The 3' Regulatory Region of the Abdominal-B Gene: Genetic Analysis Supports a Model of Reiterated and Interchangeable Regulatory Elements

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ABSTRACT

The Abdominal-B (Abd-B) gene is one of three genes in the bithorax complex, a cluster of homeotic genes in Drosophila. During embryogenesis Abd-B is expressed in a complex pattern, producing four different transcript classes, each of which exhibits a unique spatial pattern of expression. Proper regulation of the class A transcripts is required for appropriate development of the fifth through eighth abdominal segments and is mediated, in part, by a 60-kb regulatory region located 3' of the gene. We have isolated a new mutation, designated Abd-B^crass, which is caused by a deletion that leaves 15 kb of the 3' regulatory sequences immediately adjacent to the gene, but removes 45 kb of the more distant 3' regulatory elements. This mutation produces an unexpected homeotic segmental transformation of the fourth through seventh abdominal segments, and has been analyzed by genetic and molecular techniques. In situ hybridization to Abd-B^crass embryos shows a uniform and moderate level of the Abd-B class A transcript in the posterior abdomen, rather than the normal graded pattern of expression. Our analysis of the Abd-B^crass mutation has prompted a model of the 3' regulatory region of Abd-B based on reiterated cell type-specific elements controlled by adjacent position-sensitive activating elements. The gradient of Abd-B expression normally observed in the posterior abdomen appears to be achieved by varying the number of reiterated elements that are active in each segment.

THE body plan of the fruit fly Drosophila melanogaster, like that of many higher organisms, is based upon a reiterated segmental or metameric unit. Within this series of homologous units an extensive diversity of function can be achieved by varying parallel paths of development. In Drosophila two large gene complexes, the Antennapedia complex (ANT-C) and the bithorax complex (BX-C), control the critical determinative events required for the establishment of correct metameric identity. Mutations within the Antennapedia and bithorax complexes result in homeotic transformations in which one or more segments develop inappropriately to resemble segments normally more anterior or posterior (Lewis 1978; Kaufman, Lewis and Wakimoto 1980; Karch et al. 1985; reviewed by Mahaffey and Kaufman 1988). The genes of the ANT-C and BX-C encode DNA-binding proteins, each containing a homeodomain, which act as transcriptional regulators (McGinnis et al. 1984; Desplan, Theis and O'Farrell 1985; reviewed by Scott, Tamkun and Hartzell 1989). In mice and humans the gross organization of these genes is also very similar to that in Drosophila: they are members of large gene complexes and they are arrayed in colinear order along the chromosome with respect to the anatomical positions of the organism in which they are expressed (Graham, Papalopulu and Krumlauf 1989; Duboule and Dolle 1989).

Recent results in both flies and mice (Celniker et al. 1990; Whiting et al. 1991) suggest that adjacent homeotic genes share regulatory sequences, and that this is why separation of the genes has rarely occurred over long periods of evolutionary time. In fact, the genes of the BX-C do not have to be together in a single complex in order to function normally (Struhl 1984; Tiong, Whittle and Gribbin 1987), but in each case in which this has been demonstrated there is extensive duplication of the sequences between the separated genes. Analysis of the large regulatory regions serving these genes may help unravel the mystery behind the preservation of colinear clusters of homeotic genes.

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The subject of this work is the large 3' regulatory region of the Abdominal-B (Abd-B) gene of the bithorax complex. The Abd-B domain occupies the distal third of the BX-C, from approximately +90 kb to +200 kb on the standard molecular map (Figure 1). The Abd-B gene is required for the proper development of the posterior abdomen: abdominal segments five to eight (A5-A8; parasegments 10-14), the genitalia, and some terminal structures (SAKONJU and LEWIS 1989; ZAVORTINK and SAKONJU 1989). Spatial distribution of the Abd-B proteins in developing embryos parallels that of the corresponding transcript classes (CELNIKER, KEELAN and LEWIS 1989; ZAVORTINK and SAKONJU 1989). As indicated by the dashed lines, the 5' ends of the C and γ classes transcripts have not been precisely mapped. At the top of the figure the approximate extents of the genetically defined iab-5, iab-6 and iab-7 regions are indicated. The genomic fragments used as probes for the embryonic in situ hybridization experiments (LC426, JJ500, etc.) are shown above the coordinate line.

Mutations which inactivate specific subfunctions of the two abdominal genes of the BX-C are designated infra-abdominal mutations (KARCH et al. 1985). Genetically, these mutations act like loss-of-function modifications and cause segments to assume a more anterior identity. Breakpoint mutations that affect A5-A7 (iab-5, iab-6 and iab-7 mutations) map within a 60-kb region 3' of the Abd-B gene (Figure 1; KARCH et al. 1985). The vast majority of the mutations that have been isolated in this region are chromosomal breakpoints; note that an iab-6 breakpoint also removes iab-5 from proximity to Abd-B, and an iab-7 breakpoint also displaces iab-5 and iab-6. When hemizygous, iab-5 mutations cause the transformation of A5 to A4: in hemizygous iab-6 mutants A5 and A6 are transformed to A4; and hemizygous iab-7 transforms A5-A7 to A4. By genetic criteria these mutations appear to disrupt elements required for the cis-regulation of the Abd-B gene in A5-A7. They do not appear to affect Abd-B function in A8, in the genitalia, or in structures posterior to A8 (KARCH et al. 1985). It has recently been shown that iab-5,7 mutations disrupt the normal pattern of expression of the Abd-B protein (CELNIKER et al. 1990) and mRNA (SANCHEZ-HERRERO and AKAM 1989; BOULET, LLOYD and SAKONJU 1991) in A5-A7; expression in A8 and more posterior appears normal.
There are two characterized gain-of-function mutations that map within the iab-5,7 region: Miscadstral pigmentation (Mcp; LEWIS 1978) and Frontabdominal 7 (Fab-7; GYURKOVICS et al. 1990). These two mutations cause segments to assume a more posterior identity. Mcp causes a homeotic transformation in which the fourth abdominal segment develops in a fashion characteristic of the normal fifth abdominal segment (LEWIS 1978) and is caused by a 3.6-kb deletion from +94 to +97.6, within the iab-5 region (KARCH et al. 1985). It has been shown that this mutant exhibits ectopic expression of the Abd-B protein in A4 (CELNIKER et al. 1990; SÁNCHEZ-HERRERO 1991). Mcp revertant mutagenesis experiments have been used to isolate both new Abd-B alleles and many of the known mutations in the regulatory regions of the abdominal domains of the BX-C (KARCH et al. 1985). Fab-7 causes a transformation of A6 to A7 (GYURKOVICS et al. 1990). Fab-7 is also caused by a small deletion, approximately 4 kb, between +123 and +127 on the BX-C molecular map. Revertants of Fab-7 have been isolated and mapped to iab-6, iab-7, and to Abd-B (GYURKOVICS et al. 1990).

