Ultrabithorax mutations in constant and variable regions of the protein coding sequence

Robert Weinzierl, J. Myles Axton,1 Alain Ghysen,2 and Michael Akam

Department of Genetics, Downing Street, Cambridge CB2 3EH UK; 2Laboratoire de Genetique, Universite Libre de Bruxelles, 1640 Rhode-St.-Genese, Belgium

The Ultrabithorax gene (Ubx) of the Drosophila bithorax complex (BX-C) gives rise to a number of spliced transcripts that differ in the utilization of microexons within the protein coding domain. Here we report on the precise structure of three Ubx mutations. Two of these mutations lie in coding sequences common to all of the major spliced transcripts, and presumably inactivate all classes of Ubx protein. The third is a single base substitution that generates a nonsense codon in one microexon. This mutation will block the open reading frame of some but not all of the spliced forms of Ubx RNA. All three of these mutations have equivalent effects on the epidermis, but the microexon mutation has little if any effect on the development of the adult nervous system.

[Key Words: Bithorax complex; nervous system; homeotic genes; splicing variants; nonsense mutation]

Received January 14, 1987; revised version received and accepted March 5, 1987.

The Ultrabithorax gene (Ubx) of the bithorax complex (BX-C) is required for the normal identities of parasegments 5-13 in Drosophila [Lewis 1978]. Within this region, it plays many roles in development. In parasegment 6, it is the prevailing homeotic gene, controlling the morphology (and presumably physiology) of the larval and adult epidermis [Lewis 1978, 1981; Minaña and Garcia-Bellido 1982; Hayes et al. 1984], the central and peripheral nervous system [Green 1981; Jiménez and Campos-Ortega 1981; Ghysen et al. 1983; Teugels and Ghysen 1983; Thomas and Wyman 1984], the tracheal tree [Lewis 1978], the muscles [Hooper 1986], and probably most other derivatives of the ectoderm and mesoderm. In parasegment 5 it acts in concert with the Antennapedia gene [Wakimoto and Kaufman 1981; Struhl 1982] to direct the development of a quite different set of structures in many of these tissues. In more posterior segments of the animal the effects of the abdominal genes of the BX-C predominate; Ubx plays only a subsidiary role [Lewis 1978; Sánchez-Herrero et al. 1985].

The functions of Ubx in parasegments 5 and 6 are under separate genetic control. Regulatory mutations affecting Ubx expression in parasegment 5 [the antero-bithorax (abx) and bithorax (bx) mutations] map within the Ubx transcription unit; those affecting its expression in parasegment 6 [bithoraxoid (bxd) and postbithorax (pbx) mutations] map 5' to the gene [Bender et al. 1983; Hogness et al. 1985]. Both classes affect the distribution of protein products encoded by the long Ubx transcription unit [White and Wilcox 1985a; Beachy et al. 1985; Cabrera et al. 1985].

The diverse effects of Ubx in these two parasegments are achieved, at least in part, by establishing within them very different patterns of Ubx transcription [Akam and Martinez-Arias 1985]. It has been observed, however, that the Ubx gene gives rise to a number of spliced transcripts that differ in the utilization of microexons within the protein coding domain [Hogness et al. 1985; K. Komfeld and D. Hogness; M. O'Connor, R. Binari, and W. Bender, pers. comm.]. It has been suggested that proteins utilizing different sets of these microexons might mediate different functions of Ubx in development [Beachy et al. 1985].

Here we report on the precise structure of three Ubx mutations, each of which disrupts the major open reading frame of these Ubx transcripts. Two of these mutations lie in sequences common to all of the major spliced transcripts, and presumably inactivate all classes of Ubx protein. The third is a single base substitution that generates a nonsense codon in one microexon. This mutation will block the open reading frame of some but not all of the spliced forms of Ubx RNA. All three of these mutations have equivalent effects on the epidermis, but the microexon mutation has little if any effect on the development of the adult nervous system.

1Present address: CRC Eukaryotic Molecular Genetics Group, Department of Biochemistry, Imperial College, London SW7 2AZ UK.
Results

The structure of two deletions that block the common exons of the Ubx open reading frame

Two of the mutations that we have characterized are X-ray-induced deletions. These have previously been mapped to the major 5' \((Ubx^{6.28})\) and 3' \((Ubx^{2.22})\) exons of the Ubx gene [Fig. 1; Bender et al. 1983; Akam et al. 1984]. We have cloned DNA fragments containing the mutations from both deletion chromosomes, and characterized the deletions by DNA sequencing [Fig. 2].

\(Ubx^{6.28}\) carries a 32-base deletion in the 5' exon. This introduces a frame shift into the open reading frame after only 27 codons, and results in the premature termination of the open reading frame [ORF] after a further 37 incorrect codons [Figs. 1 and 2]. The end points of the deletion lie at equivalent positions within direct repeats of the sequence GGCC.

The \(Ubx^{2.22}\) chromosome carries a deletion of 1587 bp with respect to the sequence of the wild-type Canton-S chromosome [Fig. 2]. This deletion removes the final 1.4 kb of the 50-kb-long Ubx intron, the splice acceptor site of the 3' exon, and 48 codons of the homeo box. A sequence of six nucleotides has been inserted between the deletion breakpoints. One good match to a splice consensus sequence remains within the truncated homeo box [Fig. 2], but splicing at this site would result in translation out of frame with the Ubx protein.

