carcinogens considered by Tomatis to have been adequately tested.

18. According to the EPA (*12*), estimates of the number of workers exposed range from 500,000 (estimate from the National Occupational Hazard Survey) to fewer than 5000 (an industry estimate prepared by Hull and Co. for the Synthetic Organic Chemical Manufacturing Association). The latter is probably more appropriate at levels of exposure close to the permissible 8-hour time-weighted average (TWA) ambient levels of 450 mg/m^3, or to the empirically observed TWA levels of 300 mg/m^3. Thus it is assumed that 5000 workers are each exposed to 300 mg/m^3 for 8 hours per day, 250 days per year. Assuming each worker inhales 1.2 m^3/hour and absorbs all of the *p*-dichlorobenzene, annual exposure would be (250 days per year)(8 hours per day)(1.2 m^3/hour)(300 mg/m^3) = 720 g/year. Converting to an average daily dose in milligrams per kilogram per day, one obtains (720 g per year)/(70 kg)(365 days per year) = 28 mg/kg-day for 5000 workers.
19. This estimate is based on the EPA's estimated frequency distribution of persons exposed to various ambient levels from 100 μg/m^3 to ≤ 0.1 μg/m^3, and a mean inhalation rate of 1.2 m^3/hour while awake for 16 hours a day, and 0.4 m^3/hour while asleep for 8 hours a day. Environmental exposures from water were calculated on the basis of EPA estimates, and were found to be negligible compared to the air (less than 0.1 percent of the amount inhaled).
20. A report prepared by the NTP for the Senate Appropriations Committee found that of 98 chemicals testing positive in at least one species prior to September 1979, 55 had proposed or final regulations on the record. However, many of these regulations were guidelines and not mandatory standards, and, with the exception of tris-BP (the flame retardant once used in children's sleepwear), it is impossible to document any change in exposure. For example, no new standards were promulgated after it was shown by means of a CBSR that ethylene dichloride is a carcinogen, but there may have been intensified voluntary efforts to reduce exposure to this high-volume industrial chemical.
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25. A 5-year trial may seem short in view of the usual latency periods in carcinogenesis. However, Peto *et al.* (*21*) interpret the evidence from animal studies as suggesting that β-carotene inhibits a late stage in carcinogenesis; if so, its effects would be seen in just a few years.
26. C. Mettlin and S. Graham, *Am. J. Epidemiol.* **110**, 255 (1979); S. Graham *et al.*, *ibid.* **113**, 675 (1981); C. Mettlin, S. Graham, R. Priore, J. Marshall, M. Swanson, *Nutr. Cancer* **2**, 143 (1981); S. Graham *et al.*, *Am. J. Epidemiol.* **116**, 68 (1982). The percent reduction in breast cancer was based on a 3:1 weighting of the relative risks for persons over 55 and under 55 years of age, reflecting the age distribution of breast cancer mortality in the United States.
27. The power estimate, β, is based on a one-tailed test and is given by

$$\beta = \Phi\left\{\frac{N^{1/2}(P_1 - P_0)}{[P_1(1 - P_1) + P_0(1 - P_0)]^{1/2}} - 1.645\right\}$$

where N = sample size per group, P_0 = placebo mortality rate, P_1 = treated mortality rate, and Φ = cumulative Gaussian distribution function. With a two-tailed test, the power estimate would be .52.
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29. With a prior probability of 0.1, a true-positive rate of 0.8, and a false-positive rate of 0.05, the probability of carcinogenicity after a positive study is (0.1)(0.8)/[(0.1)(0.8) + (0.9)(0.05)] = 0.64. Hence, the cost-effectiveness ratio is ($30 × 10^6)/(21)(0.64), or $2.2 × 10^6 per cancer death averted.
30. Based on an average retail price of $20 per hundred at discount pharmacies in the Boston area.
31. β-Carotene may be taken safely in large doses, up to 180 mg/day [U.S. Food and Drug Administration, *Evaluation of the Health Aspects of Carotene (Beta-Carotene) as a Food Ingredient*, prepared by the Federation of American Societies for Experimental Biology (PB80-119837, National Technical Information Service, Springfield, Va., 1979)].
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RESEARCH ARTICLE

Molecular Genetics of the Bithorax Complex in *Drosophila melanogaster*

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The bodies of insects are divided into a series of segments. The segments are formed very early in the development of the embryo, and cells from one segment do not, in general, mix with cells from other segments throughout the rest of development (*1*). In the fruit fly *Drosophila melanogaster*, there are mutations that transform parts of segments or entire segments into the form of other segments. These homeotic mutations define genes that direct cells into different developmental pathways in different segments. The bithorax complex in *Drosophila* is one of the best studied clusters of such genes (*2*); these genes determine the developmental fate of many of the thoracic and abdominal segments of the animal. When the whole bithorax complex is deleted, the animal dies late in embryonic development and shows striking changes in the segmental pattern of the embryonic cuticle. The third segment of the thorax and all eight abdominal segments resemble the normal second thoracic segment (*2*). Thus the second thoracic segment, which gives rise to the pair of wings and the second pair of legs in the adult fly, can be considered the developmental ground state, and the bithorax complex directs the more posterior segments to specialized developmental pathways. Individual recessive mutations within the complex give less extreme segmental transformations than those resulting from deletions of the whole complex. These mutations transform part of a segment or segments into tissue appropriate to a more anterior segment, toward the ground state. There are also dominant mutations, which transform a segment or part of a segment into more posterior structures, away from the ground state (*3*). These dominant mutations seem to upset the regulation of genes within the complex and turn on functions in an inappropriate segment.