Most of the mutations within the 3′ regulatory region of Abd-B, the iab-5,7 region, are caused by chromosomal rearrangement breakpoints. More mutations caused by small deletions would be extremely informative and useful for the genetic and molecular characterization of this region. Using the bifunctional mutagen diepoxybutane (DEB), we have attempted to isolate mutations caused by small deletions within the Abd-B domain. The one mutation recovered within the iab-5,7 region is a deletion which removes 75% of the 3′ regulatory sequences controlling the Abd-B gene. Our analysis of this novel mutation, designated Abd-B^Df4^, supports a model of interchangeable cell type-specific regulatory elements organized in a redundant manner within the iab-5,7 region. In order to function, these elements appear to require in close proximity a second type of regulatory element that is responsive to positional information within the embryo.

**MATERIALS AND METHODS**

*Drosophila* culture and mutagenesis: Flies were maintained on standard cornmeal, molasses and yeast medium at 21–25°C. *Drosophila* stocks were kindly provided by EDWARD B. LEWIS (Caltech, Pasadena, CA). Mutations used in the mutagenesis experiments are described in LINDSLEY and ZIMM (1992) under the entry "BX-C.

Preliminary experiments with the bifunctional mutagen diepoxybutane (DEO) produced unsatisfactory results. DEO was tested at concentrations up to 100 mM for the ability to produce sex-linked lethals. One- to four-day-old (posteclosion) adult males were fed DEO in a 1% sucrose solution for 24 or 46 hr, those that survived were cross to compound-X females. Most flies subjected to the higher doses or longer feedings died; survivors were still fertile. In the F₁, evidence of sex-linked lethal induction was assayed by comparing the frequency of males to females, vs. that of control crosses. No mutagenic activity was detected.

A related compound, DEB, has been shown to be an effective mutagen in flies (CROSBY and MEYEROWITZ 1986; REARDON et al. 1987). For these mutagenesis experiments several different multiply marked third chromosome stocks were used as sources of parental males (see Table 1). A 10 mM solution of DEB in 1% sucrose was fed to 1–4-day-old adult males for approximately 24 hr. Different stocks were found to vary widely in their sensitivity to the sterility induced by higher doses of DEB. Due to this problem, in initial experiments 8 mM DEB was used.

DEB-treated males were mated en masse (approximately 20 pairs) in bottles to females heterozygous for a mutation within the Abd-B domain; the parents were transferred after 3 days and 6 days, and discarded after 9 days. The most effective chromosomes over which to screen were iab-4, iab-5, and Abd-B^Df4^ Mc (see Table 1; Abd-B^Df4^ is listed as iab-4^Df4^ in LINDSLEY and ZIMM 1992). The iab-4, iab-5 deletion was somewhat less satisfactory, since a disproportionate number of the putative new mutations recovered in trans to this chromosome were sterile in the F₁. The Abd-B^Df4^ Mc chromosome was ideal, since it allowed the detection of weak mutations and in addition covered the variable sterility associated with strong mutations within the Abd-B domain. (The Mc mutation is a small tandem duplication which includes Abd-B; LINDSLEY and ZIMM 1992.) Parental females from balanced stocks were used, so approximately half the F₁ adult males were scored over a balancer chromosome, usually TM1. A total of approximately 67,400 F₁ males were screened. An additional 5,380 males were screened in an attempt to recover DEB-induced revertants of the dominant mutation, Miscadstral pigmentation (Mcp).

F₁ males that exhibited changes in posterior abdominal pigmentation or bristle patterns were mated individually to females of the genotype Df(3R)P9/Dp(3;3)P5 or Abd-B^Df4^ Mc/TM1. The F₂ were screened for transmission of the abdominal phenotype, then used to establish stocks maintained over Dp(3;3)P5 or TM1. The complementation interactions of each new mutation over iab-7 and different classes of Abd-B alleles were analyzed; in some cases additional trans combinations were observed.

**Mounting adult abdominal cuticles:** Adult abdominal cuticles were mounted as described by DUNCAN (1982). Flies were preserved for one to ten days in a solution of one part glycerol in three parts ethanol. Abdomens were cut off, split mid-dorsally with a razor blade and incubated in a drop of 10% KOH on a slide for 3–5 min to partially digest the internal tissues. The cuticles were spread out using fine forceps, gently flattened under a coverslip, and placed on a slide warmer at 50°C for 3 hr. The cuticles were then finished, dehydrated in n-propanol and mounted in Euparal mounting medium (Carolina Biological).

