of the additional loci identified here have also been shown to have homoeotic phenotypes.**

The determination of body segment identity in *Drosophila melanogaster* is a relatively simple developmental decision that provides a good system for analysis of the genetic basis of pattern formation in a multicellular organism. Extensive genetic and molecular analyses have identified a number of genes and gene products required for proper segmentation and segmental identity (see refs. 1–4 for recent reviews); however, it is clear that many other genes required for proper segmental identity to be initiated and maintained have yet to be identified. Our goal is to identify additional genes necessary for the determination of segmental identity and to understand the function of their gene products.

The majority of genes involved in the determination of segmental identity have been identified by the existence of homoeotic mutants, mutants in which structures characteristic of one body part are replaced by structures characteristic of another body part. Genes in which homoeotic mutations have been recovered are often referred to as homoeotic genes. The first homoeotic genes were identified due to rare adult-viable dominant or recessive alleles. As mutations in most genes involved in the determination of segmental identity might be expected to be recessive-lethal mutations with little or no heterozygous effect, zygotic-lethal mutations with homoeotic phenotypes have been sought in collections of lethal mutations that die as embryos (5–7), larvae (8–10), or pupae (11, 12). Although this approach has identified many genes involved in the process, the screens applied are not likely to have discovered all or even a majority of homoeotic genes, as many known homoeotic genes were not identified in any of the experiments. The failure to isolate mutations in homoeotic genes on the basis of a lethal homoeotic phenotype may stem from a number of problems. At the time at which segmental identity is determined (the cellular blastoderm stage), the zygotic genome has only begun transcription, and the maternal contribution of genetic information is probably still very important. The maternal contribution has been shown to mask the zygotic phenotype for a number of homoeotic genes (13–15). As the majority of zygotic-lethal mutations exhibit a significant maternal contribution (16–18), maternal effects in the determination of segmental identity are not a trivial concern. Another major problem in the search for zygotic-lethal mutations with homoeotic phenotypes is the difficulty in effectively distinguishing the differences between many of the larval segments. For example, the embryonic-lethal Polycomblike alleles have a weak, but clear, homoeotic phenotype when examined carefully (13, 19), but the phenotype is so subtle that no Polycomblike alleles were recovered in a large screen for embryonic-lethal mutations that affect the larval segmental pattern (5). Another problem can arise if a gene product is required more than once during development. If a gene required for segmental identity is also required for some other developmental process, the phenotype of a mutation may be complex and not easily discernible as affecting segmental identity. A good illustration of this problem is the fushi tarazu (*ftz*) gene. The ftz protein is detectable at the cellular blastoderm stage in a pattern of seven transverse stripes perpendicular to the anteroposterior axis of the embryo and again at a later embryonic stage in specific cells of the nervous system (20, 21). The loss of ftz function at either time leads to a mutant phenotype, but the loss of function of ftz at blastoderm leads to such a severe phenotype that the nervous system phenotype cannot be discerned and was missed even in a very careful analysis of the ftz mutant phenotype (22).

As a different approach to the identification of genes involved in the determination of segmental identity, we have chosen to screen for dosage-dependent interactions with previously characterized genes. It was an approach of this type that first identified the homoeotic genes Polycomblike (23) and trithorax (24). The rationale for the approach derives from the observation that, in *Drosophila*, the amount of gene product is usually proportional to the number of wild-type copies of the gene in the genome such that flies with only one copy of a gene have only 50% of the gene product when compared to normal flies bearing two copies of the gene, whereas flies bearing a duplication for the gene (three copies) have 50% more gene product than wild type (25–29). It should, therefore, be possible to vary the amount of gene product from a regulatory locus by varying the number of copies of the wild-type gene. The variation in the level of gene product from the regulatory locus should then be reflected as variation in the level of activity of the gene that it regulates.

By screening for genes that, when present in fewer copies, affect the expression of the homoeotic genes Polycomb or Antennapedia, we have identified at least 18 genes involved in the determination of segmental identity. Six of the 18 genes are known homoeotic genes, whereas the remainder were not previously known to be involved in the determination of segmental identity.

