Effect of abx, bx and pbx Mutations on Expression of Homeotic Genes in Drosophila Larvae

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ABSTRACT

We have examined the patterns of expression of the homeotic gene Ubx in imaginal discs of Drosophila larvae carrying mutations in the abx, bx and pbx regulatory domains. In haltere discs, all five bx insertion mutations examined led to a general reduction in Ubx expression in the anterior compartment; for a given allele, the strength of the adult cuticle phenotype correlated with the degree of Ubx reduction. Deletions mapping near or overlapping the sites of bx insertions, including three abx alleles and the bxΔg20(bx-prv) allele, showed greatly reduced Ubx expression in parts of the anterior compartment of the haltere disc; however, anterior patches of strong Ubx expression often remained, in highly variable patterns. As expected, the px allele led to reduced Ubx expression in the posterior compartment of the haltere disc; surprisingly, px also led to altered expression of the en protein near the compartment border in the central region of the disc. In the metamorphic leg, all the bx alleles caused extreme reduction in Ubx expression in the anterior regions, with no allele-specific differences. In contrast, abx and bx-prv alleles resulted in patchy anterior reductions in third leg discs. In the larval central nervous system, abx but not bx alleles affected Ubx expression; the bx-prv deletion gave a wild-type phenotype, but it could not fully complement abx mutations. In the posterior wing disc, the bx-prv allele, and to a much lesser extent the bxΔg20 chromosome from which it arose, led to ectopic expression of Ubx. Unlike other grain-of-function mutations in the BX-C, this phenotype appeared to be partially recessive to wild type. Finally, we asked whether the pbx transformation, which results from early lack of Ubx expression in the mesothorax and is seen in abx animals, is due to ectopic Scr expression. Some mesothoracic leg and wing discs from abx† larvae displayed ectopic expression of Scr, which was variable in extent but always confined to the posterior compartment.

EARLY in development, the Drosophila embryo is divided into a series of metameric units termed parasegments. Following segmentation, the homeotic genes of the bithorax and Antennapedia gene complexes (the BX-C and ANT-C) help to specify the identities of the individual parasegments (for review see INGHAM 1988). In each parasegment, only one or a few homeotic genes are expressed. It is believed that the products of these genes, several of which are known to be specific DNA-binding proteins, then control the expression of particular batteries of genes whose products give each parasegment its particular identity. Accordingly, the proper spatiotemporal regulation of homeotic genes is crucial for their function. Much work over the past few years has established the wild-type patterns of homeotic gene expression in embryos, as well as the disruptive effects of many types of mutations on these patterns, leading to working models for regulation of these genes in early embryogenesis (e.g., PEIFER, KARCH and BENDER 1987; SCOTT and CARROLL 1987; INGHAM 1988).

A number of mutations in the BX-C do not exhibit major defects in early embryogenesis, but do lead to transformations in the cuticle secreted by the adult epidermis (LEWIS 1978; PEIFER and BENDER 1986). Among these, mutations such as abx, bx and pbx appear not to alter the structure of a protein product directly (PEIFER and BENDER 1986), but rather to act by altering the spatial pattern of Ubx expression (WHITE and WILCOX 1985; CABRERA, BOTAS and GARCIA-BELLIDO 1985). The properties of these mutations indicate that the regulation of homeotic gene expression is an ongoing process that continues throughout the course of development. It is not yet clear whether the later stages of BX-C gene regulation follow the same rules as those operating in early development. To address this question, it is necessary to examine the effects of these mutations on expression of the Ubx protein in the imaginal discs, which are the primordia of the affected adult tissues.