**DNA methods:** DNA was prepared from adult flies of an appropriate genotype (homozygous, hemizygous or heterozygous with a characterized chromosome) by a miniprep procedure involving proteinase K digestion followed by several rounds of ethanol precipitation. Each DNA sample was used for at least two, usually three, different restriction digests and separated by gel electrophoresis. Whole genome Southern blots were prepared, using Hybond-C (Amer sham) nylon membranes. These were subjected to successive proings with radioabeled whole λ clones spanning the proximal portion of the Abdominal-B domain (+80 to +163; see Figure 1). Radiolabeled probes were prepared with the BRL random priming kit (Life Technologies, Inc.). Location of a chromosomal breakpoint was identified by consistent
polymorphisms observed with a least two restriction en-
zymes, when compared with the chromosome of origin. The
\(\text{Abd-B}^{C_{\alpha}}\) deletion simply lacked sequences corresponding to
several of the \(\lambda\) clones; a novel fusion fragment was identi-
\(\text{Abd-B}^{C_{\alpha}}\) also recovered. An additional duplication which ap-
pears to cover the entire BX-C was recovered in a
DEB mutagenesis experiment designed to recover rever-

\(\text{Abd-B}^{C_{\alpha}}\) acts like both a loss-of-function and

\(\text{Abd-B}^{C_{\alpha}}\) DEB mutagenesis: In an attempt to recover muta-
tional lesions of a moderate size (100 bp to 10 kb)
within the \(\text{iab}-5,7\) region, we conducted a series of
mutagenesis experiments using the bifunctional mu-
tagens DEB and DEO. Our results indicate that DEO
is not an effective mutagen in flies when administered
by feeding (see MATERIALS AND METHODS).

Following DEB mutagenesis \(F_1\) adult males were
examined for changes in the pigmentation pattern of
the abdominal tergites or for changes in the bristle
pattern of the posterior abdominal sternites. A varied
collection of new mutations was obtained, but only

\(\text{iab}-5,7\) region. Seven new alleles were recovered that act, by genetic
criteria, like lesions in the \(\text{Abd-B}\) transcripts, four of
the group 1 type and three of the group 3 type (Table
1). As defined by \textsc{Sanchez-Herrero} and \textsc{Crosby}
(1988), Group 1 \(\text{Abd-B}\) alleles complement \(\text{Abd-B}\)
mutations caused by breaks in the region from approxi-
mately +170 to +200 and \textit{appear} to affect the class A
transcripts only; group 3 alleles show a slightly more
extreme embryonic phenotype, fail to complement all
mutations in the \textit{Abdominal-B} domain, and are
thought to fall in exons shared by all \(\text{Abd-B}\) transcripts.

Newly recovered alleles were subjected to whole ge-

\(\text{iab}-5,7\) region, \(\text{T}^{Y}Y;3\text{iab}-8,9^{G_{\alpha}p215}\),. It is caused
by a lesion sufficiently large to be detected by this

\(\text{iab}-5,7\) region. A chromosomal breakpoint in
the \(\text{iab}-8,9\) region, \(\text{T}^{Y}Y;3\text{iab}-8,9^{G_{\alpha}p215}\), was isolated
due to the fact that, when heterozygous, it produces
variable abnormal pigmentation patterns and a reduc-
tion in the size of the \(\text{A6}\) tergite in adult males. One
large deficiency that removes the abdominal region of
the bithorax complex and one duplication that

covers the haploinsufficient phenotype of \(\text{Abd-B}\) were
TABLE 1

Summary of DEB mutagenesis experiments

<table>
<thead>
<tr>
<th>Chromosome screened over</th>
<th>No. of F1 males screened</th>
<th>Mutations recovered</th>
<th>Chromosome induced on</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abd-B^{14}Mc</td>
<td>20,700</td>
<td>Abd-B^{15}</td>
<td>sbb^{1} ss bx^{2m}</td>
<td>Group 3 allele</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abd-B^{40}</td>
<td>p^{a} bx sr e^{a}</td>
<td>Group 3 allele; 200-300-bp deletion at +154</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abd-B^{40}</td>
<td>p^{a} bx ss e^{a}</td>
<td>Group 1 allele</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abd-B^{40}</td>
<td>sbb^{1} ss bx^{2m}</td>
<td>Group 3 allele</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Df(3R)StP234</td>
<td>sbb^{1} ss bx^{2m}</td>
<td>Uncovers Abd-A and Abd-B</td>
</tr>
<tr>
<td>iab-4,508</td>
<td>9,200</td>
<td>Dp(3;3)StP522</td>
<td>sbb^{1} ss bx^{2m}</td>
<td>Covers Abd-B haploinsufficient phenotype</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abd-B^{62}</td>
<td>sbb^{1} bx d</td>
<td>Group 1 allele; very weak, homozygous viable and fertile</td>
</tr>
<tr>
<td>iab-5</td>
<td>2,500</td>
<td>Abd-B^{40}</td>
<td>sbb^{1} bx d</td>
<td>Group 1 allele; weak</td>
</tr>
<tr>
<td>iab-7</td>
<td>1,000</td>
<td>Abd-B^{40}</td>
<td>sbb^{1} bx d</td>
<td>44.5-kb deletion from +92 to +136.5</td>
</tr>
<tr>
<td>TM1</td>
<td>27,200</td>
<td>Abd-B^{40}</td>
<td>sbb^{1} bx d</td>
<td>Group 1 allele</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abd-B^{40}</td>
<td>sbb^{1} bx d</td>
<td>Group 4 Abd-B allele; breakpoint approximately +170-175</td>
</tr>
<tr>
<td>Other</td>
<td>6,800</td>
<td>Dp(3;3)StP563</td>
<td>p^{a} Mep</td>
<td>Covers Ubx and Abd-B haploinsufficient phenotypes</td>
</tr>
</tbody>
</table>

* Abd-B alleles categorized according to SANCHEZ-HERRERO and CROSBY (1988).

animals (Figure 2, 1 and J). Heterozygous Cst females appear normal (Figure 2, K and L). These results are consistent with the interpretation that the transformations of A4 and A5 result from a gain-of-function type of misregulation, but that the transformation of A7 is due to a loss of function. The heterozygous Corset phenotype may offer an explanation for why a gain-of-function mutation comparable to Mep and Fab, but effecting a transformation of A5 to A6, has never been recovered. Such a transformation may be recessive, or nearly so, and thus would not have been detected in the types of screens that have been conducted.