\(Ubx^{195}\) is a nonsense mutation in the \(-50\) kb Ubx microexon

Within the Ubx domain, the \(Ubx^{195}\) chromosome shows no DNA lesion detectable by genomic blotting. Thus, it was provisionally classified as a true point Ubx mutation [Bender et al. 1983]. In genetic mapping experiments with this and other Ubx point mutations, \(Ubx^{195}\) behaved anomalously. All of six other alleles mapped to the 5' end of the Ubx unit, but \(Ubx^{195}\) failed to recombine with the bx\(^{24e}\) mutation [Akam et al. 1984], which lies near the middle of the Ubx transcription unit. However, it recombined at a relatively high frequency with the bx\(^{d4}\) mutation [M.E. Akam, data not shown], which lies only 10 kb upstream of the Ubx 5' exon.

Using restriction fragment polymorphisms we were able to show that each of three recombination events between \(Ubx^{195}\) and bx\(^{d4}\) had occurred 5' to 46 kb [N. Vineall and M.E. Akam, unpubl.]. These results make it unlikely that the \(Ubx^{195}\) mutation maps to either the 5' or 3' major exons, and prompted us to clone and sequence genomic fragments containing the \(-40\) kb and \(-50\) kb Ubx microexons from the \(Ubx^{195}\) chromosome.

The sequence of the \(-40\) kb microexon in the \(Ubx^{195}\) chromosome is identical to that from a wild-type chromosome [Fig. 2], and to the corresponding region of a Ubx cDNA prepared from wild-type flies [data not shown]. The \(-50\) kb microexon shows a single G to A base substitution with respect to the Ubx' chromosome, a change consistent with the known effects of ethyl methanesulfonate [EMS] mutagenesis [Fig. 2]. This change generates a nonsense codon in the open reading frame of those Ubx transcripts that utilize the \(-50\) kb microexon. Proteins encoded by such transcripts should terminate just seven amino acids before the start of the homeo box.

Ubx protein expression in mutant embryos

Beachy et al. [1985] have previously shown that embryos homozygous for the mutation \(Ubx^{6.28}\) lack all Ubx protein detectable with antibodies raised against a Ubx fusion protein.

The other two mutations that we have sequenced do not affect the 5' protein coding sequences of Ubx. Using a monoclonal antibody raised against determinants encoded in the Ubx 5' exon [White and Wilcox 1984], we find that embryos homozygous for \(Ubx^{2.22}\) or \(Ubx^{195}\) still express detectable levels of Ubx protein [Table 1], even though in the case of \(Ubx^{2.22}\), the Ubx messages must be abnormally spliced, and the protein must lack entirely the normal carboxy-terminal homeo box structure. The segmental distribution of Ubx proteins detected in \(Ubx^{195}\) and \(Ubx^{2.22}\) homozygotes is similar to that in wild type, the precise distribution of these proteins will be described in detail elsewhere [R. White, I. Davis, and M. Akam, in prep.]. The anti-Ubx antibody does not allow us to distinguish between full-length and truncated proteins that might both be synthesized from the \(Ubx^{195}\) allele.

We have also examined the expression of Ubx proteins in embryos homozygous for two other Ubx alleles. \(Ubx^{1}\) carries a "doc" element inserted into the 5' exon of the Ubx gene [Bender et al. 1983], in the 5' untranslated region of the mRNA [W. Bender, pers. comm., I. Davis and M.E. Akam, unpubl.]. \(Ubx^{409}\) carries a deletion of approximately 110 bp in the 5' protein coding sequences of Ubx [Beachy et al. 1985]. Both of these mutations eliminate all detectable Ubx proteins in homozygous mutant embryos [Table 1].

The microexon mutation disrupts Ubx functions throughout parasegments 5 and 6

The \(Ubx^{6.28}\) and \(Ubx^{2.22}\) genes are unable to direct the synthesis of intact Ubx proteins, but \(Ubx^{195}\) may synthesize normally any proteins that do not utilize the \(-50\) kb microexon. Therefore we have compared the phenotypes of these three mutations, with one another and with other Ubx alleles, to determine which if any Ubx functions are not impaired by the \(Ubx^{195}\) mutation.

Individuals homozygous for each of the mutations complete embryogenesis, and occasionally survive as third instar larvae. These show the characteristic Ubx larval phenotype previously described for \(Ubx^{1}\) [Lewis 1978], with transformed denticle belts and three pairs of anterior spiracles.