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MATERIALS AND METHODS

Unless otherwise noted, mutations and chromosome aberrations have been previously described (30–34). Pc^{T1} is an ethyl methanesulfonate-induced allele of Polycomb isolated by Stanley Tiong (University of Alberta, Edmonton). *Dp(3;Y)-Antp*$^+$ is a Y chromosome carrying a duplication for salivary chromosome region 83DE–84D (including all of the Antennapedia complex) constructed and kindly provided to us by R. Denell (Kansas State University, Manhattan). Flies were reared on yeast/sucrose or yeast/cornmeal/molasses medium at 25°C. Mutations or growth conditions that slow development of the fly affect the penetrance and expressivity of most of the dominant homoeotic mutations used (see ref. 30). To minimize nonspecific effects due to delays in development, care was taken with all crosses to avoid overcrowding, and mutations that as heterozygotes slowed development of the fly were discarded. The mutations discarded included a large number of Minute mutations, a class of dominant mutations that slow development of the fly and are probably mutations in ribosomal protein genes (35).

The five different crosses used to isolate modifier mutations were cross 1, Oregon R males mated to *TM3/Pc*^{2} *Antp*^{Ns} *sr e*^{s} females; cross 2, *cn bw sp* males mated to *TM3/mwh Pc*^{T1} *Antp*^{Scx} *p*^{p} *e*^{s} females; cross 3, *TM3/ru h th st cp in ri Antp*^{Scx} *p*^{p} *e*^{s}; *Dp(3;Y)Antp*$^+$, *y*$^+$ males mated to *TM3/Df(3R)Antp17* females; cross 4, *y*; *TM3/Pc*^{R1} males mated to *C(1)M4, y*; *Dp(2;Y)L124, B*^{S} females; and cross 5, *cn bw sp*; *Dp(2;Y)L124, B*^{S} males mated to *TM3/Pc*^{R1} *p*^{p} *e*^{s} females. For mutagenesis, males were given 4000 rad (1 rad = 0.01 Gy) of γ-irradiation from a ^{60}Co source or were fed ethyl methanesulfonate for 24 hr according to the methods of Lewis and Bacher (36) and were mated to virgin females of the appropriate genotype. Mutagenized males were discarded after 4 days to ensure that only postmeiotically treated chromosomes were tested. The inseminated females were returned to new cultures for subsequent brooding. Progeny were scored for dominant suppressors or enhancers of the known homoeotic mutations in the crosses. Individual flies carrying putative suppressor or enhancer mutations were backcrossed to flies carrying the known homoeotic mutations, and verified suppressor or enhancer mutations were subsequently recovered as balanced stocks.

The meiotic map positions were determined for the dominant suppression or enhancement effects on the Polycomb or Antennapedia mutations. With the exception of the two Polycomb alleles, a recessive-lethal mutation mapped to the same position on the chromosome and was assumed to be due to the modifier mutation. The cytological locations were determined for the recessive-lethal characters of the mutations by complementation analyses with existing duplication and deficiency-bearing chromosomes. Salivary chromosome analysis followed the methods of Lefevre (37). For complementation tests, at least 200 progeny were scored from each cross. Allelism to previously named loci was determined by failure to complement known alleles in all cases except Sex comb on midleg (*Scm*) and Polycomb, in which allelism was inferred from the phenotypes and locations (19).

