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 recombinated 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, bx, lab-2, lab-5, and lab-6 affect the first, second, third, fifth, and eighth abdominal segments.

where \( N = \) sample size per group, \( P_1 = \) placebo mortality rate, \( P_2 = \) treated mortality rate, and \( \Phi = \) cumulative Gaussian distribution function.

With a two-tailed test, the power estimate would be 0.52.

28. E. Crouch and R. Wilson, Toxicol. Environ. Health 8, 1095 (1981). 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 \( (300 \times 10^3)/(21)(0.64) \), or 52.2 \( \times 10^3 \) per cancer death averted.

30. Based on an average retail price of $20 per hundred at discount pharmacies in the Boston area.

31. \( \beta \)-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)).


34. J. D. Graham and J. W. Vaupe1, Risk Analysis 1, 211 (1981).


36. Supported by grants from the Alfred P. Sloan Foundation and the Mobil Foundation. I thank D. M. Eddy for discussions of the data and T. M. Bailer III, P. Braun, J. Cairns, M. Thompson, and two anonymous reviewers for suggestions.

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RESEARCH ARTICLE
Collecting Bithorax DNA

Our initial toehold in bithorax DNA was gained by “jumping” across an inversion. The inversion, called Cbx"88", 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 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") 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 natal 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. bx1 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. 1). Homologous 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 bx44 is another spontaneous allele, intermediate in its pheno-
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^3 reverses 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 insert in this revertant confirms our presumption that the gypsy element is responsible for the bx^3e 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^3e 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^2, bx^4, and bx^6) 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 and haltere transformations like bx^3, but it produces the more anterior presutural notal tissue in the third thoracic segment. abx^1 is 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 mutation; the insertion alone gives the wild type.

There is another x-ray-induced mutation (21), called bx^2^T, 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^2 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.

**phbx and Cbx Mutations**

The mutations Cbx^1 (Contrabithorax) and phbx^1 (postbithorax) arose together after x-ray exposure and were subsequently separated by recombination (22). The recessive phbx 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^3 phbx 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 phbx 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^1 insertion are identical to the end points of the phbx deletion, to within about 0.5 kb, as judged by restriction mapping and heteroduplex analysis of the clones derived from the Cbx and phbx mutants.

The molecular events of the Cbx and phbx 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 phbx 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^1pbx flies have normal halteres and lack any postnotal tissue in the third thoracic segment, as if the insertion rescues the deletion. The phbx 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^3 or Ubx^3 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, was also x-ray-induced and is associated with a large deletion from about -14 to +1 kb. The phenotype is similar to pbx, except in transvection (T7). 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 flies.

There are several other dominant mutations analogous to Cbx, including Cbx, Haltere-mimic (Hm), Cbx, and Cbx, although each of these is phenotypically quite distinct (T7). Cbx and Cbx 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 partially transformed to posterior second thoracic, which has been described as cis-inactivation of the adjacent bxd region (T6). 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 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 bxa and bxt gypsies but is in the opposite orientation. bxd has spontaneously reverted to wild type twice. In both instances (T3), 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, has an insertion of the gypsy mobile element at -23 kb, identical in restriction map and orientation to the bxd gypsy (Fig. 3). The mutation bxd, also spontaneous, is associated with an
insertion of the gypsy element at −17.5 kb, opposite in orientation to that of bxd' (Fig. 3). bxd^d 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' but opposite in orientation. We presume that the element at −6.5 kb is silent because bxd^d is completely suppressible by su(Hw) (see below). Other spontaneous bxd alleles (bxd^a and bxd^b) 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^d 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^59, 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^d 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^50 again removes the first abdominal tergite, the posterior haltere is more swollen than in bxd^d, 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 halteres 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^d, bx^c, bx^d^A2, bx^d^V, bx^d^H, bx^d^K, and bx^d^F 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 become 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^c, bx^d^A2, and bx^d^F; 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 bx^d or hemizygous for bx^d^A2, and was examined by...

Fig. 4. Photographs of bx 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^100 (Fig. 1); the second chromosome is (from top to bottom) Canton S wild type, bxd^F, bxd^V, bxd^K, bxd^H, 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. Ubx70 and Ubx82 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+, 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 bx2 chromosome.

Ubx840 is also similar in phenotype to Ubx2; 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. Ubx840 is one of a group of nine Ubx mutations induced by EMS (25); the Ubx800 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 Ubx1 (26). (The remainder were stronger alleles associated with the rearrangements mentioned above.) One of these, Ubx628, 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 Ubx1. The other, Ubx822, 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 (Ubx1, Ubx840, Ubx628, and Ubx822) 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 Ubx822 at the left end has been confirmed by genetic mapping that places it 0.02 unit to the left of the bx34r mutation.