To test whether \(Ubx^{195}\) affects segment- or compartment-specific functions of Ubx differentially, we have crossed this and a set of other Ubx alleles to \(abx, bx, bx^{d4}\).
Figure 1. The location of Ubx<sup>6.28</sup>, Ubx<sup>195</sup>, and Ubx<sup>9.22</sup> mutations within the Ubx transcription unit, and the predicted structures of the resulting Ubx proteins. (Above) The spliced structure shown in solid lines is that most frequently found among Ubx cDNAs derived from embryonic RNA [class I, Hogness et al. 1985]. The DNA scale is marked in kilobases (kb) using the coordinates of Bender et al. (1983). The open reading frame is shaded. The diagram does not indicate alternative splice donor sites 27 bases apart which may be used to terminate the 5′ exon [see Fig. 2]. The two microexons, located at approximately -40 kb and -50 kb, are each 52 bases long, and are not drawn to scale. Rarer cDNAs representing alternative spliced forms of this transcript lack the first [class II] or both [class IV] of the microexons, [dotted lines, K. Kornfeld and D. Hogness; M. O’Connor, R. Binari, and W. Bender, pers. comm.]. (Below) The structures of wild type, Ubx<sup>*</sup>, proteins of classes I and IV are indicated. Exons are separated by vertical lines. The solid box indicates the homeo box. Hatched boxes indicate homopolymeric tracts of glycine, encoded by the GGX-A repeat, and alanine, encoded by the GGX-C repeat [R. Weinzierl and M. Akam, in prep.]. Below these are shown the predicted structures for the same protein classes in the three Ubx mutations. Cross-hatching indicates out-of-frame translation of Ubx sequences downstream of the Ubx<sup>6.28</sup> deletion.
Ultrabithorax mutations

A) 5' (-30 kb) Protein Coding Region:

```
1 MetAsnSerTyrPheGluInAlaSerGlyPheTryGlyHisProHisInAlaThrGlyMetAlaGlySerGlyGlyHisThrAlaArgAlaAlaAlaAlaAla
ATAAATCGACTTGGGATACGGGCTTCTGCTTGTTatatGCATCAACCCACGATTGGCCACATTGGGATTGCACTGTCCTGCTGACCTGCTGACCTGCTGACCTGCTG
Ubx 6.28 MetAsnSerTyrPheGluInAlaSerGlyPheTryGlyHisProHisInAlaThrGlyMetAlaGlySerGlyGlyHisThrAlaArgAlaAlaAlaAlaAla
121 ArgGlyPheLeuSerLeuGlnValAlaSerGlnValAlaSerGlyLeuSerGlyLeuSerGlyLeuSerGlyLeuSerGlyLeuSerGlyLeuSerGlyLeuSerGlyLeuSerGlyLeuSerGlyLeuSer
```

B) -40 kb Microexon:

```
LysIleArgSerAspLeuThrGlnTyrGlyGlyIleSerThrAspMetGly
```

C) -50 kb Microexon:

```
LysArgTyrSerGluSerLeuAlaGlySerLeuLeuProAspTrpLeuGly
```

D) 3' (-103 kb) Exon:

```
1 G GATCCTGTATTTTTGCTACC A TTTCGTTAAGACTTTCTGAGAGATATGGCCG AC AAATTGCCATAAACTG ACGC A TCGC A AATC TTGTG A CCTG TC ACTGGCC A ATTTTCTGGCACATT
```

Figure 2. (See next page for legend.)
bxd, and ppx mutations. With respect to transformations of the epidermis, we have not been able to detect any major difference between \textit{Ubx}\textsuperscript{195} and the protein null alleles \textit{Ubx}\textsuperscript{6.28} and \textit{Ubx}\textsuperscript{628}, or \textit{Ubx}\textsuperscript{22}, which is effectively null [Fig. 3]. All of these mutations behave very like the original \textit{Ubx}\textsuperscript{1} mutation. [See Morata and Kerridge (1980) and Kerridge and Morata (1982) for a careful documentation of the effects of \textit{Ubx}\textsuperscript{1}, \textit{Ubx}\textsuperscript{6.28}, and other \textit{Ubx}.] All provide significantly more \textit{Ubx}\textsuperscript{1} function when in \textit{trans} with the recessive alleles than does a \textit{Ubx} deficiency or rearrangement, but this effect is probably not mediated by \textit{Ubx} protein synthesized from the \textit{Ubx} mutant chromosome [see Discussion].

We were particularly concerned to determine whether \textit{Ubx}\textsuperscript{195} inactivates the \textit{postprothorax} (\textit{ppx}) function of \textit{Ubx}. This function differs from all other activities of \textit{Ubx} in that it is required only early in development [Morata and Kerridge 1981], and in that it is required not for normal morphogenesis, but to suppress the activity of another homeotic gene, \textit{Sex combs reduced} [Struhl 1982]. The \textit{Ubx}\textsuperscript{195} mutation does disrupt the \textit{ppx} function. In \textit{trans} with \textit{abx}\textsuperscript{2}, it results in extensive transformations of the second thoracic leg to give first leg structures. Such transformations are never observed in the genotype \textit{abx}\textsuperscript{2}/+. The penetrance of this transformation is low [see the legend to Fig. 3]. In part, this is because the \textit{abx}\textsuperscript{2} mutation does not inactivate the \textit{ppx} function completely.

The microexon mutation differs from other \textit{Ubx} alleles with respect to time and tissue specificity

While testing the ability of \textit{Ubx}\textsuperscript{195} to complement the recessive alleles of the \textit{abx} and \textit{bxd} classes, we noticed
that heterozygotes between these mutations and Ubx^{195} were strikingly more viable than the equivalent combinations with otherwise comparable Ubx alleles. Most flies of the genotype Ubx^{+/abx} or Ubx^{+/bxd} die in the late pupal period and never initiate eclosion. For all Ubx^{1}-like alleles tested, except Ubx^{195}, more than 90% of the expected flies of these genotypes fail to eclose (Table 2). By contrast, under the same conditions 90% of Ubx^{195}/abx individuals eclose, as do a significant fraction of Ubx^{195}/bxd.