All Polycomb alleles have low penetrance as heterozygotes and are difficult to use in a screen for suppressor mutations. The presence of *Dp(2;Y)L124*, a duplication for 21A–21C, greatly enhances the heterozygous Polycomb phenotype (30) and results in essentially complete penetrance of the dominant extra sex combs phenotype. Suppressors are more easily identified and recovered in the enhanced background. To increase the penetrance of *Antp*^{Ns} and *Antp*^{Scx} and facilitate the isolation of suppressor mutations, the *Antp*^{Ns} and *Antp*^{Scx} flies were also heterozygous for a Polycomb allele. A second genotype with enhanced expressivity of *Antp*^{Scx} [*Antp*^{Scx}*/Df(3R)Antp17*; *Dp(3;Y)Antp*$^+$] was also used to facilitate isolation of suppressor mutations. Suppressors isolated in the double-mutant backgrounds may suppress only one of the mutations in the background or may specifically suppress the interaction. To determine if the mutations would interact with Polycomb mutations in an otherwise normal genotype and to compare the effects of the different mutations, the dominant effects of the modifier mutations on the extra sex combs phenotype of *Pc*^{R1}/+ males were quantified. For the quantification, mutant females were crossed to *TM3/Pc*^{R1} males. All progeny were allowed to eclose (to control for variations due to differences in developmental rate within a genotype), and the numbers of sex comb teeth were counted (×100 power under the dissecting microscope) on the second and third legs of at least 20 randomly selected males carrying both *Pc*^{R1} and the mutation of interest. All mutations were tested in combination with *Pc*^{R1} with the exception of *Scr*^{E1}, *Scr*^{E2}, and *Scr*^{E3}.

RESULTS AND DISCUSSION

Over 67,000 progeny from mutagenized flies were screened for dominant suppressor and/or enhancer mutations of homoeotic alleles of the Polycomb and/or Antennapedia loci. The number of progeny of each genotype examined, the phenotypes suppressed or enhanced, and the 64 modifier mutations recovered are given in Table 1.

Table 2 shows the 18 loci identified by the 64 dominant suppressor and enhancer mutations isolated. The enhancers show between a two- and four-fold enhancement of the extra sex combs phenotype of *Pc*^{R1}/+. The suppressors range from 50% to 100% suppression of the *Pc*^{R1}/+ phenotype, with the majority showing >95% suppression. Some of the mutations were also tested for interactions with dominant Antennapedia alleles. Alleles of *Pc, Pcl, Scm, Ba, brm, kto,*

Table 1. Genetic screens utilized and the mutations recovered

Cross*	Mutagen	Flies, no.	Transformation phenotype scored	Mutations recovered
1	EMS	7,851	Enhancement of extra sex combs	*Pcl*^{K1},*Pc*$^{K1-K2}$,*Scm*^{K1}
1	EMS	10,507	Suppression of antenna to leg	*brm*^{1},*trx*^{E3},*mor*$^{1-2}$,*osa*$^{1-3}$
2	EMS	10,638	Suppression of extra sex combs	*kis*^{5},*vtd*^{1},*urd*^{2}
3	EMS	11,006	Suppression of extra sex combs	*Scr*$^{E1-3}$
4	γ-rays	10,188	Suppression of extra sex combs	*urd*^{1},*l(3)87Ca*^{E1}, *Su(Pc)37D*^{1},*trx*$^{E1-E2}$
5	EMS	11,765	Suppression of extra sex combs	*kis*$^{1-4}$,*kis*$^{6-8}$,*Ba*^{E1},*dev*$^{1-2}$, *brm*$^{2-3}$,*kto*^{1},*vtd*^{2},*Scr*^{E4}, *mor*$^{3-4}$,*l(3)87Ca*^{E2},*osa*^{4}, *skd*^{1},*trx*$^{E4-E12}$,*sam*^{1}
5	γ-rays	5,934	Suppression of extra sex combs	*kis*^{9},*brm*$^{4-6}$,*vtd*$^{3-5}$,*mor*$^{5-6}$, *trx*^{E13},*trx*^{B14},*skd*^{2}

EMS, ethyl methanesulfonate; γ-rays, 4000 rad of γ-irradiation.
*The genotypes of the five crosses are given in *Materials and Methods*.