Most animals of the genotype Cst/Df(BX-C) [using Df(3R)P69 or Df(3R)StP234] die prior to pupation, thus we have observed only a few escapers of each sex. The phenotype of these animals is similar to that of Cst homozygotes, however, the transformed segments are less clearly A6 in character, usually appearing intermediate between an A5 phenotype and an A6 phenotype. In males A4 and A5 show some characteristics of A5, with 6–8 bristles on the A4 and A5 sternites; A6 appears almost normal, but displays a few bristles on the sternite; A7 shows a moderate to extreme transformation to A6 (data not shown).

Complementation behavior of Cst heterozygous with Abd-B was analyzed using Abd-B^{15} (a group 1 allele, believed to affect only the class A transcripts; see Table 1) and Abd-B^{40} (a group 3 allele, probably a null mutation, affecting all Abd-B transcripts; see Table 1). In males, Abd-B/+ has a haploinsufficient phenotype resulting in 2–8 bristles on the A6 sternite, the appearance of a very small A7 tergite, and genitalia which are protruding and rotated; heterozygous Abd-B females appear wild type. Males of the genotype Cst/Abd-B^{15} or Abd-B^{40} show a much more extreme transformation of A6–A7 to A5, including a large seventh abdominal segment; in these animals A4 also develops like A5 (Figure 2, M and N). In females this transformation can be observed only in A7, which is larger than normal and the sternite of which exhibits a bristle pattern characteristic of A3–A6 (Figure 2, O and P).

Corset abolishes the spatial gradient of Abd-B mRNA in the posterior abdomen: Embryos from Cst stocks were analyzed by in situ hybridization to determine if the distributions of Abd-B transcripts are altered in this mutant. Wild-type patterns of Abd-B expression have been described in detail elsewhere (SANCHEZ-HERRERO and CROSBY 1988; KUZIORA and MCGINNIS 1988) and will be reviewed only briefly here. In all descriptions both the affected parasegment and the segment whose anterior portion corresponds to that parasegment will be designated. (In the abdomen the visible adult structures are primarily derived from the anterior of each segment, for example, the visible parts of the fourth abdominal tergite are derived entirely from the ninth parasegment.) The probes that were used are indicated in Figure 1. The Abd-B class A transcripts are detected by the common probe, LC426, and by the class A transcript-specific probe, JJ500. In wild-type embryos these two probes reveal a graded pattern of expression in parasegments (PS) 9–12 (segments A4–A7). In PS9 (A4) Abd-B expression is detected at low levels only in some parts of the mesoderm during stage 13 (retracted germ band) and slightly later; in PS10 (A5) there is a low level of expression observed in various cell types,
starting early in stage 12 (beginning of germ band retraction); in PS11 (A6) an intermediate level of expression is observed starting slightly earlier during stage 11; and in PS12 (A7) a moderate level (at early stage 11, extended germ band) to a high level (after stage 14 in the condensing ventral nerve cord) of expression is observed (Figure 3, A–E). Both the level of labelling and the number of cells labeled decrease from PS12 to PS9 (A7 to A4). During stages 11–12 the pattern of cells that are labeled in each parasegment is successive in nature: cells surrounding the developing tracheal pits are labelled first, next a band of mesodermal cells appears labeled, and slightly later additional cells appear labeled (Figure 3, A and B). In
FIGURE 3.—Analysis of Corset by in situ hybridization to embryos. Probed with JJ500, specific for the class A Abd-B transcripts (see Figure 1). Embryos oriented anterior to left, and unless stated otherwise, dorsal at the top. Numbers indicate parasegments. (A, F) Fully extended germ band (stage 11). (B, G) Beginning of germ band retraction (early stage 12). (C, H) End of germ band retraction (stage 13/14). (D, I) Lateral views of the condensing ventral nerve cord (stage 16); staining of visceral mesoderm around hindgut is also conspicuous. (E, J) Ventral views of the condensing ventral nerve cord (stage 16). Expanded region of Abd-B expression is apparent as soon as expression anterior to PSI3 is visible (stage 11; F). At stage 16, the reiterated pattern in PSI9-12 typical of Corset can be clearly seen in the ventral nerve cord (J).

PS10 (A5) the cells surrounding the trachael pits are the only cells in which label is detectable at this stage (and only with high specific activity probes). At stage 13, there is detectable Abd-B expression in mesodermal cells underlying PS9 (A4) and PS10 (A5) (out of the plane of focus in Figure 3C), but expression in the ventral nerve cord appears to extend anteriorly only to PS11 (A6) (Figure 3C). Shortly after, labeling is observed in cells of the ventral nerve cord derived from PS10 (A5) (Figure 3D). It should be noted that the apparent temporal differences in Abd-B expression observed in the posterior abdomen could reflect an
actual temporal difference in the initiation of the Abd-B expression or, alternatively, could be the result of varying levels of transcription. A lower level of transcription would lengthen the lag time before the transcript accumulates to a detectable level.