No similar effect is observed with bx or pbx alleles (Table 2). Extreme alleles of these classes \( e.g., bx^3, pbx^3 \) result in significant lethality with all Ubx mutations, but the number of flies dying correlates well with the extent of epidermal transformation, and is similar for Ubx^{195} and other Ubx alleles. It often results from mechanical difficulties occurring after eclosion has been initiated.

The unexpected survival of these mutant combinations suggested that the effects of the Ubx^{195} mutation on internal tissues may not be the same as those of other Ubx alleles. More particularly, they suggested a differential effect on the nervous system. All strong abx and bxd mutations result in segment transformations of the nervous system, when homozygous or in \( trans \) with Ubx mutations, but even the strongest bx and pbx alleles have little or no effect in this tissue (Teugels and Ghyesen 1985; White and Wilcox 1985a).

We therefore analyzed the neural phenotypes of Ubx^{195} and other Ubx alleles. In the larval nervous system, the effects of Ubx^{195} are similar to those of other Ubx protein null alleles [Fig. 4]. However, in the adult nervous system there is a clear difference. Typically, Ubx mutations, in \( trans \) with abx or bxd alleles, transform the pattern adopted by specific fascicles in parasegments 5 and 6 of the ventral ganglion [Teugels and Ghyesen 1985]. The Ubx^{195} mutation in the same genotypes is virtually without effect on the development of these adult structures [Fig. 5 and Table 2].

To show that this is not simply a threshold effect, revealing slight leakiness of the Ubx^{195} mutation, we have examined for comparison the effects of Ubx^{61d}. This allele inactivates Ubx only weakly; it results in much less extreme epidermal transformations than Ubx^{1}, and is even homozygous viable [Lewis 1982]. Yet it indisputably transforms the adult nervous system.

We conclude that the Ubx^{195} allele is essentially wild type with respect to some Ubx functions required during metamorphosis of the nervous system. We presume that these are mediated by protein variants that do not contain the \(-50\text{-}kb\) microexon.

### Discussion

The three Ubx mutations that we have sequenced, together with a number of less well-characterized alleles, all result in epidermal phenotypes very similar to that of the original Ubx^{1} mutation. These have been classed as “moderate” Ubx alleles, for in many mutant combinations they result in less severe segment transformations than those associated with Ubx deficiencies [E. Lewis, pers. comm., Kerridge and Morata 1982]. A similar effect can be detected in the central nervous system (CNS) as well (Table 3). Nonetheless, our data indicate that many of these mutations are effectively null mutations for the Ubx proteins. Three of the alleles, Ubx^{1} itself, Ubx^{6-28}, and Ubx^{499}, lack any protein detectable with antibodies to the Ubx 5’ exon. A fourth, Ubx^{22}, must, from its structure, inactivate all Ubx products that utilize the homeo box.

The existence of many “stronger than null” Ubx mutations requires some explanation. Most strong Ubx mutations are associated with visible chromosome rearrangements or deficiencies [Lindsley and Grell 1968]. In cases where strong Ubx mutations appear cytologically normal [e.g., Kerridge and Morata 1982], molecular analysis has invariably revealed a deletion of many kilobases, or a cytologically invisible chromosome inversion [Bender et al. 1983].

One explanation for the difference between Ubx null and rearrangement mutations would be that the Ubx domain of the bithorax complex synthesizes products other than the Ubx proteins, which the Ubx null alleles can still express. The only identified candidate for such a product is a protein encoded within the bxd region of the Ultrabithorax domain, on a transcript expressed during larval and pupal stages [Hogness et al. 1985; Lipshitz et al. 1987].

A second possibility is that the strong Ubx mutations make antimorphic products. This cannot account for the differences between protein null alleles and chromosomal deficiencies, but may be relevant when the Ubx gene is broken by a rearrangement. The great majority of these rearrangements leave 5’ coding sequences of Ubx intact. Some express detectable Ubx proteins [M.E. Akam, unpubl.] that must be truncated or fused to novel sequences. These may interfere with the functions of Ubx, particularly if the Ubx protein acts as a dimer or multimer.

### Table 1. Ubx protein expression in mutant embryos

| Ubx allele | Expected proportion of mutant homozygotes (%) | Number of embryos examined | Number (%) of embryos lacking Ubx protein |
|-----------|-----------------------------------------------|---------------------------|------------------------------------------|
| Ubx^{1}   | 25                                            | 81                        | 19 (23%)                                  |
| Ubx^{640} | 25                                            | 145                       | 35 (24%)                                  |
| Ubx^{22}  | 25                                            | 86                        | 1 (1%)                                    |
| Ubx^{195} | 25                                            | 75                        | 0 —                                      |