We have examined in detail the effects of abx, bx and pbx mutations on Ubx expression in the imaginal discs and central nervous system of mature larvae,
and specifically set out to answer a number of questions raised by previous work. First, it is known that each segment of adult cuticle is divided by lineage restrictions into anterior and posterior compartments. Previous examination of the adult phenotypes and imaginal discs of abx, bx and pbx mutants implied that they affect the expression of Ubx in either the anterior or the posterior compartment, but not both, of a particular segment (Lewis 1978; White and Wilcox 1985). By doubly staining imaginal discs with anti-Ubx and the posterior-specific anti-en antibodies, we examined directly the compartment specificity of the various mutant phenotypes. Second, it had been reported that alterations in Ubx expression in haltere and leg discs of abx and bx34' mutants indicated that they affect the expression of Ubx in either the anterior or the posterior compartment, but not both, of a particular segment (White and Wilcox 1985; Cabrera, Botas and Garcia-Bellido 1985). We have extended this study to other alleles of abx and bx and find that this difference is general. Third, we have characterized the effects of early loss of Ubx+ function on the expression of Ser in the mesothorax, since Ser derepression has been implicated in the so-called pbx transformation of posterior meso- to prothorax. Finally, we describe several unexpected regulatory interactions, including aberrant expression of en protein in discs of pbx' mutants and ectopic expression of Ubx in a partial revertant of bx34'.

MATERIALS AND METHODS

Mutant alleles: Molecular analysis of abx, bx and pbx mutations is detailed by Peifer and Bender (1986). The abx alleles and the bx34-prv allele are deletions in an intron of the Ubx transcription unit (see Figure 1, adapted from Peifer and Bender 1986). The bx alleles studied here are insertions of the gypsy transposable element into the same intron. The pbx' allele is a deletion in the bx5 region, upstream of the Ubx transcription unit.

Fly stocks: Wild type was the Barton strain or stocks carrying the multiply marker ruprica chromosome. In all figures, wild-type controls are of the ruprica/Df(3)Ubx106 genotype. Mutant alleles were maintained over the TM6B balancer (Craymer 1984), which is Ubx+ and carries a dominant Tubby (Tb) marker. Larvae were the progeny of crosses between various balanced stocks, and the desired larvae were identified by their Tb+ phenotype. Fly strains carrying ruprica/TM6B, or Df(3)Ubx106, bx1', bx2', bx1'bx2', bx34Kb, bx34-prv, abx', abx2 (also termed abx34), abx' and abx2' were obtained from Welcome Bender; Ubx' and bx34 were from the Drosophila Stock Center, Bloomington, IN. Flies were reared on the food described by Condie and Brower (1989).

Experimental approach: Unless otherwise noted, all alleles were assayed for Ubx expression as hemizygotes in trans to Df(3)Ubx106 (hereafter termed ‘Df’), a deletion that removes the entire Ubx transcription unit and the sites of all the mutations studied here (Peifer and Bender 1986) (Figure 1). Control animals were either wild type or contained the multiply marked ruprica chromosome over Df(3)Ubx106.

Imaginal discs and ventral ganglia from mature third instar larvae were stained with a monoclonal antibody against Ubx protein (White and Wilcox 1984) and visualized by indirect immunofluorescence microscopy as previously described (Brower 1987). In some experiments, rabbit polyclonal antibody against en protein, the generous gift of Steve Dinaro (Dinaro et al. 1985), was used. In most cases, we also employed an image processing subtraction method (Brower 1986), whereby the nonspecific background staining was subtracted from the specific staining (e.g., Figure 6). Such images were often useful in determin-
ing the distribution of Ubx protein in cases where specific staining was obscured by a high background, such as halteres from weak bx alleles (see RESULTS). Data were collected using a video camera and recorder.

RESULTS

Haltere imaginal discs: To determine if the alterations in Ubx expression seen in bx/

mutant haltere discs (WHITE and WILCOX 1985; CABRERA, BOTAS and GARCIA-BELLIDO 1985) are characteristic of all bx and abx mutations, we examined these and five additional alleles. It has been reported that the bx3 and bx34 alleles result in decreased Ubx expression in the anterior haltere disc, while the abx2 allele displays variability in its effect on Ubx expression in this disc (WHITE and WILCOX 1985; CABRERA, BOTAS and GARCIA-BELLIDO 1985). This correlates well with the findings that the abx and bx mutations cause similar adult transformations of anterior T3 to anterior T2, and that the abx alleles produce a more variable adult phenotype (PEIFER and BENDER 1986).