Table 2. Loci identified by dominant suppressor or enhancer mutations

Locus	Map position	Cytological location	Alleles recovered	r/n* in Pc^{R1}/+ males
Suppressor loci				
kismet (*kis*)	2–0	21B6–8	9	0/80 to 79/80
Su(Pc)37D	2–	37D2–38C1	1	82/160
Brista (*Ba*)	2–107.8	60D14–E2	1	8/80
devenir (*dev*)	3–41		2	9/80 and 17/80
brahma (*brm*)	3–43.0	72AB	6	0/80 to 26/80
kohtalo (*kto*)	3–46	76B–D	1	4/80
verthandi (*vtd*)	3–46		5	1/80 to 9/80
Sex combs reduced (*Scr*)	3–47	84B1–2	4	103/80
l(3)87Ca	3–51.7	87C4–5	2	6/80 and 7/80
urdur (*urd*)	3–53	87F12–15	2	27/160 and 105/80
trithorax (*trx*)	3–54.2	88B1–3	14	0/80 to 23/80
moira (*mor*)	3–58.1	88F9–89B4	6	0/80 to 23/160
osa (*osa*)	3–60.0	90B1–D1	4	22/80 to 85/80
skuld (*skd*)	3–		2	14/80 and 26/80
sallimus (*sam*)	3–		1	1/80
Enhancer loci				
Polycomblike (*Pcl*)	2–84	55BC	1	707/80
Polycomb (*Pc*)	3–47.1	78E	2	395/80 and 760/80
Sex comb on midleg (*Scm*)	3–49	85F	1	485/80

*r, total sex comb teeth counted; n, total number of second and third legs scored. r/n is given for the alleles with the weakest and strongest dominant interactions with Pc^{R1}. r/n for the control crosses is 1087/400.

Scr, and *trx* show clear dominant enhancement or suppression of $Antp^{Scx}$, whereas alleles of *vtd*, *l(3)87Ca*, *Su(Pc)37D*, *urd*, *mor*, *skd*, and *osa* do not (unpublished data). All of the loci identified by modifier mutations are required for zygotic viability; however, not all of the alleles are lethal in homozygous mutant flies. Both Polycomb alleles were recovered in trans to Pc^{R1} and are homozygous viable alleles. Allelism to Polycomb was judged by the map position (between *ri* and *eg*) and recessive extra sex combs phenotype. Complementation tests between all pairwise combinations of alleles within each locus were done, with the exception of three kismet alleles induced on *Dp(2;Y)L124* (kis^6, kis^7, and kis^8), three *Scr* alleles induced on a chromosome carrying $Antp^{Scx}$ (Scr^{E1}, Scr^{E2}, and Scr^{E3}), and vtd^1, which was lost before complementation tests were completed. All combinations failed to complement for zygotic viability with the exception of the *vtd* locus. Progeny containing heteroallelic combinations of *vtd* alleles eclosed as adults with frequencies from 0% to 90% of the expected total. Many of the surviving adults were abnormal, with a common syndrome of thin bristles, rough eyes, reduced sex combs, and incised wings.

The types of genes we would expect to identify vary. In screening for mutations that interact with Antennapedia mutations, for example, one might expect several levels of interaction. Genes that regulate the Antennapedia locus should have dosage-dependent effects on expression of Antennapedia alleles. In addition, target genes that are regulated by Antennapedia protein should show dosage-dependent interactions under conditions where Antennapedia expression is limiting (exactly the types of conditions chosen for the experiments reported here) by binding Antennapedia protein to a target gene that cannot produce functional products. A third type of gene expected to show dosage-dependent interactions with Antennapedia mutations includes genes whose products (either RNA or protein) interact with Antennapedia gene products to regulate other genes. Again, the dosage-dependent interactions will only be seen when Antennapedia gene function is limiting. Examples of all three types of genes are probably represented in the sample of mutations described in this work. Polycomb mutations have been shown to affect the pattern of transcription from the wild-type Antennapedia gene (38) and, thus, Polycomb regulates Antennapedia expression either directly or indirectly. Sex combs reduced expression is altered in Antennapedia mutations (39), suggesting that the Sex combs reduced gene is a target, either directly or indirectly, of Antennapedia gene regulation. Alleles of Polycomb and Sex combs reduced interact with some alleles of Antennapedia (unpublished data). No gene products have yet been shown by biochemical methods to functionally interact with Antennapedia gene products; the genes identified here provide good candidates for such an approach.