Embryos from parents heterozygous for each Ubx mutation were stained with anti-Ubx antibody FP3.38 [White and Wilcox 1984], using either fluorescently labeled second antibody [White and Lehmann 1986] or the ‘Vectastain’ ABC peroxidase system [Vector Labs]. The populations are expected to contain three classes of progeny, \(+/+, +/Ubx, \) and \(Ubx/Ubx\). Embryos from germ band shortening stages onwards were scored for the presence of detectable Ubx protein expression. A proportion of embryos in these populations showed weak staining, but it was not possible to distinguish embryos unambiguously into distinct classes.
Figure 3. Cuticular phenotypes of the $Ubx^{195}$ mutation in trans with recessive alleles of the Ultrabithorax domain: (a) $Ubx^{195}/abx^{2}$. Second leg showing large bristles [arrowheads] characteristic of first leg [the ppx transformation]. (b) $Ubx^{195}/bx^{3}$. Haltere showing extensive transformation to anterior wing [the bx transformation]. (c) $Ubx^{195}/pbx^{1}$. Haltere showing extensive transformation to posterior wing [the pbx transformation]. The penetrance of bx and pbx transformations is complete, and the examples illustrated are typical of most individuals. The extent of the ppx transformation is variable, and the penetrance is low (4% of legs, $n = 198$), but significantly different from that of the control genotype $+/abx^{2}$ (0%, $n = 356$). Comparable figures for other Ubx alleles in the same experiment are $Ubx^{s49}$, 9% ($n = 126$), $Ubx^{I}$, 16% ($n = 48$), $Ubx^{130}$, 42% ($n = 32$). Scale bar, 50 μm.

Table 2. Exceptional viability of $Ubx^{195}$ allelic combinations

| Percent eclosion of adult mutant flies [number expected] | $abx^{2}/Ubx$ [18°C] | $bx^{I00}/Ubx$ [25°C] | $bx^{3}/Ubx$ [25°C] | $pbx^{I}/Ubx$ [25°C] |
|--------------------------------------------------------|-----------------------|------------------------|-----------------------|------------------------|
| $Ubx^{I}$-like alleles                                  |                       |                        |                        |                        |
| $Ubx^{195a}$                                           | 90 [341]              | 36 [85]                | 38 [80]                | 46 [71]                |
| $Ubx^{s49a}$                                           | 1 [268]               | 0 [193]                | 35 [62]                | 34 [79]                |
| $Ubx^{1}$                                              | 1 [300]               | 0 [179]                | 27 [62]                | 70 [77]                |
| $Ubx^{922}$                                            | <1 [353]              | <1 [235]               |                        |                        |
| $Ubx$ rearrangements and deficiencies                   |                       |                        |                        |                        |
| $Inv Ubx^{130}$                                         | 2 [297]               | 1 [73]                 |                        |                        |
| $Df Ubx^{42}$                                           | 8 [269]               | 0 [100]                |                        |                        |
| $Df Ubx^{109}$                                          | 0 [376]               | 0 [114]                |                        |                        |

*a $Ubx^{195}$ and $Ubx^{s49}$ are most directly comparable, in that they were induced on the same chromosome background and show indistinguishable epidermal phenotypes in all of these mutant combinations.

*b Indicates that particular combinations were not tested.
A third possibility is that the presence of a structurally normal \textit{Ubx} chromosome may enhance the expression of \textit{Ubx} proteins from its synapsed homolog, even when it can encode no active protein itself. A mechanism of this type is favored by several observations:

1. The difference between point alleles and rearrangements is only observed when \textit{Ubx} mutations are heterozygous with bx, bxd, and pbx mutations. All of these mutations alter the distribution of \textit{Ubx} proteins, but do not themselves prevent the synthesis of at least some normal \textit{Ubx} products. Little or no difference is observed in the effects of protein null point alleles and deficiencies when these are made homozygous, either throughout whole embryos, or as clones of cells in adults [Lewis 1963; Morata and García-Bellido 1976; Kerridge and Morata 1982; Mañá and García-Bellido 1982; Hayes et al. 1984]. Under such circumstances \textit{trans} interactions can have no effect on \textit{Ubx} protein expression.

2. The transvection effect has long been taken to support the idea that chromosome pairing may affect functions of the \textit{Ubx} domain [Lewis 1954, 1982]. Transvection is the enhancement of a mutant phenotype by rearrangements that disrupt the pairing of homologous chromosomes without directly affecting the mutant gene. It is observed when regulatory alleles of the Ultrabithorax domain (e.g., bx, pbx) are heterozygous with one another, or with \textit{Ubx} alleles affecting protein coding. It is not observed between even the most distant pair of \textit{Ubx} alleles, \textit{Ubx}^{195} and \textit{Ubx}^{222} [Lewis 1985].

All of these points lead us to suspect that the major function retained by \textit{Ubx} protein null alleles is the ability to stabilize normal conformation or regulation of a synapsed homolog. Conversely \textit{Ubx} rearrangements may result in phenotypes more extreme than those of deficiencies by actively disrupting the configuration of a homolog. M. Ashburner et al. [in prep.] have evidence for such an effect acting on the \textit{white} gene.

The \textit{Ubx} microexon mutation

In most respects, the phenotype of the \textit{Ubx} microexon mutation appears very similar to that of the protein null alleles. This is consistent with the apparent rarity of processed \textit{Ubx} transcripts lacking the second microexon. Only a small fraction (~2%) of the \textit{Ubx} cDNA clones which have been characterized lack this microexon [K. Kornfeld and D. Hogness; M. O’Connor, R. Binari, and W. Bender, pers. comm.]. The great majority of these, however, have been recovered from early embryonic cDNA libraries, and so this fraction may not be representative of the spectrum of \textit{Ubx} products throughout development.