We found that the five bx alleles—bx6, bx34, bx3, bx83ka and bx83ks—and the three abx alleles—abx1, abx2 and abxCAGA—examined displayed the same general phenotypes as those previously reported (examples in Figures 2 and 3). All of the five bx mutations led to a reduction in Ubx expression throughout the anterior region of the haltere disc. The degree of reduction correlated roughly with the strength of the adult cuticle phenotype of the allele in question [compare the disc from a rather weak allele (bx34) in Figure 2B with that from a strong allele (bx3) in Figure 3A]. In contrast, the three abx mutations led to extreme reduction of Ubx expression in parts of the anterior haltere disc, but typically some anterior regions continued to express Ubx at high levels. Thus, the anterior haltere epithelium in abx larvae was often a mosaic of Ubx expressing and nonexpressing regions, sharply defined but variable in pattern (Figure 2, C and D). This mosaicism was seen in about 60% of the abx2/Df discs examined. Other abx discs resembled those from a strong bx allele, in that staining extended from the posterior margin of the disc to a sharp boundary beyond which no staining was observed (Figure 3C—but see below). Mosaicism was also seen in about 40% of the abx1/Df discs, although the separated patches of anterior staining were generally smaller and fewer in number; only about 10% of the abxCAGA/Df discs showed mosaic patterns of Ubx expression. Thus, the frequency of mosaic expression correlated inversely with the size of the deletion (see Figure 1). It also correlates with the degree of variability observed in the adult phenotypes (PEIFER and BENDER 1986), in that abx2 gives much more variable transformations than do the other two alleles.

To test whether the boundaries of Ubx staining in these mutants corresponded well with the boundary between anterior and posterior compartments, we stained discs with antibodies to Ubx and to the en
protein, which is expressed only in the posterior compartments (DiNardo et al. 1985; Kornberg et al. 1985; Brower 1986, 1987). By this criterion, the region affected by the bx\(^+\) mutation corresponded well with the anterior compartment defined by lack of en expression (Figure 3, compare A and B). In contrast, for discs of the one abx allele tested, abx\(^-\), the affected region was confined to the anterior compartment, but many anterior regions continued to express high levels of Ubx (Figure 3, panels C and D). Even in abx\(^-\) discs that did not display an obvious mosaic pattern, and therefore looked superficially like bx discs, strong anti-Ubx staining generally extended into the anterior compartment.

These results raise the question of whether the qualitative difference between the bx and abx patterns is related to the types of genetic lesions (insertions vs. deletions), or to the locations of the genetic lesions (see Figure 1). To test this, we examined a partial revertant of bx\(^{34}\), termed bx\(^{34+\text{prv}}\) (or bx\(^{34+\text{prv}^\text{7246}}\)), and here denoted bx\(^{+\text{prv}}\) for brevity. This allele deletes about 10 kb of DNA, primarily in the "bx region." Thus we expected that, if the type of lesion was responsible for the variability and anterior staining, the bx\(^{+\text{prv}}\) allele should show these properties, whereas, if the location of the lesion was the important factor, the bx\(^{+\text{prv}}\) allele should behave like a bx allele, since it deletes most of the region containing the bx insertions. We found that bx\(^{+\text{prv}}\) resulted in a high frequency of mosaic staining patterns in the haltere disc (Figure 2, E and F), indicating that, at least in this tissue, this mutation behaved most like an abx allele.