The Cst mutation abolishes the gradient of Abd-B expression in these parasegments (PS9–12/A4–A7). Embryos from two different balanced Cst stocks were analyzed; of the embryos suitable for analysis at stage 11 approximately one quarter to one third (assumed to be the homozygous mutant animals) exhibit a uniform, intermediate level of Abd-B expression from PS9 (A4) to PS12 (A7) (Figure 3, F and G) in which the cells surrounding the tracheal pits are conspicuously labeled and mesodermal cells less conspicuously labeled; the level of expression in PS9 (A4) may be slightly lower than in the other three segments. The temporal gradient of expression is also no longer observed: expression in PS12 (A7) to PS10 (A5) appears simultaneously; expression in PS9 (A4) may lag slightly. Again, whether the temporal differences in expression are real or simply due to our inability to detect very low levels of expression is impossible to determine. In later stages in Cst animals expression of Abd-B in the ventral nerve cord (VNC) appears in a reiterating pattern from PS9 (A4) to PS12 (A7); the normal steep gradient of expression in the VNC is not observed (Figure 3, H–J).

Slightly less than half the embryos from the balanced Cst stocks exhibit a pattern of Abd-B expression that is less dramatically altered. These we assume to be heterozygous animals. In these animals Abd-B expression appears normal posterior to PS10 (A5), but extends forward to PS9 (A4), and is clearly visible in that segment and in PS10 (A5) in all preparations (Figure 4, D–F). Since in these in situ hybridization experiments normal Abd-B expression in PS10 (A5) is only barely detectable, expression in PS9 (A4) and PS10 (A5) appears to be higher in Cst/+ animals than the wild-type level in PS10 (A5).

In wild type, the class A transcripts are also expressed in PS13 (A8) to PS14 (A9) and in other posterior structures. Their expression in these segments appears essentially unaffected by the Cst mutation; expression in the visceral mesoderm of the hindgut may be somewhat reduced in homozygous animals. The specific spatial expression of the class B, C and γ transcripts of Abd-B can also be observed by in situ hybridization using transcript-specific probes. These three transcripts are normally confined to PS14 (A9) and PS15 (A10) (Sánchez-Herrero and Crosby 1988; Kuziora and McGinnis 1988; DeLorenzi et al. 1988). Using genomic probes that detect the class B, C and γ transcripts (Figure 1) the patterns of expression in embryos from balanced Cst stocks are indistinguishable from wild type (data not shown).

**Heterozygous Corset animals resemble homozygous Mcp:** The Miscadastral pigmentation (Mcp) mutation causes a transformation of A4 and A5 to a segment primarily A5 in character, with some characteristics of A6 (Lewis 1978; Duncan 1986). This mutation is caused by a much smaller deletion, approximately 3.6 kb, from +94.0 to +97.6 (Figure 1; Karch et al. 1985). The phenotype of Cst/+ animals is almost indistinguishable from that of homozygous Mcp: the A4 tergite of males is completely pigmented and there is a slight abnormality of fusion along the midline of the A4 and A5 sternites.

The embryonic pattern of Abd-B expression as observed in in situ hybridizations to heterozygous Cst is very similar, but not identical, to that of Mcp homozygous animals. In Mcp animals the level of expression in PS9 (A4) is comparable to or slightly exceeds that in PS10 (A5); the level of expression in PS11 (A6) is clearly higher than in the preceding two parasegments (Figure 4, A–C). In heterozygous Cst animals the level of expression in PS9 (A4) is slightly lower than in PS10 (A5); the level of expression in PS10 appears closer to that in PS11 (A6) (Figure 4, D–F). Expression of Abd-B in PS9 (A4) and PS10 (A5) is also detected slightly earlier in Cst heterozygous animals than in Mcp homozygotes. Abd-B protein distribution in Mcp mutants has been described by Celniker et al. (1990) and Sánchez-Herrero (1991) and parallels that of the Abd-B transcript, except that detectable levels of the protein are not observed in PS9 (A4) and PS10 (A5) until after germ band retraction.

**Distal iab-7 breakpoints result in ectopic expression of Abd-B:** For purposes of comparison, four chromosomal rearrangements which break in the iab-7 region, the region of the distal Cst breakpoint, were analyzed in detail. The molecular breakpoints within the BX-C of two of these, iab-7\textsuperscript{C66} and iab-7\textsuperscript{MN2}, were known (Karch et al. 1985); the breakpoints of iab-7\textsuperscript{64} and iab-7\textsuperscript{770} were determined by whole genome Southern analysis (see Figure 1). In these four mutants an amount of the iab-5,7 region comparable to or exceeding that in Cst remains in cis 3' of the Abd-B gene. Genetic analyses of the phenotypes and complementation behavior of the iab-7 mutations suggest that Abd-B is no longer expressed in segments A5–A7 (PS10–12), but is normally expressed in segment A8 (PS13) and more posterior (Karch et al. 1985; Duncan 1987; Celniker et al. 1990; M. Crosby and R. Tautvydas, unpublished results). This prediction is supported by analyses of Abd-B protein distribution in iab-7 mutants (Celniker et al. 1990; Boulet, Lloyd and Sakonju 1991; Sánchez-Herrero 1991). These results suggest that iab-7 mutations affect expression of the class A transcripts, since this is the only class normally expressed in PS10–12 (A5–A7); our results confirm that only the class A transcripts
are affected in these mutants (see below).

The typical *iab-7* phenotype is the transformation of the fifth through seventh abdominal segments to a pattern of development normally observed for the fourth or fifth abdominal segment (KARCH et al. 1985; CELNIKER et al. 1990). In males carrying *iab-7* mutations, either as hemizygotes, as trans-heterozygotes, or in trans-combinations with *Abd-B*, there is a wide variability in the amount of pigment observed on the fourth through seventh tergites. It has been postulated that this is due to misexpression of *abdominal-A* in these animals, rather than that of *Abd-B* (CELNIKER et al. 1990). However, by genetic criteria most aspects of the *iab-7* phenotype appear to be the result of complete loss of *Abd-B* expression in A5–A7. Despite the fact that their breakpoints span a region of 11–15 kb, the four *iab-7* mutations analyzed produce very similar phenotypes; even *iab-7*^{164} and *iab-7*^{270}, which break over 20 kb downstream from the *Abd-B* gene, produce extreme *iab-7* phenotypes.