A genetic map of the complex is shown in Fig. 1. Most of the recessive mutants and several dominant mutants show no cytologically visible rearrangements in the salivary gland polytene chromosomes, and they can be recombined with each other. The recombination distances between some pairs are shown. The recessive mutations *bx* and *pbx* affect development of the anterior and posterior halves, respectively, of the third thoracic segment. In the abdomen, *bxd*, *iab-2*, *iab-5*, and *iab-8* affect the first, second, fifth, and eighth ab-

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Abstract. *The bithorax complex in* Drosophila melanogaster *is a cluster of homeotic genes that specify developmental pathways for many of the body segments of the fly. The DNA of the bithorax complex has been isolated, and a region of 195,000 base pairs that covers the left half of the complex is described here. The lesions associated with many of the bithorax complex mutants have been identified, and most are due to DNA rearrangements. Most of the spontaneous mutants have insertions of a particular mobile element named "gypsy." This element affects the functions of sequences removed from the site of insertion. Mutant lesions for a given phenotypic class are distributed over large DNA distances of up to 73,000 base pairs.*

dominal segments, respectively. With the exception of *pbx*, the alleles are arranged left to right in the complex in the order, anterior to posterior, of the segments which they affect. Some of the recessive alleles and most of the dominant ones are associated with cytologically visible rearrangements, which all have a breakpoint within the pair of doublet bands designated 89E1-4. These bands are in the middle of a prominent constriction, and so it is difficult to distinguish the cytological positions of various breaks within the complex. Some of the rearrangements, such as the bxd^{100} transposition, can be located on the genetic map by testing the resulting fragments of the complex for complementation with the various recessive mutants.

At present we know little of the molecular processes by which genes such as those of the bithorax complex control the developmental fates of cells. However, the large polytene chromosomes in the salivary glands of *Drosophila* have permitted the isolation of such genes when nothing is known of the gene products. We have used techniques called chromosome walking and jumping to isolate a small fragment of the bithorax complex in recombinant DNA molecules (*4*). That fragment was used to isolate adjacent DNA fragments in a chromosomal walk through the bithorax complex. The first objective of the molecular analysis of the complex has been to identify the DNA changes in the various bithorax mutants. For the mutations in the complex associated with cytologically visible rearrangements, the rearrangement breakpoints can easily be mapped on the DNA (*5*). However, many of the best studied mutations are cytologically normal. When such mutations are due to single base changes in the DNA sequence, they are very difficult to locate. As it happened, the majority of these cytologically normal mutations in the bithorax complex are associated with easily detectable insertions and deletions.

Collecting Bithorax DNA

Our initial toehold in bithorax DNA was gained by "jumping" across an inversion. The inversion, called Cbx^{+R1}, breaks in the bands 87E1,2 and 89E1,2; the latter breakpoint is within the bithorax complex, as judged by its cytology and by its *Ubx* phenotype (see *Ubx* mutants below). One of the inversion fusion fragments was cloned and identified by homology to sequences in 87E, and the bithorax complex sequences from the fusion fragment were used as the starting point of a chromosomal walk (*4*). Figure 2 shows a composite restriction map covering 195 kb (1 kb is 1000 base pairs), marked off in kilobases from the starting point of the walk. This DNA region covers the left half of the bithorax complex, from *abx* through *pbx*, plus sequences to the left of the complex. The walk has also been extended to the right through the region of abdominal mutations, but that half of the complex is less well characterized genetically and molecularly, and it will not be discussed here. The chromosomal orientation of the walk was initially determined by in situ hybridizations of bithorax complex probes to four rearrangements with *bxd* phenotypes (see *bxd* mutants below), and has been amply confirmed by the positions of the lesions associated with several genetically mapped mutants (*6*).

The entire map of Fig. 2 consists of single-copy DNA, as can be judged by Southern blot analysis at standard criteria with representative recombinant phage from along the walk. (Repeated sequences that are poorly matched or shorter than a few hundred base pairs might be missed by this analysis.) Thus, there is as yet no evidence for large repeating DNA units corresponding to segments of the fly. DNA was collected from libraries representing two wild-type strains of *Drosophila*, Canton S and Oregon R, and restriction maps from the two strains are compared in Fig. 2. The number of restriction site differences between the two strains does not suggest tight evolutionary sequence conservation for the overall region (*7*).

As the overlapping DNA segments were collected in the walk, we began to look for the breakpoints of cytological rearrangements associated with various mutations. Such breakpoints can be located unambiguously on the DNA map by in situ hybridizations. Probes from along the walk were hybridized to a chromosome with an inversion, for example, to see where the probes switched from labeling one inversion end point to the other end point (*8*). Once the breakpoint site was identified to within about 15 kb, Southern blots were done with genomic DNA from the inversion strain to find the anomalous restriction fragments associated with the inversion breakpoint. We also began to examine, by Southern blot analysis, the DNA from cytologically normal, spontaneous, and x-ray–induced mutations. Most had anomalous restriction fragments indicative of DNA insertions or deletions. For many of these mutations we have constructed libraries of recombinant phage from the mutant DNA and isolated the region of interest (*9*). The mutant lesions were then characterized directly by restriction mapping and by electron microscopy of heteroduplex molecules. The descriptions of the mutant lesions that follow are based on such clones from mutant libraries, unless otherwise noted.

bx and *abx* Mutations

Calvin Bridges found the first spontaneous *bithorax* mutant (bx^1) for which the complex is named. Mutations of *bx* transform the anterior third thoracic segment into anterior second thoracic, so that anterior haltere becomes anterior wing tissue, anterior third leg resembles second leg, and anterior notal tissue appears on the dorsal surface of the third thoracic segment. Various alleles of *bx* differ markedly in the strength of the transformation, and also in which regions of the third thoracic segment are most strongly transformed. bx^1 is the weakest of the alleles considered here; its expression is variable and sometimes overlaps wild type (*10*). It is associated with an insertion of the mobile repetitive element named "412" (*11*) at the map position −60 kb (Fig. 3). Homology to 412 was suggested by the restriction map of the insert, and it was confirmed by comparison to a prototypic copy of 412 (*12*).