To test for complementation between abx and bx\(^{+\text{prv}}\) or bx alleles, we examined discs of transheterozygotes. About two-thirds of the haltere discs from bx\(^{+\text{prv}}\)/abx larvae were grossly transformed morphologically, and many of these showed mosaic staining patterns. About a third of the discs had fairly normal morphology and staining patterns. Adults also displayed wide variability in morphological transformations of halteres and metanotum (not shown; Peifer and Bender 1986). These data indicate that bx\(^{+\text{prv}}\) and abx alleles could not fully complement one another (see also below). Similar complementation tests were carried out for two bx\(^{34}\)/abx combinations, bx\(^{34}/abx\(^-\) and bx\(^{34}\)/abx\(^{-}\); a similar pattern of partial and highly variable complementation was observed (see also Peifer and Bender 1986).

We have also examined the compartmental specificity of Ubx expression in haltere discs of a different type of mutant, pbx\(^-\). In contrast to bx and abx mutations, pbx mutations have been reported to reduce Ubx expression specifically in the posterior compartments of haltere discs (White and Wilcox 1983; Carrera, Botas and Garcia-Bellido 1985). In pbx\(^-\) haltere discs stained with both anti-Ubx and anti-en antibodies, we found that the domain of Ubx expression slightly overlapped the domain of en expression in the central pouch of the haltere disc epithelium. Even more surprisingly, the pattern of en expression appeared to be altered in the mutant. Although there was a distinct boundary of en expression corresponding to the Ubx boundary, in the haltere pouch there was also a stripe of cells to the anterior side of this boundary and somewhat separated from it that expressed both the en and Ubx proteins. This stripe was not observed in wild-type haltere discs (data not shown).

Metathoracic leg discs: We also compared the effects of abx and bx mutations on third leg discs (data not shown). As in the halteres, the abx and bx\(^{+\text{prv}}\) lesions often resulted in patchy expression of Ubx in the anterior regions of the leg discs. The patches of
Ubx-positive cells were not always so well defined in the leg disc; however, this impression probably resulted in large part from the fact that Ubx expression in parts of the anterior metathoracic leg disc is at somewhat low levels in wild type (White and Wilcox 1984; Brower 1987). In bx animals, Ubx expression was reduced to essentially undetectable levels in the anterior part of the disc, with no patches of significant expression. All of the five bx alleles tested resulted in large part from the fact that Ubx expression in parts of the anterior metathoracic leg disc is at somewhat low levels in wild type (White and Wilcox 1984; Brower 1987). In bx animals, Ubx expression was reduced to essentially undetectable levels in the anterior part of the disc, with no patches of significant expression. All of the five bx alleles tested resulted in this extreme phenotype, including alleles that give only weak haltere phenotypes. No detectable change was seen in the posterior compartment. Previous evidence (Peifer and Bender 1986) also indicated that all these bx alleles give equally strong transformations of T3a leg to T2a leg in the adult. We cannot, of course, rule out the possibility that different alleles have different residual levels of Ubx expression, but that all these levels are below our limit of detection.

Central nervous system: We examined the effects of bx, bx-prv, and abx alleles on Ubx expression in the CNS. In larvae, the wild-type CNS shows expression of Ubx at high levels in parasegment 6 (posterior T3 and anterior A1), at somewhat lower levels in parasegment 5 (posterior T2 and anterior T3), and in a small nest of midline cells in the anterior part of parasegment 4 (Brower 1987; Figure 4A). The effects of BX-C mutants on Ubx expression have only been reported for the CNS of the developing embryo. Here, the bx° mutation has no detectable effect on Ubx expression, whereas abx° leads to elimination of Ubx in almost all of the most anterior parasegment in which it is normally expressed, parasegment 5 (White and Wilcox 1985).

All the bx alleles examined had no effect on Ubx expression in the larval ventral ganglion (not shown), whereas all the abx alleles eliminated Ubx expression in parasegment 5, except for the nest of midline cells (Figure 4B). Accordingly, the effects of the mutations on Ubx expression in the late larval CNS paralleled those previously seen in the embryonic CNS.