The presence or absence of the ~50-kb \textit{Ubx} microexon is not used to distinguish compartment- or segment-specific functions of \textit{Ubx}. However, its absence may be used to distinguish a specific class of \textit{Ubx} proteins that function during development of the adult nervous system. Our results imply that \textit{Ubx} proteins which lack the second microexon provide sufficient \textit{Ubx} activity in the developing adult nervous system to establish normal morphology, whereas such proteins have no detectable effect, or are not produced, in the epidermis.

This does not necessarily mean that different \textit{Ubx}
Figure 5. [See facing page for legend.]
protein variants have different functions. It may simply reflect a difference between the splicing apparatus of the CNS and the epidermis, such that significant levels of complete Ubx protein are produced from the Ubx195 gene in the former but not the latter. A more appealing possibility is that the differential expression of splicing variants relates to the different developmental roles of Ubx. Although there is no direct evidence to support this, we note that the microexons encode a stretch of amino acids that lie immediately adjacent to the homeo box, and that vary the separation of this DNA-binding domain from another highly conserved region of the Ubx protein (Wilde and Akam 1987). We guess that the alterations in this sequence that result from alternative splicing will significantly affect the biological properties of the Ubx protein.

Materials and methods

Fly genetics

Ubx and other mutations used in this work are described as follows: Ubx195 and Ubx409 from E.B. Lewis (see Akam et al. 1984). Ubx409 from E.B. Lewis [see Sánchez-Herrero and Morata 1984]. Ubx428 and Ubx292 (Kerridge and Morata 1982). abx2 from S. Kerridge and G. Morata, see Casanova et al. 1985). Other BX-C mutations are described in Lindsley and Grell (1968) and Lewis (1978, 1981).

Figure 5. Effects of Ubx alleles on the adult nervous system. Panels show whole mounts of the adult CNS, stained with the antibody 5D12. This antibody stains a subset of fascicles in the nervous system. In the embryo, all segments show the same staining pattern, but during adult development, segment-specific patterns appear (Teugels and Ghysen 1985). Genotypes: [a,d] bxdr100/Ubx195, [b,e] bxdr100/Ubx428, [c,f] bxdr100/Ubx292, [a-c] Posterior dorsal commissure (pd) in T3 (parasegment 5, arrowhead) and A1 (parasegment 6, arrow). [d-f] Pictures of the same three ganglia taken at a more ventral focal plane, to show the posterior intermediate commissures (pi) of T2 (parasegment 5, arrowhead) and T3 (parasegment 6, arrow). The leg neuromeres are outlined by a dotted line. With Ubx195(a and d) the pattern of commissures is indistinguishable from wild type (compare Fig. 1 of Teugels and Ghysen 1985). The commissures of parasegment 6 are not transformed. With Ubx292(b and e), there is essentially complete transformation of commissures in parasegment 6 to resemble those in parasegment 5. Ubx428(c and f) results in a partial transformation. There is a small additional neuromere in a and d, presumably a remnant of the extensive larval transformation, while no such neuromere can be detected in c and f.

For comparison of each Ubx allele, parallel crosses were set on yeast glucose medium and reared at 25°C, except for crosses with abx2, which were reared at 17–18°C throughout embryogenesis [to increase the penetrance of ppx phenotypes; Casanova et al. 1985], and 25°C thereafter.

Cloning of DNA fragments carrying Ubx mutations

DNA was prepared from adult flies carrying Ubx mutations, balanced over the chromosome TM1 [Lindsley and Grell 1968]. EcoRI-cut genomic DNA was cloned into λ vectors by standard techniques [Maniatis et al. 1982], and the resulting libraries probed for homology to clones spanning the appropriate regions of the Ubx transcription unit [Bender et al. 1983; Beachy et al. 1985, Hogness et al. 1985]. Isolated clones carrying the Ubx428 and Ubx292 deletion fragments were distinguished from those carrying fragments of the balancer chromosome by the reduction in size of restriction fragments spanning the deletion.

For Ubx195, restriction polymorphisms identified by genomic blotting were used to distinguish clones deriving from the Ubx mutant and balancer chromosomes.

Table 3. Ubx transformations in fascicles of the adult nervous system

| T2 | pd | pi | pv | T3 | ad | ai | av | pd | pi | pv | A1 | ad | ai | av | pd | N |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---|
| abx/Df | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 8 |
| abx/Inv Ubx50 | + | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | 6 |
| abx/Ubx2 | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | 6 |
| abx/Ubx428 | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | 6 |
| abx/Ubx414 | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | 6 |
| abx/Ubx195 | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | 6 |
| bxdr100/Df | [±] | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 7 |
| bxdr100/Inv Ubx50 | [±] | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 9 |
| bxdr100/Ubx2 | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | 11 |
| bxdr100/Ubx414 | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | 12 |
| bxdr100/Ubx195 | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | [±] | 13 |

Terminology and symbols are as in Teugels and Ghysen [1985]. T2, T3, A1 indicate segments affected. pd, pi, etc., indicate particular fascicles. N is the number of nervous systems examined. (+) Complete or near-complete transformations; (±) partial transformation; (+, ±) seen in all cases examined; (+, ±) seen in majority of cases; [(+, ±)] seen occasionally. Blank spaces indicate that fascicles were untransformed. Df indicates a chromosome deleted for the entire Ubx gene (= Df bxdr100 for abx2/4Df; Df Ubx195 for bxdr100/Df).
Weinzierl et al.

and at one site corrected by the data of Beachy [1986], which we have used as our primary standard.