The mutation bx^{34e} is another spontaneous allele, intermediate in its pheno-

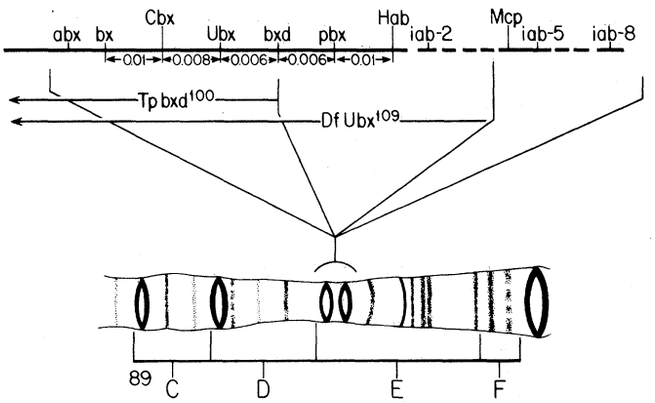


Fig. 1. Genetic map of the bithorax complex. The alleles *abx* (*anterobithorax*), *bx* (*bithorax*), *Cbx* (*Contrabithorax*), *Ubx* (*Ultrabithorax*), *bxd* (*bithoraxoid*), and *pbx* (*postbithorax*) are mutants in the left half of the complex covered by the DNA map. The mutants *Hab* (*Hyperabdominal*), *iab-2* (*infraabdominal-2*), *Mcp* (*Miscadestral pigmentation*), *iab-5*, and *iab-8* define the abdominal half of the complex. *Mcp* and *iab-5* were isolated and mapped by Crosby (*35*). The dominant alleles are raised above the recessive ones. Recombination distances between some pairs of mutations are shown. The diagram also shows the approximate position of a *bxd* transposition breakpoint and the extent of a deficiency, Ubx^{109}, that removes the left half of the complex. The entire cluster maps on the third chromosome to the pair of doublet bands at the 89E constriction, as shown.

type; it usually produces a thin band of notal tissue in the third segment and halteres that are enlarged and bent downward (*13*). It is associated with the insertion of another mobile element at −63.5 kb (Fig. 3). This element is 7.3 kb in length and has direct terminal repeating sequences of about 0.5 kb; we have named the element "gypsy" (*14*). bx^{34e} reverted spontaneously to a less extreme phenotype. The partial revertant (*15*) was examined by Southern blot analysis and the restriction pattern of the gypsy insert had changed. The partial revertant has not yet been cloned and examined directly, but a change in the gypsy insertion in this revertant confirms our presumption that the gypsy element is responsible for the bx^{34e} mutation.

The mutation bx^3 is the strongest of the spontaneous *bx* alleles; homozygotes always show strong haltere to wing transformation and a wide band of notal tissue in the third thoracic segment (*16*). bx^3 also has a subtle dominant phenotype of slight shrinkage of the presutural region of the notum in the second thoracic segment (*17*). There are two mobile repetitive elements inserted in the bx^3 chromosome, a gypsy at −57 kb, identical in restriction map and orientation to the bx^{34e} gypsy, and a 4.3-kb element named "Doc" (*4*) at −53 kb (Fig. 3). We know the Doc element is irrelevant to the phenotype for two reasons. First, bx^3 was recombined onto a chromosome carrying the Cbx^1 mutant (see below); the Doc element was lost in the exchange. The bx^3 mutation was subsequently reisolated by recombination away from *Cbx*, and this laundered copy (*18*) of bx^3, with only the gypsy insertion, appears identical in phenotype to the original. Second, bx^3 reverted spontaneously with loss of the gypsy (*19*). DNA cloned from the revertant still contained the Doc element at −53 kb plus one 0.5-kb terminal repeat of the gypsy element at −57 kb (Fig. 3), which is consistent with excision of the gypsy by recombination between its terminal repeats. The reversion in this chromosome appears to be complete; no *bx* phenotype is present even when the revertant chromosome is heterozygous with a deficiency for the whole complex.

Other spontaneous *bx* alleles (bx^9, bx^{AV}, and bx^G) are associated with apparent insertions in the −75 to −55 kb region, but the mutant lesions have not yet been examined in cloned DNA.

The mutation abx^1 (*anterobithorax*) was initially designated bx^7, but was renamed when it could be distinguished from other *bx* alleles by a number of criteria (*17, 20*). abx^1 gives anterior leg and haltere transformations like bx^3, but it produces the more anterior presutural notal tissue in the third thoracic segment. abx^1 was x-ray–induced, and is associated with an insertional transposition of 6 kb of bithorax complex DNA (−79 to −73 kb) into the 97D region near the right end of the third chromosome, as determined by in situ hybridization (Fig. 3). The deletion in the bithorax complex at 89E and the insertion in 97D have been separated by recombination, and each has been made homozygous. The deletion alone gives a phenotype apparently identical to the initial abx^1 mutant; the insertion alone gives the wild type.