In Corset animals, the same downstream region, the 15 kb immediately 3' of the *Abd-B* gene, appears able to direct expression of the gene at levels high enough to have a clear phenotypic effect. The only *iab-7* mutation for which there is phenotypic evidence that the elements in *iab-7* are active at all is *iab-7*^{49982}, in which heterozygous adult animals display patches of abdominal or genital cuticle on the head (AWAD et al. 1981; KARCH et al. 1985). The appearance of ectopic abdominal tissue in this mutant argues that, at least in later stages, *iab-7* elements are active and directing some appropriate cell type-specific expression of *Abd-B*. Obviously, correct spatial bounds of expression have not been maintained.

We have extended previous analyses of *Abd-B* mRNA distribution in *iab-7* mutants (SÁNCHEZ-HER-
and Akam 1989; Boulet, Lloyd and Säkonju 1991), using nonradioactive in situ hybridization to embryos. We have used high specific activity probes in order to detect relatively low levels of Abd-B expression. We have also distinguished between the different transcript classes by using genomic probes specific to a single class (see Figure 1). The normal expression of the Abd-B class A transcripts is dramatically altered in these mutants. This is most easily observed in the ventral nerve cord (VNC) of late embryos, at stages 14–17; the normal expression of Abd-B in PS10 (A5) to PS12 (A7) is absent in mutant embryos (Figure 5A; Sánchez-Herrero and Akam 1989; Boulet, Lloyd and Säkonju 1991). Usually, there is absolutely no detectable Abd-B expression in the VNC in these segments. As described below, in the case of iab-7^2M^2, there is a very low level of ectopic expression in a few cells within each neuromere of the ventral nerve cord detected by probes for the class A transcripts. For all four mutants only the distribution of the class A transcripts is atypical; distributions of the B, C and γ classes appear normal (data not shown). The distribution of the class A transcripts in A8/PS13 and more posterior also appears normal (Figure 5A).

Two of the iab-7 breakpoints result in detectable ectopic expression of Abd-B. For iab-7^SG^62 we suspected that this might be the case, since iab-7^SG^62 mutants show phenotypic evidence of ectopic expression. Embryos carrying iab-7^SG^62 exhibit Abd-B expression during stage 13–16 in the anterior midgut and the proventriculus (Figure 5B). In stage 16 animals a low level of expression is also observed in several discrete spots dorsal to the pharynx, possibly elements of the stomatogastric nervous system (Figure 5B). In iab-7^2M^2 embryos ectopic Abd-B expression is detected only with high activity probes, but is rather extensive: after germ band retraction in mesodermal tissues (Figure 5, C and D); and during stages 16–17 in the VNC, in a few cells in each neuromere (Figure 5E). These tissues are sites of normal Abd-B expression in the posterior abdomen of the embryo.

In animals carrying either iab-7^164 or iab-7^770 the 3' regulatory elements remaining in cis to the Abd-B gene appear to be inactive, or very nearly so, despite the fact over 20 kb of the 3' sequences remain intact downstream of the Abd-B gene. In both mutants Abd-B expression is usually observed only in PS13 (A8) and more posterior, with no detectable expression in PS10 (A5) to PS12 (A7) even in later stages (Figure 5A). The exception is that occasional embryos carrying iab-7^164 exhibit a low level of ectopic expression during stages 13–14 in some mesodermal cells in the thorax and abdomen; this is most conspicuous in hemizygous animals (Figure 5F). However, this ectopic labeling is not detected in all stage 13–14 embryos carrying iab-7^164, perhaps because it is at the limits of the sensitivity of the technique, or possibly because it is artificial.

Thus the effect of the iab-7 chromosomal breakpoints upon Abd-B expression is dramatically different from that of Corset, despite the fact that 10 to 20 kb of the 3' regulatory sequences remain in normal proximity to the gene in all of these mutations. In the case of the iab-7 mutations, separation of these 3' regulatory elements from downstream iab-5,7 elements appears to render them dysfunctional. In Corset, the same 3' regulatory elements are again separated from the downstream iab-5,7 elements, but they are now adjacent to a different portion of the BX-C, the iab-4 region. This configuration appears to permit their nearly normal activity.

The iab-4,5^DB deletion results exclusively in loss-of-function transformations in the posterior abdomen: The effects of an existing deletion within a similar size range (28 kb) which removes part of iab-4, all of iab-5, and part of iab-6 (Figure 1; Karch et al. 1985; Busturia et al. 1989), were also compared to those of Cst. In homozygous animals the iab-4,5^DB deletion appears to cause exclusively loss-of-function transformations in the posterior abdomen. Externally A4 appears normal, however, that iab-4 elements are affected is indicated by the sterility of homozygous animals of both sexes (Cumberledge, Szabad and Säkonju 1992; Szabad and Nothiger 1992). The sterility phenotype is complemented by Abd-B domain mutations (iab-5^AB; Abd-B^M2, Abd-B^M2), but not by deletions of abd-A [Df(3R)P2; Df(3R)Ubx^100]. It has been shown that the distribution of Abd-A protein in these animals is altered in a few cell types, including the gonads (Karch, Bender and Weiffenbach 1996).

In homozygous iab-4,5^DB males the fifth abdominal segment has the characteristics of a normal fourth abdominal segment and the sixth abdominal segment appears to be partially transformed toward A4; this phenotype is also observed in animals of the genotype iab-4,5^DB/Abd-B (Karch et al. 1985; Busturia et al. 1989).