The effects of the bx-prv allele on Ubx expression in the larval CNS were somewhat anomalous. Although ventral ganglia from hemizygous bx-prv/Df' larvae expressed Ubx in a pattern similar to the wild type and bx pattern, bx-prv/abx transheterozygotes showed wide variability in the pattern of Ubx expression. Many of the ganglia examined were similar to wild-type or showed only minor reductions in Ubx expression (12/22 examined for abx°; 11/15 for abx°/bx° and 3/13 for abx°/bx°). The remaining ganglia showed greatly reduced numbers of staining cells in parasegment 5; in most of these cases, only a few cells stained in positions lateral to the nest of midline cells (Figure 4C). Accordingly, the abx alleles were partially dominant to bx-prv in this tissue.

In contrast, abx alleles appeared to be largely recessive to wild type and to bx. The abx°/wild-type ganglia looked like wild-type or ruPrica/Df (12/12 examined); a small fraction of abx°/wild-type ganglia (2/10 examined) showed somewhat reduced staining in the posterior portion of PS5. In the two abx/bx transheterozygote combinations tested, abx°/bx° and abx°/bx°, slight reductions in Ubx expression in parasegment 5 were observed in most of the ganglia examined.

Ectopic Ubx expression in bx-prv wing discs: We asked whether any of the alleles affect expression of Ubx in wing discs. In wing discs of wild-type flies, Ubx is expressed only in the cells of the peripodial membrane, and not in the columnar epithelial cells (White and Wilcox 1984; Brower 1987). In flies hemizygous for most of the mutants examined here, Ubx expression in the wing disc was unaffected. However,
hemizygous and homozygous bx-pru flies showed high level expression of Ubx in patches in the presumptive posterior notal region of the wing disc epithelium (Figure 5, A and B).

The precise pattern of this ectopic expression was highly variable. In most discs, one to several large, brightly staining patches of cells were seen. In a minority of discs, the patches were small; among these, some were not very bright. Finally, a few discs showed no detectable staining in this region. We have categorized the staining patterns into large, bright patches; small, bright patches; small, faint patches; and no patches. The distribution of discs in these categories for various genotypes is listed in Table 1. In addition, a substantial proportion of the bx-pru/Df wing discs contained a few staining cells at variable positions in the wing pouch (example in Figure 5A). We did not see any obvious morphological alterations in the dorsal mesothorax of adult bx-pru/Df flies.

Hemizygotes carrying bx-pru over Ubx\textsuperscript{1}, a pseudo-point mutation (BEACHY, HELFAND and HOGNESS 1985) that inactivates Ubx function, also displayed ectopic expression in the wing (Table 1). This finding indicates that this phenotype is uncovered by the loss of Ubx function, rather than by the loss of some other element (e.g., the abx/bx region) deleted in the large Ubx\textsuperscript{109} deficiency.

For a given larva, the extent of morphological transformation and patchy anterior expression of Ubx in bx-pru/Df haltere discs tended to correlate inversely with the extent of the ectopic expression in the wing discs; that is, larvae giving wing discs with the largest, brightest Ubx patches tended to have morphologically normal haltere discs with patterns of Ubx expression that were close to wild type, whereas larvae giving wing discs with small patches tended to have large, morphologically transformed haltere discs with very abnormal Ubx patterns. This correlation was not absolute, but we found few examples of larvae giving small wing patches and relatively normal halters.

Although the deletion mapped in the bx-pru mutation is the only gross rearrangement found by restriction mapping (PEIFER and BENDER 1986), it was possible that the partial revertant has a second mutation that was actually responsible for the ectopic Ubx expression. To test for the presence of other defects, we examined animals hemizygous for the original bx\textsuperscript{34e} allele from which the bx-pru mutation had arisen. A small proportion of bx\textsuperscript{34e} discs contained small, usually faint patches of Ubx staining in the same posterior notal region of the wing disc (Figure 5C, Table 1). Therefore, it is possible that the original bx\textsuperscript{34e} chromosome contains some unidentified lesion that is in part responsible for the ectopic expression. Nonetheless, it is clear that the phenotype is much stronger for the chromosome carrying the bx-pru allele.