All other DNA sequences were determined by the dideoxy method on subclones in M13. The sequence across the UbxB28 deletion was determined on both strands of the PstI-Sau3A fragment flanking the deletion. The sequences of the -40- and -50-kb microexon regions of the UbxB28 and UbxB+ (TMI balance chromosomes were determined by shotgun cloning of TaqI and HinfI fragments from the cloned genomic EcoRI fragments spanning the microexons, and sequencing those clones homologous to the UbxB cDNA 3712 (Beachy et al. 1985; Beachy 1986).

The sequence spanning the UbxA 3' exon in the wild type {Canton-S} was determined by sequencing random subclones from the 3.2-kb BamHI fragment spanning the UbxA homeo box [derived from λ 2296; Bender et al. 1983; coordinates −102 to −105 kb]. The final sequence was assembled using the DB programs [Staden 1982].

The equivalent BamHI fragment spanning the UbxA22 deletion [now 1.6 kb] was cloned directly into M13, and sequenced across the deletion from the BamHI site.

Acknowledgments

Drs. Ed Lewis, Steven Kerridge, and Gines Morata generated and made available to us the UbxA mutations. We thank them also for invaluable discussions. David Hogness and many members of his laboratory provided materials and information essential to us—our thanks notably to Rob Saint for subclones of the UbxA region, Phil Beachy and Debra Peattie for the exchange of sequence information, and Kerry Kornfeld for details of the structure of variant UbxA cDNAs. We also thank Welcome Bender for providing details of additional variant UbxA cDNA clones and a clone of the UbxA mutant allele. Drs. Y.N. and L.Y. Jan generously provided the SD12 antibody, and Dr. Rob White the anti-UbxA antibody. We thank Iain Dawson for assistance in sequencing the UbxA22 deletion, Nick Vineall and llan Davis for allowing us to quote their unpublished results, Helen Moore and Elena Reoyo for carrying out much of the genetics, and Rosemarie Baines for preparing the manuscript.

Alain Ghysen is chercheur qualifie of the Fonds National de la Recherche Scientifique {Belgium}. J.M. Axton thanks the Durham fund of King’s College, Cambridge, for financial support. This work was supported by the Medical Research Council of Great Britain.

References

Akam, M.E., H. Moore, and A. Cox. 1984. Ultrabithorax mutations map to distinct sites within the bithorax complex of Drosophila. Nature 309: 635–637.

Akam, M.E. and A. Martinez-Arias. 1985. The distribution of Ultrabithorax transcripts in Drosophila embryos. EMBO J. 4: 1689–1700.

Beachy, P.A., S.L. Helfand, and D.S. Hogness. 1985. Segmental distribution of bithorax complex proteins during Drosophila development. Nature 313: 545–551.

Beachy, P.A. 1986. “The Ultrabithorax domain in the Bithorax complex of Drosophila.” Ph.D. Thesis, Stanford University.

Bender, W., M. Akam, F. Karch, P.A. Beachy, M. Peifer, P. Spierer, E.B. Lewis, and D.S. Hogness. 1983. Molecular genetics of the bithorax complex in Drosophila melanogaster. Science 221: 23–29.

Biggin, M.D., T.H. Gibson, and G.F. Hong. 1983. Buffer gradient gels and 35S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. 80: 3963–3965.

Cabrera, C.V., J. Botas, and A. García-Bellido. 1985. Distribution of Ultrabithorax proteins in mutants of Drosophila bithorax complex and its trans-regulatory genes. Nature 318: 569–571.

Casanova, J., E. Sánchez-Herrero, and G. Morata. 1985. Prothoracic transformation and functional structure of the Ultrabithorax gene of Drosophila. Cell 42: 663–669.

Green, S.H. 1981. Segment specific organization of leg motorneurons is transformed in bithorax mutants of Drosophila. Nature 292: 152–154.

Ghysen, A., R. Janson, and P. Santamaria. 1983. Segmental determination of sensory neurons in Drosophila. Dev. Biol. 99: 7–26.

Ghysen, A., L.Y. Jan, and Y.N. Jan. 1985. Segmental determination in Drosophila central nervous system. Cell 40: 943–948.

Hayes, P.H., T. Sato, and R.E. Denell. 1984. Homeosis in Drosophila: The Ultrabithorax larval syndrome. Proc. Natl. Acad. Sci. 81: 545–549.

Hogness, D.S., H.D. Lipshitz, P.A. Beachy, D.A. Peattie, R.B. Saint, M. Goldschmidt-Clermont, P.J. Harte, E.R. Gavis, and S.L. Helfand. 1985. Regulation and products of the UbxA domain of the bithorax complex. Cold Spring Harbor Symp. Quant. Biol. 50: 181–194.