There is another x-ray–induced mutation (*21*), called bx^{SK}, with a phenotype like that of abx^1; we suggest that it be renamed abx^2. This mutation is associated with a 1.5-kb deletion (−79.5 to −78 kb) that overlaps the abx^1 deletion (Fig. 3). The abx^2 deletion was mapped by Southern blot analysis in which the mutant chromosome was compared with the marked chromosome on which it was induced.

pbx and *Cbx* Mutations

The mutations Cbx^1 (*Contrabithorax*) and pbx^1 (*postbithorax*) arose together after x-ray exposure and were subsequently separated by recombination (*22*). The recessive *pbx* mutation transforms posterior haltere to wing, posterior third leg to second leg, and gives dorsal postnotal tissue in the third thoracic segment (*22*). Double mutant flies *bx pbx* have both anterior and posterior transformations of the third thoracic segment, and so they develop four wings (*16*). The dominant Cbx^1 mutation transforms posterior wing into posterior haltere, and the posterior notal tissue is reduced. Thus the *pbx* and *Cbx* transformations are complementary, although not completely so (*17*). pbx^1 is associated with a deletion of 17 kb from −3 kb to +14 kb (see Fig. 3). Cbx^1 has an insertion of that same 17-kb segment, with its orientation inverted, into the map at position −44 kb (Fig. 3). The limits of the *Cbx* insertion are identical to the end points of the *pbx* deletion, to within about 0.5 kb, as judged by restriction mapping and heteroduplex analysis of the clones derived from the *Cbx* and *pbx* mutants.

The molecular events of the *Cbx* and *pbx* mutations suggest a simple model for the observed phenotypes. The −3 to +14 kb region encodes the information to specify posterior third thoracic segment. The loss of that information in *pbx* homozygotes causes the developmental path for the posterior third thoracic segment to mimic the second thoracic ground state (*2*). When this sequence is inserted at −44 kb, it is expressed in the second thoracic segment, so that third thoracic structures are produced. This interpretation is reinforced by the phenotype of the double mutant. *Cbx pbx/pbx* flies have normal halteres and lack any postnotal tissue in the third thoracic segment, as if the insertion rescues the deletion. The *pbx* transformation in the third leg is not altered by *Cbx*, however. An alternative model (*2*) postulates that the *Cbx* insertion causes inappropriate expression of the bx^+ or Ubx^+ products, which causes the dominant *Cbx* transfor-

mation, and substitutes for the loss of pbx^+ in the double-mutant animal.

The only other *pbx* allele, pbx^2, was also x-ray–induced and is associated with a large deletion from about −14 to +1 kb. The phenotype is similar to pbx^1, except in transvection (*17*). This deletion has not yet been cloned, but analysis of genomic DNA by the Southern technique is unambiguous, since restriction fragments from within the deletion show no homology to the DNA of pbx^2 flies.

There are several other dominant mutations analogous to Cbx^1, including Cbx^2, *Haltere-mimic* (*Hm*), Cbx^3, and Cbx^{Twt}, although each of these is phenotypically quite distinct (*17*). Cbx^3 and Cbx^{Twt} are discussed below.

bxd Mutations

The *bxd* (*bithoraxoid*) mutations transform the first abdominal segment to third thoracic; the first abdominal tergite is reduced or absent, and strong alleles, when hemizygous, generate an extra leg or pair of legs from the first abdominal segment and, rarely, an extra haltere. In addition, the posterior third thoracic segment is partly transformed to posterior second thoracic, which has been described as *cis*-inactivation of the adjacent *pbx* region (*16*). Like *bx* alleles, *bxd* alleles vary in the severity of the segmental transformation and somewhat in the spectrum of transformed structures observed.

The mutation bxd^1 occurred as spontaneously and is associated with an insertion of the gypsy mobile element at −21 kb (Fig. 3). The gypsy is identical in restriction map to the bx^3 and bx^{34e} gypsies but is in the opposite orientation. bxd^1 has spontaneously reverted to wild type twice. In both instances (*23*), Southern blots of the whole genome show that 0.5 kb of extra DNA remains at about −21 kb, which presumably represents a copy of the gypsy terminal repeat.

Another spontaneous mutation, bxd^{55i}, has an insertion of the gypsy mobile element at −23 kb, identical in restriction map and orientation to the bxd^1 gypsy (Fig. 3). The mutation bxd^{51j}, also spontaneous, is associated with an

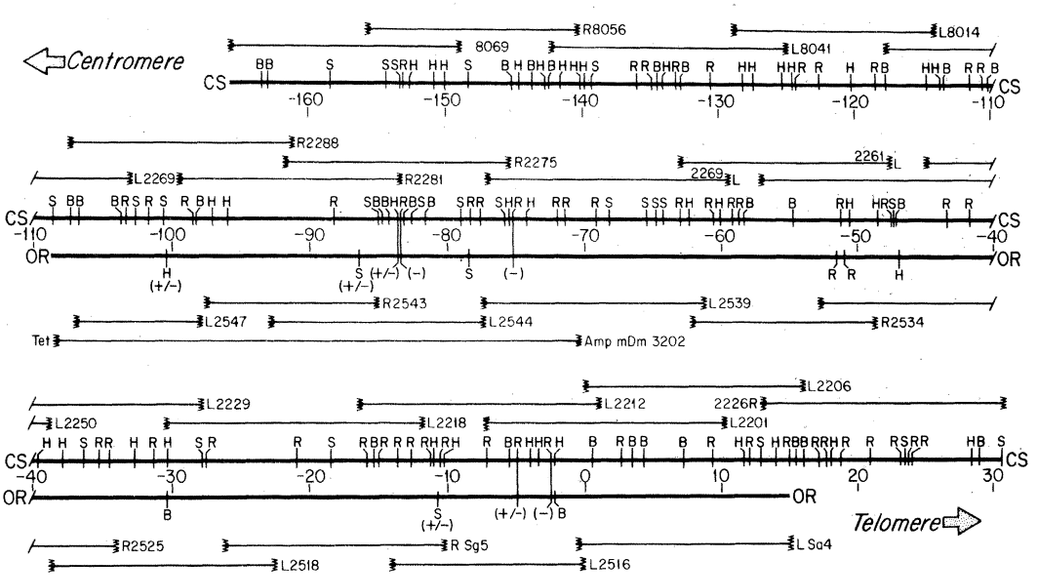


Fig. 2. Composite restriction map of the DNA walk. The boldface lines marked *CS* show the map from the Canton S wild type for the four restriction enzymes Bam H1, Hind III, Eco RI, and Sal I (*B*, *H*, *R*, and *S*, respectively). The coordinates show the distance in kilobases from the starting point of the walk. The thinner lines above the *CS* map indicate the cloned DNA segments from the Canton S library of recombinant phage. The *L* and *R* designations indicate the orientation of the left and right arms of the lambda phage vector. The boldface lines marked *OR* show the map for the Oregon R wild type. The restriction sites are identical to *CS* except where indicated. The +/− symbol indicates that the site is present in some Oregon R chromosomes but not in others. Representative cloned segments from the Oregon library of recombinant phage are shown below the *OR* line, as well as one Oregon R segment cloned into a cosmid vector (mDm 3202).