We screened larvae hemizygous for other bx and abx alleles for ectopic wing expression (Table 1). Discs from the three abx deletions did not show patches; these alleles included abx\textsuperscript{CAG4}, which may partially overlap with the bx-pru deletion. Likewise, discs from four bx alleles showed no evidence of these patches. These alleles included bx\textsuperscript{8K}, two alleles containing gypsy insertions at sites covered by the bx-pru deletion (bx\textsuperscript{8K} and bx\textsuperscript{4}), and bx\textsuperscript{1}, whose site of insertion may also be covered by this deletion (Figure 1, see PEIFER and BENDER 1986).

Gain-of-function mutations in the BX-C that cause ectopic, anterior expression of Ubx, such as Cbx and Hm, are generally dominant to wild-type (WHITE and AKAM 1985; CARRERA, BOTAS and GARCIA-BELLIDO 1985). In contrast, wing discs from bx-pru/wild-type heterozygotes showed a wide range in the extent of ectopic expression (Table 1). Accordingly, a wild-type allele could often, but not invariably, complement the bx-pru defect, indicating that bx-pru is partially but not completely recessive to wild-type for this phenotype. In addition, some wing discs from abx/bx-pru transheterozygotes also exhibited ectopic expression, the proportion differing among the three abx alleles (Table 1).

We also examined wing discs from bx\textsuperscript{34e}/bx-pru and bx\textsuperscript{34e}/wild-type larvae. The bx\textsuperscript{34e}/bx-pru discs showed a distribution of patches rather similar to that shown.
by bx^{34r} hemizygotes, suggesting that bx-prv is recessive to bx^{34a}. Discs from bx^{34a}/wild-type flies showed no evidence of patches, indicating that bx^{34r} is recessive to wild-type with regard to ectopic Ubx expression.

Finally, we asked whether bx-prv hemizygotes showed ectopic expression in the mesothoracic leg disc. Most of these discs (14/19 examined) showed the same low level of expression in the posterior compartment as seen in the wild type (BROWER 1987). A few showed increases in staining along the posterior margin of the disc, and in one of these the increase was substantial. One disc showed a small patch of bright staining in the posterior compartment of T2 legs [Figure 6, B and C; in these images, which were obtained using the subtraction method, specific staining appears dark on a lighter background (see MATERIALS AND METHODS)]. Often, Scr protein was observed in a band along the disc margin, which included the cells that give rise to the posterior femur; however, expression in more central regions was also seen, sometimes in the absence of significant peripheral expression (Fig. 6C). Scr expression was not observed in the T3 leg; this is consistent with the observation that the ppx transformation is observed only in T2 with this genotype.

Casanova, Sánchez-Herrero and Morata (1985) also reported finding a humerus-like structure in the
posterior mesonotum of abx^2/Ubx flies. Although we did not notice this dorsal T2 to T1 transformation in adults, we did observe Scr expression in the posterior dorsal region of 3 of 26 wing discs from abx^2/Df animals that were raised at 17° (Figure 6A).

**DISCUSSION**

**Contrast between bx and abx:** In both the haltere and third leg discs, bx alleles led to a general reduction in anterior Ubx expression. Alleles with weak effects in the haltere led to strong leg phenotypes in both the adult cuticle (Peifer and Bender 1986) and in the pattern of Ubx expression in the disc. Thus, dorsal and ventral discs of the same segment can be differentially affected by these regulatory mutants. This is seen also when comparing the effects, in dorsal and ventral discs, of mutations in the esc gene, which regulates the homeotic genes of the BX-C and ANT-C (Glickman and Brower 1988).

The effects of abx mutations on Ubx expression were qualitatively different from those of bx alleles. Within the anterior compartment of abx metathoracic discs, regions of strong Ubx expression were often intermingled with regions of essentially no expression. The boundaries of such mosaics were well defined, and, although some trends seemed to emerge, the patterns were highly variable. This variability is also observed in the adult phenotype of abx, and is one of the features distinguishing abx from bx adults (Peifer and Bender 1986).