Hooper, J. 1986. Homeotic gene function in the muscles of Drosophila larvae. EMBO J. 5: 2321–2329.

Jiménez, F. and J.A. Campos-Ortega. 1981. A cell arrangement specific to thoracic ganglia in the central nervous system of the Drosophila embryo: Its behaviour in homeotic mutants. Wilhelm Roux’s Arch. Dev. Biol. 190: 370–373.

Kerridge, S. and G. Morata. 1982. Developmental effects of some newly induced Ultrabithorax alleles of Drosophila. J. Embryol. Exp. Morphol. 68: 211–234.

Lewis, E.B. 1954. The theory and application of a new method of detecting chromosomal rearrangements in Drosophila melanogaster. Am. Nat. 88: 225–239.

—. 1963. Genes and developmental pathways. Am. Zool. 3: 33–56.

—. 1978. A gene complex controlling segmentation. Nature 276: 565–570.

—. 1981. Developmental genetics of the bithorax complex in Drosophila. ICN-UCLA Symp. Mol. Cell. Biol. 23: 189–205.

—. 1982. Control of body segment differentiation in Drosophila by the bithorax gene complex. In Proceedings of the 9th Congress of the international society of Developmental Biologists (ed. M.M. Burger), p. 269.

—. 1985. Regulation of the genes of the bithorax complex in Drosophila. Cold Spring Harbor Symp. Quant. Biol. 50: 155–164.

Lindsley, D. and E.H. Grell. 1968. Genetic variations of Drosophila melanogaster. Carnegie Inst. Wash. Publ. No. 627.

Lipshitz, H.D., D.A. Peattie, and D.S. Hogness. 1987. Novel transcripts from the Ultrabithorax domain of the Bithorax complex. Genes Dev. 1: 307–322.

Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Maxam, A.M. and W. Gilbert. 1980. Sequencing end labelled DNA with base-specific chemical cleavages. Methods Enzymol. 65: 499–560.

Minaña, F.J. and A. García-Bellido. 1982. Pre-blastoderm mosaics of mutants of the bithorax complex. Wilhelm Roux’s Arch. Dev. Biol. 191: 331–334.

Morata, G. and A. García-Bellido. 1976. Developmental analysis of some mutants of the Bithorax system of Drosophila. Wilhelm Roux’s Arch. Dev. Biol. 179: 125–143.
Morata, G. and S. Kerridge. 1980. An analysis of the expressivity of some bithorax transformations. In Development and neurobiology of Drosophila. (ed. O. Siddiqi, P. Babu, C. Hall, and J. Hall), pp. 141–154. Plenum Press, London.
——. 1981. Sequential functions of the bithorax complex of Drosophila. Nature 290: 778–781.
Sánchez-Herrero, E. and G. Morata. 1984. The Ubx syndrome of Drosophila: The prothoracic transformation (ppx) is independent of bx, bxd and pbx. Wilhelm Roux’s Arch. Dev. Biol. 193: 263–265.
Sánchez-Herrero, E., I. Vernós, R. Marco, and G. Morata. 1985. The genetic organization of the bithorax complex of Drosophila. Nature 313: 108–113.
Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. 74: 5463–5467.
Staden, R. 1982. Automation of the computer handling of gel reading data produced by the shotgun method of DNA sequencing. Nucleic Acids Res. 10: 4731–4751.
Struhl, G. 1982. Genes controlling segmental specification in the Drosophila thorax. Proc. Natl. Acad. Sci. 79: 7380–7384.
Teugels, E. and A. Ghysen. 1983. Independence of the numbers of legs and leg ganglia in Drosophila bithorax mutants. Nature 304: 440–442.
——. 1985. Domains of action of bithorax genes in Drosophila central nervous system. Nature 314: 558–561.
Thomas, J.B. and R.J. Wyman. 1984. Duplicated neural structure in bithorax mutants of Drosophila. Dev. Biol. 102: 531–533.
Walimoto, B.H. and T. Kaufman. 1981. Analysis of larval segmentation in lethal genotypes associated with the Antennapedia gene complex. Dev. Biol. 81: 51–64.
White, R.A.H. and M. Wilcox. 1984. Protein products of the bithorax complex in Drosophila. Cell 39: 163–171.
——. 1985a. Regulation of the distribution of Ultrabithorax proteins in Drosophila. Nature 318: 363–366.
——. 1985b. Distribution of Ultrabithorax proteins in Drosophila. EMBO J. 4: 2035–2043.
White, R.A.H. and R. Lehmann. 1986. A gap gene, hunchback, regulates the spatial expression of Ultrabithorax. Cell 47: 311–321.
Wilde, C.D. and M. Akam. 1987. Conserved sequence elements in the 5’ region of the Ultrabithorax transcription unit. EMBO J. (in press).
*Ultrabithorax* mutations in constant and variable regions of the protein coding sequence

*Genes Dev.* 1987, 1:
Access the most recent version at doi:10.1101/gad.1.4.386

References

This article cites 38 articles, 7 of which can be accessed free at:
http://genesdev.cshlp.org/content/1/4/386.full.html#ref-list-1

License

Email Alerting Service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.