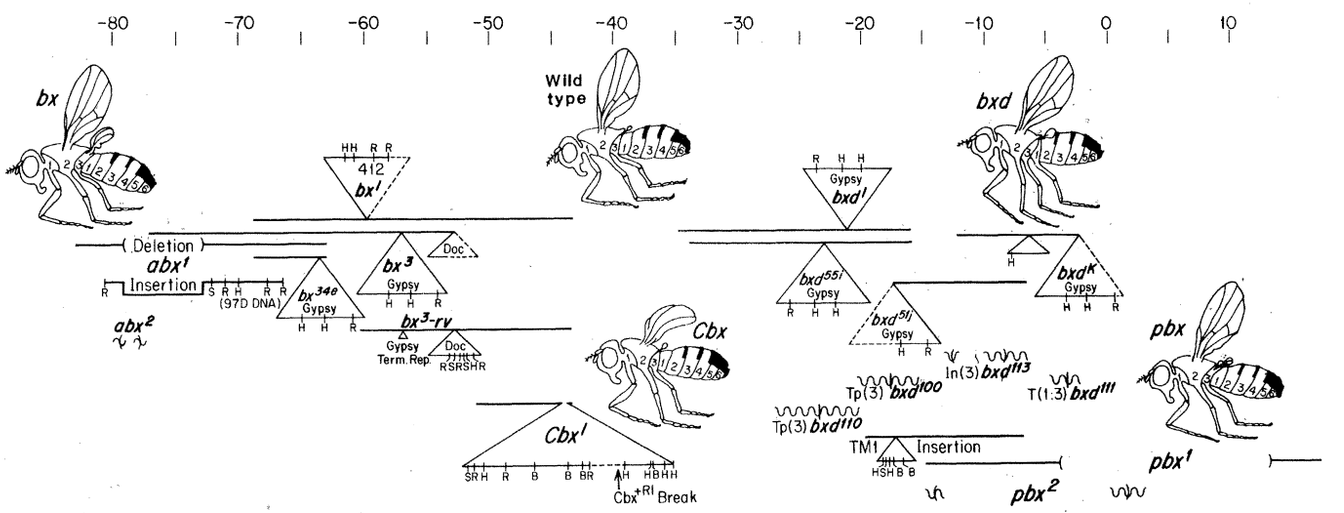
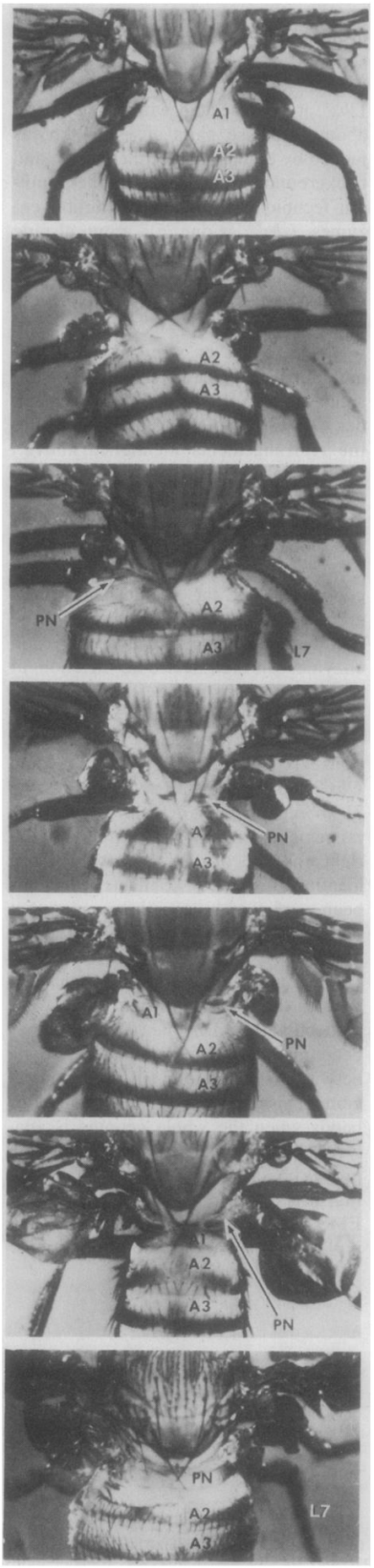


Fig. 3. Lesions of the *abx*, *bx*, *Cbx*, *bxd*, and *pbx* mutations. The maps of recombinant clones derived from mutant libraries are shown on the coordinate scale of Fig. 2. The thin horizontal lines indicate DNA sequences cloned from the various mutants that are identical to the wild-type map. Triangles touching these lines show DNA insertions; parentheses show deletions. Dotted lines indicate sequences not recovered in recombinant clones but inferred from genomic Southern blots. Horizontal wavy lines indicate the limits of uncertainty in the positions of breakpoints identified by Southern blots. The drawings of male flies indicate the phenotypes of *bx*, *Cbx*, *bxd*, and *pbx* mutants. Three thoracic and six abdominal segments are numbered.