Since the bx and abx mutations result from different types of genetic lesions (insertions vs. deletions), and also occupy neighboring regions of DNA (see Figure 1), it was of interest to determine if the differences seen were due to the different locations or the different molecular nature of the genetic lesions. The results with the bx-prv mutation, a deletion lying primarily in the bx region, indicate that it is the type of molecular lesion that determines whether one sees a mosaic pattern or a uniform diminution of Ubx expression in the anterior metathorax.

We also found that bx and abx alleles result in different phenotypes in the larval CNS (Figure 4), as previously seen in the embryonic CNS (White and Wilcox 1985). The pattern of Ubx expression in ganglia from bx animals is essentially wild type, while abx mutants show a lack of Ubx protein in parasegment 5, except for continued expression in the midline cells. In this tissue, however, the behavior of bx-prv did not clearly reveal whether it is the location or the type of lesion that lies behind the difference. The pattern of Ubx expression in the CNS of bx-prv larvae was essentially wild type, like the bx mutants. However, this mutation did not fully complement the abx alleles, indicating that the apparent wild-type pattern of Ubx staining in bx-prv does not actually reflect "wild-type" regulation. Previous studies with a number of homeotic and segmentation genes have also shown that regulatory processes and relationships in the CNS are different from those in the epidermis (see, for example, Carroll and Scott 1985; Doe et al. 1988), as observed here.

**Compartmental specificity of effects:** The bx, abx and phbx mutations appear to affect particular compartments, as judged initially by their effects on adult cuticle (Lewis 1978). Direct examination of Ubx expression in imaginal discs, and comparison of the affected regions with fate maps (Bryant 1978) of wild-type discs, led to the suggestion that the effects of these mutations on Ubx expression are specific to particular compartments in the imaginal disc epidermis as well. However, fate maps provide only a crude indication of where compartment boundaries lie, and detailed comparisons of homeotic gene expression with the expression of the posterior compartment-specific en protein have shown that conclusions based
only on fate maps can be misleading (e.g., Brower 1987). This problem is compounded when the morphology of the disc, and the size of the compartments, is distorted due to a homeotic transformation.

By double-staining bx3 halttere discs with anti-Ubx and anti-en antibodies, we found that the Ubx and en boundaries were coincident, at least to a resolution of 2–3 cell diameters. This finding indicates that the bx3 allele affects Ubx expression specifically in the anterior compartment of the halttere disc. This was not true for abx2 halttere discs, however. Even in abx2 discs in which the Ubx expression boundary lay near the compartment boundary, there was no clear coincidence in most cases. In no disc, though, did we see the region of reduced Ubx expression extend into the posterior compartment. In contrast to these observations, White and Wilcox (1985) reported that expression in Ubx was reduced in the posterior compartment of abx2 discs. It seems likely that their interpretation resulted from distortions of the disc morphology as discussed above.

The pattern of anti-en staining in the pouch region of pbx1 halttere discs also revealed that the regulatory effects of this mutation are more complex than had been expected. In contrast to the bx and abx mutations, pbx is purported to reduce Ubx expression in discs of the posterior metamorhax. As expected, we found the pbx effects in the halttere disc to be confined primarily to the posterior compartment, but there was a central stripe near the compartment border that stained with both anti-Ubx and anti-en (Figure 3, E and F). Although this may suggest that some posterior cells remain able to express Ubx, it is also possible that the stripe represents ectopic expression of en in cells that otherwise would be typically anterior. There is no a priori reason to expect the posterior reduction in Ubx to alter the en pattern, and the effect may be indirect, perhaps acting through regulatory genes of the segment polarity class (e.g., Ish-Horowicz et al. 1989). Examination of other alleles, such as pbx2 and a bx pbx double mutant, may help to resolve this issue.