The majority of mutations isolated in this work on the basis of dominant interactions with Polycomb and/or Antennapedia mutations identify genes in regions of the genome that were previously identified in a systematic screen of the autosomes for genes that, when duplicated, affect the expression of Polycomb and/or Antennapedia mutations (30). The localization of the genes identified by mutations in the work described here and the regions that when duplicated interact with Polycomb and/or Antennapedia mutations are shown in Fig. 1. With the exception of the Sex combs reduced locus, the effects of increasing the gene dosage of a region are the opposite of the effects of decreasing the gene dosage for the mutation identified in that region. For example, mutations that suppress the phenotype of Polycomb mutations are in regions that, when duplicated, enhance the phenotype of Polycomb mutations. The failure of Sex combs reduced duplications to behave as expected is probably a consequence of other genes of the Antennapedia complex that are also present in the duplications. Eleven genes probably show dosage-dependent interactions with Polycomb and/or Antennapedia when gene copy number is either increased or decreased, whereas four of the genes appear to only show dosage effects when the amount of gene product is decreased and not when the amount of gene product is increased above the wild-type level.

The conclusion that the screens used here to identify genes involved in the determination of segmental identity are relatively specific is suggested by the high frequency of homoeotic mutations identified. One-third of the genes that we identified by modifier mutations are previously identified homoeotic genes. Several of the additional loci identified here appear to be homoeotic genes as well. For two of the loci,