insertion of the gypsy element at −17.5 kb, opposite in orientation to that of bxd^1 (Fig. 3). bxd^K is also spontaneous and has two insertions, an unidentified 3.4-kb repetitive element at −6.5 kb and a gypsy element at −2.5 kb (Fig. 3). The gypsy is identical in its restriction map to that of bxd^1 but opposite in orientation. We presume that the element at −6.5 kb is silent because bxd^K is completely suppressible by *su(Hw)* (see below). Other spontaneous *bxd* alleles (bxd^9 and bxd^{SR}) are also associated with apparent insertions in the −20 to −5 kb region, but these mutant lesions have not yet been cloned. The balancer chromosome TM1 has an insertion of about 3 kb of unidentified repetitive DNA at about the same position as the bxd^{51j} insertion (Fig. 3), but the TM1 insertion has no detectable *bxd* phenotype.

Another large class of strong *bxd* alleles are x-ray–induced and are associated with cytological rearrangements that split the bithorax complex. Four such breakpoints have been mapped by in situ hybridizations and Southern blots of genomic DNA; their positions are shown in Fig. 3. bxd^{100} is a transposition of the left half of the complex into 66C, bxd^{110} is a transposition of the 91D–92A region into the bithorax complex, bxd^{111} is a translocation of the right half of the complex into 4D, and bxd^{113} is an inversion to 69 (apparently associated with a small deletion of bithorax material at the breakpoint). bxd^{111}, which maps farthest to the right, can be distinguished from the other rearrangement alleles by its larval phenotype (*20*).

The phenotypes of the different *bxd* alleles can be correlated somewhat with their position on the DNA map. Figure 4 shows the more extreme phenotypes of several *bxd* alleles, all heterozygous with a deficiency. Farthest to the left is bxd^{55i}, which causes complete loss of the first abdominal tergite (rarely with a seventh leg) but very little transformation of the posterior third thoracic segment. bxd^1 also always removes the first abdominal tergite, and there is slightly more enlargement of the haltere, with an occasional thin band of postnotal tissue in the dorsal third thoracic segment. About 20 percent of flies with this allele have seven or eight legs. bxd^{51j} again removes the first abdominal tergite, the posterior haltere is more swollen than in bxd^1, and there is consistently a band of dorsal postnotal tissue. About 40 percent of these flies have extra legs. bxd^K shows a variable reduction of the first abdominal tergite, rarely complete. Flies with this mutation never have extra legs, but the posterior haltere is variably enlarged, sometimes like a *pbx* haltere, with occasional patches of postnotum in the third thoracic segment. pbx^1 and pbx^2 give consistent transformation of posterior haltere to wing with extra dorsal postnotal tissue, and both give variable slight reduction of the first abdominal tergite. Thus, the insertions of the gypsy elements, going from left to right, show a graded effect on the first abdominal segment (strongest on the left) and on the posterior third thoracic segment (strongest on the right). A *bxd* breakpoint consistently gives strong expression of all of the above transformations (the flies usually die before eclosion), as if causing complete inactivation of the whole region.



Suppressor of Hairy-Wing and Gypsies

Many of the spontaneous *bx* and *bxd* alleles are suppressed by the recessive second-site suppressor, *suppressor of Hairy-wing* [*su(Hw)*]. This suppressor affects particular spontaneous mutations at several other loci, such as *scute*, *cut*, *forked*, and *lozenge*. Of the bithorax complex mutations we have recloned, bx^3, bx^{34e}, bxd^1, bxd^{51j}, bxd^{55i}, and bxd^K are suppressible. These are all of the mutations that have insertions of the gypsy element, suggesting that a gene product of *su(Hw)* might interact specifically with this element. Thirteen other suppressible alleles at other loci have since been checked for gypsies, and all except two alleles of *rudimentary* have the gypsy element at the site of the mutation (*14*). Several other spontaneous mutations in the bithorax complex are also suppressed, including bx^9, bx^G, and bxd^9; we expect the cloning of their DNA will reveal gypsy insertions.

The gypsy element is apparently not excised in suppressed animals. DNA was extracted from suppressed adult flies homozygous for bxd^1 or hemizygous for bx^3, and was examined by

Fig. 4. Photographs of *bxd* and *pbx* mutants. All pictures show the backs of female flies with the wings extended to reveal the dorsal abdomen. All flies have one chromosome with deficiency Ubx^{109} (Fig. 1); the second chromosome is (from top to bottom): Canton S wild type, bxd^{55i}, bxd^1, bxd^{51j}, bxd^K, pbx^1, and Tp bxd^{100}. Some parts of the cuticle are designated as follows: *A1*, *A2*, and *A3*, the first, second, and third abdominal tergites; *PN*, new postnotal tissue appearing in the third thoracic or first abdominal segments; *L7*, a seventh leg from the first abdominal segment. The bottom fly (Tp bxd^{100}) had to be dissected out of the pupal case; it shows a band of cuticle between *A2* and *PN* which lacks hairs and pigment. This cuticle is presumed to be scar tissue which fills the space normally taken by the first abdominal tergite.

Southern blot analysis. The band from the gypsy insertion remained unchanged in both cases. We have little other information on the mechanism of suppression since most of the suppressed alleles are in "complex loci" (*24*) for which the gene products have not been identified.

Ubx Mutations

Ubx (*Ultrabithorax*) mutations fail to complement with *bx*, *bxd*, and *pbx* alleles, and the *Ubx* recessive phenotype is equivalent to the sum of these three phenotypes (*3*). Animals homozygous for *Ubx* die as larvae or early pupae, but it is clear from larval cuticular structures that the third thoracic and first abdominal segments are both transformed to copies of the second thoracic segment (*2*).