Ectopic gene expression and bx-pru: Ectopic Ubx expression was observed in wing discs of hemizygous and homozygous bx-pru larvae (Figure 5). Unlike several dominant gain-of-function mutations affecting cis-acting sites and conferring ectopic expression (e.g., White and Akam 1985; Carrera, Botas and Garcia-Bellido 1985), bx-pru was partially or completely recessive to each of three different wild-type chromosomes and to the three abx alleles examined. These findings suggest that the defect can be complemented in trans to some extent. However, the situation is complicated both by the fact that the recessiveness was not complete, and by the finding that discs from bx3+/Df also showed some ectopic expression. Thus it is possible that background variation can influence the levels of ectopic expression seen in different genetic backgrounds, and the conclusion that bx-pru is not dominant to wild-type must be viewed with some caution.

Nonetheless, if we grant this conclusion for the sake of discussion, one other finding suggests how a wild-type chromosome might be dominant to bx-pru. The pseudopoint mutant Ubx1 failed to eliminate ectopic wing expression in trans to bx-pru, indicating that the dominance of the wild-type chromosome is unlikely to be due to transvection, and suggesting that it is due to the loss of Ubx function. Thus, the signal that acts in trans to down-regulate Ubx from the bx-pru chromosome during the course of development is likely to be the Ubx protein itself, although it is uncertain whether the effect is direct or indirect. As discussed below, other evidence also suggests that Ubx is normally expressed in the precursors of the posterior notum at an earlier developmental time, and it is possible that the bx-pru ectopic expression in the wing is the result of an inability to turn off Ubx, rather than an aberrant activation of the gene; this is consistent with the recessive nature of the effect.

The bx-pru allele also displayed a complex dominance relationship to abx alleles. In halttere discs, both abx and bx-pru gave similar extents of morphological transformation and patterns of Ubx expression. A large proportion of abx/bx-pru transheterozygotes also gave these phenotypes, indicating that the two types of alleles could not fully complement one another. In the ventral ganglion, the bx-pru allele could not fully complement abx lesions in abx/bx-pru transheterozygotes, even though the Ubx expression pattern of bx-pru/Df larvae appeared identical to wild-type (Figure 4). Finally, in wing discs, abx alleles were roughly similar to wild-type in their ability to suppress the bx-pru-related patches of ectopic Ubx expression; bx1 appeared to be more effective than the other two alleles (Table 1).

Scr expression and the pxpx effect: As outlined in the results, various genetic experiments (Morata and Kerridge 1981; Casanova, Sanchez-Herrero and Morata 1985) have led to the proposal that the pxpx effect results from an early lack of Ubx expression, leading to stable expression of the Scr gene product in posterior regions of the meso- and metathoracic segments. This effect can be observed in clones of Ubx+ tissue (generated by somatic recombination), or, in the mesothorax, in animals mutant for some alleles of abx or bx. We have confirmed that Scr is indeed expressed in posterior regions of T2 imaginal discs in a sizable fraction of abx2/Df larvae. Although it was not possible to stain for Scr protein and assay for the pxpx cuticle phenotype in the same animal, the penetrance of both of these effects displayed a similar temperature dependence.
It was possible that Scr expression would be increased generally in the T2 discs, with the adult phenotype confined to posterior compartments because of a possible involvement of other homeotic genes in determining the cuticle pattern. We found, however, that T2 expression of Scr protein was confined to the posterior regions of each disc. The pattern of expression within the posterior region was variable. This may account for the higher percentage of leg discs as compared to adult legs that showed a "ppx" effect, since only those discs that expressed Scr protein in the presumptive femur would be scored as ppx adults.

Previous studies have indicated that embryonic or early larval Ubx expression is necessary to turn off Scr in posterior T2 (Casanova, Sánchez-Herrero and Morata 1985). In this respect, it is interesting that the ppx expression of Scr protein in the wing disc is in the same region as the ectopic Ubx expression seen in bx-prv animals, and this ectopic expression also is repressed by a wild-type copy of Ubx.

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LITERATURE CITED


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