There are several Ubx alleles that are weaker than Ubx1. Most of them were induced with EMS. One (Ubx21) (27) has been mapped by recombination to be between Cbx and bxd in the Ubx1 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 Cbx3 (also called Cbx-like) (17) and Cbx2/T (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 abx3, bx3, Cbx1, Ubx1, bxd1, and pbx1 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-

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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 21986B are a Ubx deficiency and three inversions induced on a Cbx1 chromosome by E. B. Lewis and T. Ramey. The Cbx1 is +R1 inversion was induced by T. Kaufman, also on a Cbx chromosome. The 780 translocation was induced by Lewis on a su(Hw) sbf chromosome. Ubx840, In 12.5, T 4.30, In 862, 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\(^{24R}\), and bx\(^d\) confirm that the gyp
sies 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 cyto-
logically normal spontaneous mutations
(bx\(^1\), bx\(^2\), bx\(^{34}\). Ubx\(^1\), bx\(^d\), bx\(^d\)\(^{40}\)
and bx\(^d\)) are associated with insertions of mobile elements. For com-
parison, 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 cytotypically
normal x-ray-induced alleles have large
DNA deletions (Ubx\(^{43}\), abd\(^1\), abd\(^2\).
Ubx\(^{28}\)\(^{3}\), 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 unex-
ected that these mutant lesions would
be so spread out, the bx alleles over 7 kb
and the bx\(^d\) 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 ele-
ments remain in place in suppressed flies.
Likewise, the revertants of bx\(^1\) and
bx\(^d\) 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
element codon sequence. Insertions of
most other mobile elements into the bx
and bx\(^d\) regions may also be silent, as
in the TM 1 insertion and the Doc element
in bx\(^3\); this might account for the pre-
domiance of gypsy insertions among
the available mutants. The failure to find
true point alleles of bx, bx\(^d\), 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
ture if the gene products are folded RNA
molecules. Alternatively, there may be
many subtle functions encoded so that
only lesions that inactivate many func-
tions can give a noticeable phenotype.
Unfortunately, this action at a distance

makes it difficult to locate the regions
responsible for normal bithorax func-
tions, or to guess how many distinct
functions there are.

The Ubx lesions have a peculiar ar-
range. The Ubx\(^{9-23}\) deletion lies 73
kb to the left of the other cytoplologically
normal Ubx alleles (Ubx\(^3\), Ubx\(^{28}\), and
Ubx\(^{28-29}\)), on the other side of the region
of abx and bx lesions. The distribution of
the Ubx rearrangement breakpoints indi-
cates a requirement for chromosome
continuity over the same 73 kb. For the
bxd function, there is a similar require-
ment 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 chromosomes (34).

Preliminary mapping of these complementar-
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 al-
les with small deletions at −105 or −32
kb, inactivate in cis the function of abx,
bx, bxd, and pbx. Long transcripts may 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

3. The dominant phenotype of Ubx mutations, a
 slight enlargement of the halteres, is entirely due to
 haploinsufficiency.
 Biol., in press. This reference describes the
recombinant libraries and procedures used for
chromosome walking.
5. P. Spierer, A. Bender, W. Bender, D. S. Hog-
ness, ibid., in press.
6. The orientation dictated by the fusion fragment
was initially misleading. The Cbx\(^{−24}\) inversion
was induced on a Chro chromosome and the
inversion breakpoint fell within the Cbx\(^+\) inser-
tion (see section on pbx and Chx). Until the
inverted orientation of the Chx 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 Can-
ton 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 se-
quence 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 B7DE region showed about 0.4
percent divergence [see (4)].
8. Such rearrangements were usually made cyto-
logically homoeogous so that the two break-
points of the rearrangement were well separated
in squashed preparations. When the rearrange-
ment had a recessive lethal Ubx phenotype, the
lethality was covered from the bithorax complex in the X or second chrono-
some.
9. DNA from mutant adult flies was partially di-
gested with Eco RI and ligated into purified
arms of the lambda vector Sep 8 (R. W. Davis,
D. Botstein, J. R. Roth, Advanced Bacterial
Genetics (Cold Spring Harbor Laboratory, Cold
Spring Harbor, N. Y., 1980). Plasmids were pack-
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259 (1979)) and the resulting plaques screened
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that in the white locus (33). The reduced fre-
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