Analysis of Abd-B transcript distribution in iab-4,5^DB embryos by in situ hybridization supports the interpretation that the A5 phenotype is due primarily to a loss of Abd-B expression. Abd-B class A transcripts are completely missing from PS10 (A5) and appear to be slightly reduced in abundance in PS11 (A6) (Figure 5G) compared to the heterozygote (Figure 5H). There is no evidence of ectopic expression. Analysis of the spatial distribution of Abd-B protein in this mutant has provided similar results (Sánchez-Herrero 1991). Despite the fact that, like Corset, the iab-4,5^DB deletion is not associated with a translocation or other rearrangement that moves the Abd-B gene away from the rest of the BX-C, its effects upon Abd-B expression
are more similar to those of the iab-5,7 chromosomal breakpoint mutations than those of Corset.

**DISCUSSION**

We have isolated a novel regulatory mutation within the iab-5,7 region of the bithorax complex which we have named Abd-B**corset**. In homozygous Corset animals the fourth through seventh abdominal segments all develop like the normal sixth abdominal segment. This mutation is a deletion which removes 75% of the 3′ regulatory sequences required for properly controlled expression of Abd-B in segments A4–A7 (PS9–12). The effect of this loss is an increase in the levels of Abd-B expression in parasegments 9 and 10 (A4 and A5) and a simultaneous decrease in expression in parasegment 12 (A7). Instead of the usual graded pattern of embryonic Abd-B expression in PS10–12 (A5–A7), a reiterating pattern of expression, comparable to the normal level and normal cell types observed in PS11 (A6), is observed in PS9–12 (A4–A7).

The Corset phenotype is unlike that of any previ-
ously known mutations within the 3' regulatory region of AbdB. Two gain-of-function mutations have been previously characterized: Mep (Lewis 1978; Duncan 1986), and Fab-7 (Gyurkovics et al. 1990). Both of these mutations are caused by relatively small deletions, less than 4 kb in size, which leave most of the iab-5,7 region intact (Figure 1; Karch et al. 1985; Gyurkovics et al. 1990). Larger lesions such as chromosomal breakpoints or the iab-4,50 deletion (Figure 1) produce primarily loss-of-function phenotypes (Karch et al. 1985; Corset is a dramatic exception.

The iab-5,7 region appears to be composed of arrays of interchangeable regulatory elements: The most surprising aspect of the Cst phenotype is that the posterior abdominal segments all develop in a manner typical of the normal A6 segment, despite the fact that the 3' cis-regulatory elements previously thought to be required for A6 development are completely missing. In the case of heterozygous animals the same mutation causes transformation to A5, even though the region thought to be required for A5 development is also deleted.

In iab-6 mutants, caused by breakpoints in the region from +105 to +125, A7 develops normally (or shows slight effects) but A5 and A6 exhibit loss-of-function transformations to A4 or A5 (Karch et al. 1985; Celniker et al. 1990). It has been previously shown that the distribution of Abd-B protein in an iab-6 mutant appears normal in PS12 (A7), but is missing entirely from PS10-11 (A5-A6) (Celniker et al. 1990; Boulet, Lloyd and Sakonju 1991). Thus, it appears that the region from +125 to +151, the iab-7 region, is sufficient for normal development of A7 but not of A5 or A6. In contrast, in Corset animals a portion of the iab-7 region, from +136 to +151, supports a pattern of Abd-B expression similar to that normally observed in A6. In other words, a subset of the cis-acting elements that normally direct development of A7 is capable of directing A6-type development. Under certain conditions, as in the fourth abdominal segment in heterozygous Cst animals, the same elements direct A5-type development.

It should be pointed out that in wild-type embryos the pattern of Abd-B expression in A6 (PS11) is a subset of that in A7 (PS12), and the pattern in A5 (PS10) is a subset of that in A6. This is unlikely to be true later in development. The development of A5, A6 and A7 are very similar in the embryo, however, in adults these segments are distinctly different. In the adult male, for example, there are virtually no cuticular structures derived from A7.

These results can be explained by a model of the organization of the iab-5,7 region in which it is assumed that many of the regulatory elements in this region are reiterated and interchangeable (Figure 6A). This has been previously suggested by Boulet, Lloyd and Sakonju (1991). Such redundant elements may be cell type-specific or tissue-specific elements. However, it is also possible that the redundant elements simply serve to increase expression and that cell type-specific elements are distinct entities. We postulate that the gradient of Abd-B expression observed in the posterior segments is achieved by varying the number of such reiterated elements that are active in each segment (Figure 6). The elements in iab-7 may not be different, there may simply be more of them. Alternatively, there may also be elements that are unique to iab-6 and iab-7 or to iab-7 alone. In the case of Corset, the same set of regulatory elements appears to be active in A4 to A7 and there is no longer a gradient of Abd-B expression (Figure 6C).

Although it is not possible to precisely quantify levels of expression observed by in situ hybridization, the levels of Abd-B expression in A4-A7 in Corset embryos appear to approximate those normally observed in A6, not the higher levels observed in A7. This is easily explained if the primary difference between iab-6 and iab-7 is simply the number of regulatory arrays. The fact that, in normal animals, the pattern of cell types expressing Abd-B also differs in A5, A6 and A7 (PS10-12) can be explained by a model of arrays of redundant elements as well, if it is assumed that in different cell types different thresholds of regulatory factors are required to elicit detectable expression of Abd-B. For example, the reduced number of iab-7 elements present in Cst may be sufficient to elicit expression during stage 11 in the cells surrounding the tracheal pits and in the mesoderm. Elements which regulate expression in other cell types may still be present, but not in sufficient numbers to induce detectable Abd-B expression in these cells. However, as mentioned above, it is possible that there are also unique elements in iab-6,7 that are deleted in the Corset mutation. Irvine, Helfand and Hogness (1991) suggest that a similar type of organization, with multiple elements that are in some cases interchangeable, may exist within the large 5' regulatory region of the Ultrabithorax gene.