Most of the available *Ubx* alleles are associated with cytological rearrangements with a break in the bithorax complex. These breakpoint alleles are nearly equivalent in phenotype to deletions for the left half of the complex; all give very strong transformations when heterozygous with *bx*, *bxd*, or *pbx*. The breakpoints of 12 such *Ubx* mutations are shown in Fig. 5; Ubx^{780} and Ubx^{882} were induced by ethyl methane sulfonate (EMS) and the rest by x-rays. All were induced on defined background chromosomes, and so most of these breakpoints were identified by comparing Southern blot patterns of their genomic DNA with those of the background chromosomes. The leftmost breakpoint (actually the end point of a cytological deficiency for 89D-E) is at about −105 kb; the rightmost breakpoint is at −32 kb. Thus the Ubx^+ function apparently requires continuity of the chromosome for a region of at least 73 kb.

Ubx^1, a spontaneous mutant, is a medium-strong allele; it gives less extreme transformations when heterozygous with *bx*, *bxd*, or *pbx* than does a deficiency for the bithorax complex. It is associated with an insertion of the Doc mobile repetitive element at −32 kb (Fig. 5). This Doc element is identical in its map but opposite in orientation to the silent Doc insertion at −53 kb in the bx^3 chromosome.

Ubx^{849} is also similar in phenotype to Ubx^1; it is associated with a deletion of about 110 base pairs at −32.4 kb, as judged by comparison of the mutant and background chromosomes by the Southern technique with four restriction enzymes. Ubx^{849} is one of a group of nine *Ubx* mutations induced by EMS (*25*); the Ubx^{780} translocation was also from this group, but no lesions were found for the others.

Among a set of x-ray–induced *Ubx* mutations, two had phenotypes very similar to that of Ubx^1 (*26*). (The remainder were stronger alleles associated with the rearrangements mentioned above.) One of these, $Ubx^{6.28}$, is associated with a deletion of about 50 base pairs at −31.5 kb, very close to the site of insertion of the Doc element in Ubx^1. The other, $Ubx^{9.22}$, has not yet been cloned, but a comparison of the mutant and background chromosomes analyzed by the Southern technique with five restriction enzymes showed a 1.6-kb deletion at −105 kb and no other detectable change in the left half of the bithorax complex.

These four cytologically normal mutations (Ubx^1, Ubx^{849}, $Ubx^{6.28}$, and $Ubx^{9.22}$) therefore map at the ends of the 73-kb region defined by the *Ubx* rearrangement breakpoints—three at the right end and one at the left end. The location of $Ubx^{9.22}$ at the left end has been confirmed by genetic mapping that places it 0.02 unit to the left of the bx^{34e} mutation.

There are several *Ubx* alleles that are weaker than Ubx^1. Most of them were induced with EMS. One (Ubx^{51}) (*27*) has been mapped by recombination to be between *Cbx* and *bxd*, in the Ubx^1 region. This allele shows no anomalous bands when the whole genome is analyzed by the Southern technique with probes covering this region. We presume that it is a true point mutant, and other EMS-induced alleles for which we found no lesions may also be point mutations.

The Left End

The mutations Cbx^3 (also called *Cbx-like*) (*17*) and Cbx^{Twt} (*Twin-thorax*) (*28*) are associated with lesions in the leftmost region of the complex. Both are x-ray–induced inversions to 89A and 87EF, respectively. Both have a dominant phenotype with a variable reduction of anterior wing and notum. Neither breakpoint has a *Ubx* phenotype and both are viable over deficiencies for the complex. Both breakpoints fall in the region of the leftmost *Ubx* lesions, between −110 and −103 kb (Fig. 5), as judged by hybridization in situ and Southern blot analysis. These breakpoints will have to be cloned and examined in detail, but the phenotypes suggest that the inversions remove from the left end of the complex a negative regulatory region which keeps the abx^+ or Ubx^+ functions repressed in the second thoracic segment.

We continued to walk farther to the left, looking for rearrangement breakpoints clearly outside the complex. One of the TE transpositions isolated by Ising (*29*), TE77, inserted the *white* to *roughest* region of the X chromosome into 89D. This translocation produces no segmental transformation and, since the insertion is so large, it is assumed to lie outside (proximal to) the bithorax complex. The site of insertion has recently been recloned by Paro *et al.* (*30*). The 35-kb region around the TE77 insertion site that they isolated is identical to that shown on our map (Fig. 2) between −154 and −119 kb. The TE77 insertion site is at −136 kb.

Concluding Remarks

We are confident that the DNA changes identified in this article are responsible for the mutant phenotypes for several reasons. The mutations abx^1, bx^3, Cbx^1, Ubx^1, bxd^1, and pbx^1 have each been recombined with the adjacent mutations in the series, and the lesions we describe cosegregated with the mutant phenotypes. The changes in the gyp-

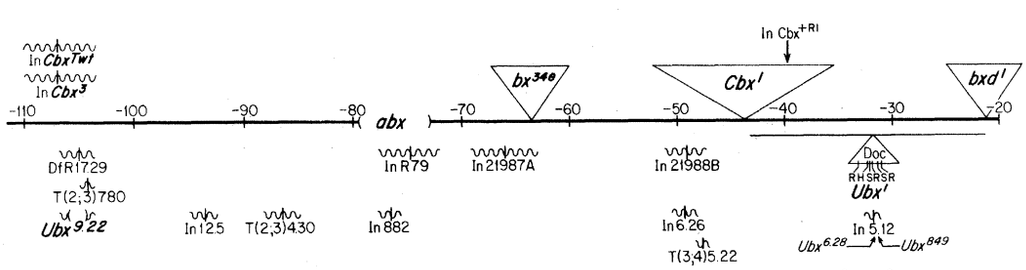


Fig. 5. Lesions of *Ubx* mutations. The boldface line represents the DNA map of Fig. 2, with some of the mutant lesions of Fig. 3 included for orientation. Df R17.29, In R79, In 21987A, and In 21988B are a *Ubx* deficiency and three inversions induced on a Cbx^1 chromosome by E. B. Lewis and T. Ramey. The Cbx^{+R1} inversion was induced by T. Kaufman, also on a *Cbx* chromosome. The 780 translocation was induced by Lewis on a $su(Hw)^2$ sbd^2 chromosome. $Ubx^{9.22}$, In 12.5, T 4.30, In 882, In 6.26, T 5.22, and In 5.12 are inversions, translocations, and a deficiency induced on a marked third chromosome by S. Kerridge and G. Morata.