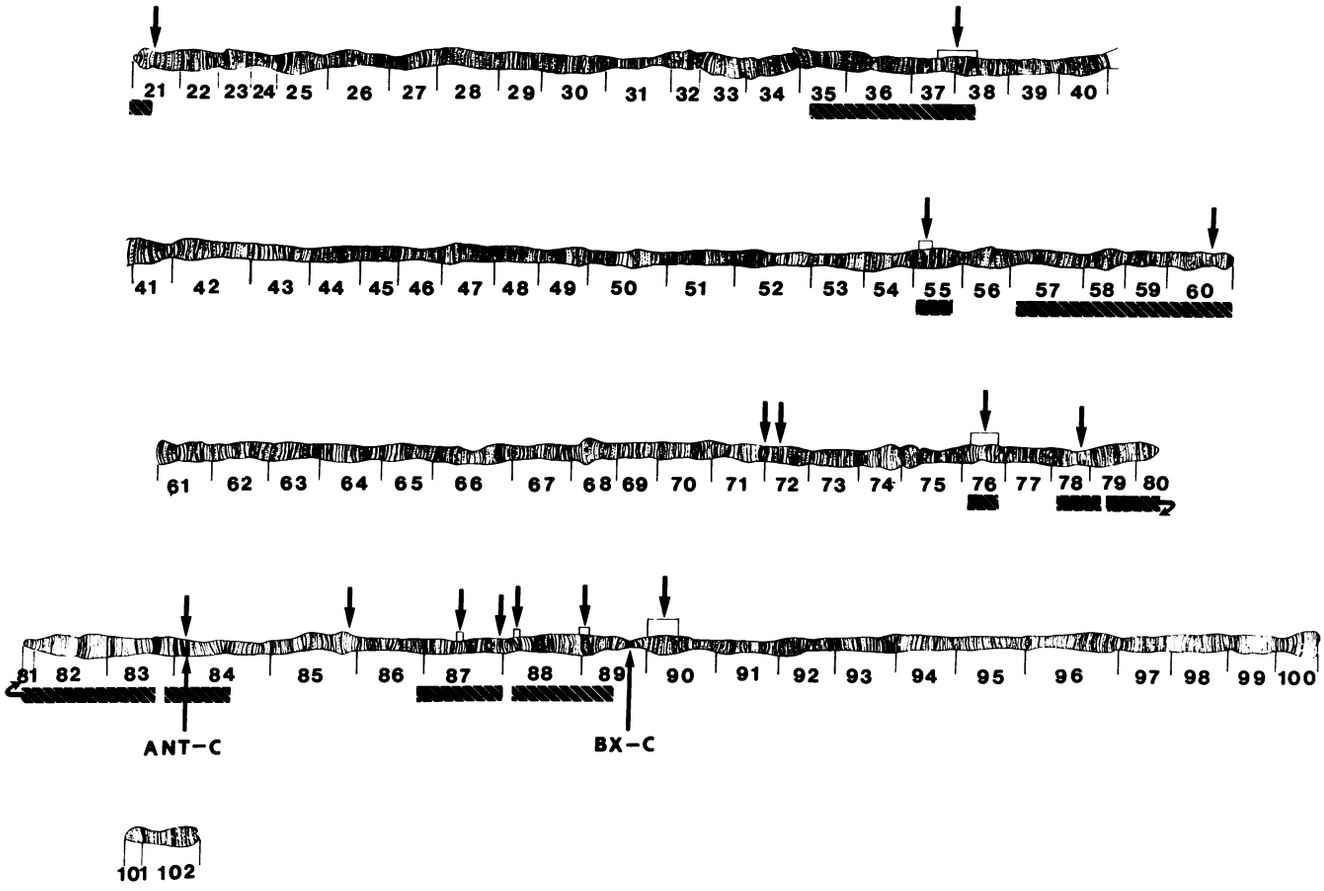

FIG. 1. Salivary chromosome map of the autosomes showing duplications and mapped mutations that affect the expression of Polycomb and/or Antennapedia mutations. The polytene chromosome banding patterns are labeled according to Bridges (40). The shaded bars below the chromosomes indicate the extents of the three duplications that suppress (55B–55E, 78A–79D, and 83E–84D) and the seven duplications that enhance the extra sex combs phenotypes of Polycomb and/or Antennapedia mutations (30). The locations of the Antennapedia complex (ANT-C) and bithorax complex (BX-C) are shown below the third chromosome. The arrows above the chromosomes indicate the genes identified by mutations that suppress or enhance the phenotypes of Polycomb and/or Antennapedia mutations (see Table 2 for the names of the genes). The positions of the *sam*, *skd*, and *vtd* loci were not determined with sufficient accuracy to place them on the cytological map. The *dev* locus is shown by the arrow at 71F, the most proximal limit for its location.

kismet and moira, homozygous patches of cells were generated in heterozygous individuals by radiation-induced mitotic recombination during larval growth of the imaginal cells. For both loci, such homozygous patches of tissue show homoeotic transformations in some segments of the adult cuticle (unpublished data). Since we have only examined two of the loci in such somatic mosaics, it is possible that many of the other mutations will also have homoeotic phenotypes when examined in homozygous clones of cells; however, the eight loci for which homoeotic phenotypes have already been demonstrated (the six known homoeotic loci plus kismet and moira) account for 38 of the 64 mutations isolated (59%). For a nonspecific screen (by nonspecific, we mean a screen to identify mutations of interest among a random sample of mutations examined), the expected frequency of homoeotic mutations is at least 200 times lower than this. Our estimate for the low frequency of expected homoeotic mutations is derived from the results of two different approaches that have previously been used by other laboratories to identify homoeotic mutations. For example, from ≈4400 embryonic-lethal mutations examined, only 12 mutations with recognized homoeotic phenotypes were identified (5–7). In addition, examination of 270 sex-linked lethal mutations in somatic mosaics revealed no mutations with a recognized homoeotic phenotype (41–43). Beyond the observation that the majority of mutations isolated in our screens are in homoeotic genes, we feel that the isolation of several alleles for each locus (an average of 3.6 alleles per gene) in a relatively small number of loci (18 total) suggests that there is specificity to the targets of mutagenesis.

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