sy elements in the revertants of bx^3, bx^{34e}, and bxd^1 confirm that the gypsies are responsible for these mutations, and we have found a general correspondence between mutations suppressible by *su(Hw)* and insertions of the gypsy element (*14*). The DNA map locations of the other lesions correlate well with their phenotypes and their positions on the genetic map (*31*).

We were surprised that all of the cytologically normal spontaneous mutations (bx^1, bx^3, bx^{34e}, Ubx^1, bxd^1, bxd^{51j}, bxd^{55i}, and bxd^K) are associated with insertions of mobile elements. For comparison, only 3 out of 5 spontaneous alleles in the *rosy* locus (*32*) and 8 out of 13 spontaneous alleles in the *white* locus (*33*) are associated with mobile element insertions. Likewise, the cytologically normal x-ray–induced alleles have large DNA deletions ($Ubx^{9.22}$, abx^1, abx^2, $Ubx^{6.28}$, pbx^1, and pbx^2) or insertions (Cbx^1), whereas the large majority of x-ray–induced *rosy* mutations are apparent point mutations (*32*). It was also unexpected that these mutant lesions would be so spread out, the *bx* alleles over 7 kb and the *bxd* alleles over 20 kb. This spread of the mutant lesions could reflect mutations that inactivate coding regions distant from the site of the mutant lesion. This hypothesis is most obvious for the gypsy insertions because the gypsy elements remain in place in suppressed flies. Likewise, the revertants of bx^3 and bxd^1 leave insertions in the DNA at the site of the original gypsy element; since the flies are completely reverted, it is unlikely that these insertions interrupt essential coding sequence. Insertions of most other mobile elements into the *bx* and *bxd* regions may also be silent, as in the TM1 insertion and the Doc element in bx^3; this might account for the predominance of gypsy insertions among the available mutants. The failure to find true point alleles of *bx*, *bxd*, and *pbx* suggests that single-base changes in these regions may be invisible. Perhaps these regions do not encode functions, or perhaps the functions are not inactivated by most single-base changes, as might be true if the gene products are folded RNA molecules. Alternatively, there may be many subtle functions encoded so that only lesions that inactivate many functions can give a noticeable phenotype. Unfortunately, this action at a distance makes it difficult to locate the regions responsible for normal bithorax functions, or to guess how many distinct functions there are.

The *Ubx* lesions have a peculiar arrangement. The $Ubx^{9.22}$ deletion lies 73 kb to the left of the other cytologically normal *Ubx* alleles (Ubx^1, Ubx^{849}, and $Ubx^{6.28}$), on the other side of the region of *abx* and *bx* lesions. The distribution of the *Ubx* rearrangement breakpoints indicates a requirement for chromosome continuity over the same 73 kb. For the *bxd* function, there is a similar requirement for continuity over some 20 kb. The need for such continuity may reflect RNA transcripts spanning these regions, or tissue-specific rearrangements of the DNA. Several complementary DNA clones generated from embryonic RNA have recently been isolated by homology to genomic bithorax clones (*34*). Preliminary mapping of these complementary DNA's indicates that exons are spread out over the 73-kb *Ubx* region in some clones and over the 20-kb *bxd* region in others. *Ubx* mutations, including the alleles with small deletions at −105 or −32 kb, inactivate in *cis* the function of *abx*, *bx*, *bxd*, and *pbx*. Long transcripts might be processed differently to give the RNA products for each of these functions, with sequences from the −105 and −32 kb regions included in all the different products.

References and Notes

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6. The orientation dictated by the fusion fragment was initially misleading. The Cbx^{+R1} inversion was induced on a Cbx^1 chromosome, and the inversion breakpoint fell within the Cbx^1 insertion (see section on *pbx* and *Cbx*). Until the inverted orientation of the *Cbx* insertion was discovered, the implied orientation of the DNA map was reversed.
7. In the DNA region recovered from both strains, 88 restriction sites have been identified in Canton S, of which five are missing in the Oregon R clones. Thus five out of 528 base pairs are changed, which implies about 1 percent sequence divergence. The reciprocal comparison is complicated by variation in the restriction maps among clones isolated from Oregon R. The same method of comparison of 167 restriction sites in the 87DE region showed about 0.4 percent divergence [see (*4*)].
8. Such rearrangements were usually made cytologically homozygous so that the two breakpoints of the rearrangement were well separated in squashed preparations. When the rearrangement had a recessive lethal *Ubx* phenotype, the lethality was covered by a duplication for the bithorax complex in the X or second chromosome.
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36. We thank R. Saint for providing subclones of many bithorax restriction fragments, R. Paro for providing clones from the TE77 insertion site, K. Wepsic for care of mutant stocks, and W. Harpain for his generous gift of half-pint milk bottles. This work was supported by NIH grants to W.B., D.S.H., and E.B.L. and by an NSF grant to D.S.H. with additional funding from a Helen Hay Whitney Foundation fellowship (W.B.)., a Damon Runyon fellowship (M.A.), Swiss National Foundation fellowships (F.K. and P.S.), and a National Science Foundation predoctoral fellowship (M.P.).

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