We postulate that the different developmental pathways followed by A5, A6 and A7 are mediated by quantitative differences in Abd-B expression (class A transcripts). The fact that animals that are Cst/+ exhibit a transformation to A5 instead of A6, resembling very closely homozygous Mep animals, supports the idea that differences in Abd-B expression in these two segments are quantitative rather than qualitative. In addition, the resemblance to Mep, which is assumed to be caused by ectopic function of regulatory elements in the iab-5 region (Figure 6B), lends support to a model postulating similar regulatory elements within iab-5 and iab-7. The homozygous Cst pheno-
A. Wild type

Regulation of the Abdominal-B Gene

A. Wild type

abd-A domain ——— Abdominal-B domain

lab-4  lab-5  lab-6  lab-7

+80  +90  +100  +110  +120  +130  +140  +150

B. Mcp

lab-4  lab-5  lab-6  lab-7

+80  +90  +100  +110  +120  +130  +140  +150

lab-4  lab-5  lab-6  lab-7

Mcp

C. Corset

lab-4  lab-5  lab-6  lab-7

+80  +90  +100  +110  +120  +130  +140  +150

Corset

Figure 6.—Model of the 3′ regulation of the Abdominal-B class A transcripts and effects of the mutations Mcp and Corset. There is some evidence that regulatory regions of abd-A and Abd-B overlap in the region of iab-4,5 (Greiner et al. 1990); this is indicated by the arrows above the maps. Below each map is indicated the theoretical state of the 3′ regulatory sequences in abdominal segments four through seven (A4–A7). Patterned ovals represent reiterated cell type-specific or tissue-specific regulatory elements. Larger white ovals represent position-sensitive regulatory elements required for the function of the cell type-specific elements; each white oval represents a unique element or group of elements, responsive to different positional information. Solid black regions represent inactive regions. In Corset animals a subset of iab-7 elements is juxtaposed to regulatory elements of iab-4, promoting a pattern of development typical of the normal A6 in segments A4–A7. See Discussion for further explanation.

type demonstrates that there are similar elements within iab-6 and iab-7.

The paucity of iab-5,7 mutations recovered in the DEB-mutagenesis experiments also implicates a high degree of redundancy (or a lot of spacer DNA) within this regulatory region. The chromosome used most successfully in the mutagenesis experiments (Abd-B$^{D14}$ Mc) allows detection of most weak transformations, and simultaneously covers the variable sterility associated with some mutations of the Abd-B domain. Over this chromosome it is not likely that many visible mutations (except possibly iab-5-types) were missed or lost. The maximum probable target size for the type of Abd-B mutations detectable in this screen (those that affect the class A transcript) is 15 kb; the size of the iab-5,7 region is approximately 60 kb. Seven Abd-B alleles were recovered, one caused by a 200–300-bp deletion and six caused by lesions too small to be detected. With a target size at least four times as large, it is reasonable to assume that the iab-5,7 region did suffer many such small lesions, but that none produced a detectable phenotype. One mutation of this type has been isolated previously: Gyrkovics et al. (1990) report that a 1-kb deletion from +143 to +144 produces a typical iab-7 loss-of-function phenotype.

An additional class of cis-acting elements is required for the activity of the cell type-specific elements: We hypothesize that on the Corset chromosome the remaining iab-7 elements are providing the information necessary to effect development appro-
priate for the posterior abdomen, but that elements within the inappropriately juxtaposed iab-4 region are providing both an activating function and a function able to confer spatial specificity. The elements in iab-7 when separated from the rest of the BX-C, as in iab-7 breakpoint mutations, appear to be inactive and/or unable to respond to appropriate positional information. However, the same elements on the Corset chromosome appear to be active. These observations have lead us to postulate the existence of specific activating elements that must be in close proximity and are required to activate the redundant and cell type-specific elements (Figure 6). These are the elements that interact with and respond to position-specific molecules, such as the gap gene products. According to our model, there is such an element or elements in the iab-4 region that is critical for the unique Corset phenotype. The interaction of a particular position-responsive activating element with adjacent redundant elements appears to prevent their interaction with more distal position-responsive elements; the loss of the normal position-specifying element allows interactions with a more distal element to occur.

Based upon the phenotypes of iab-6 and iab-7 mutants, elements normally required for activity of the iab-7 cell type-specific elements are located at approximately +125. A portion of the iab-4 region appears able to replace that function, promoting the activity of the iab-7 elements remaining on the Corset chromosome, but in an inappropriate parasegmental pattern. Random rearrangements may occasionally break close to a comparable element: the iab-7SGA62 mutation appears to reposition the distal iab-7 region in proximity to a comparable element that promotes the activity of the remaining iab-7 cell type-specific elements in the head of the animal, producing adults with abdominal cuticle originating from the posterior part of the head. In the cases of iab-7MS2 and iab-7154, a subset of the iab-7 cell type-specific elements may be slightly active.

The same region in iab-4 may be responsible for the extension of Abd-B expression in both Mep and Corset (Figure 6, B and C). It is interesting that the iab-4,5DB deletion, which extends further into the iab-4 region, does not result in the extension of Abd-B expression into A4. Thus, these postulated elements appear to be between +85 (the left breakpoint of iab-4, 5DB) and +92 (the left breakpoint of Corset).

Although analysis of the iab-7 breakpoint mutations suggests the existence of such a position-responsive activating element or elements at approximately +125, it appears that the Fab chromosome retains most elements necessary for normal function of the iab-7 region. Several iab-6 breakpoint mutations have been isolated as Fab revertants; animals carrying these mutations exhibit a nearly normal seventh abdominal segment (GYURKOVICS et al. 1990).

**Interactions between different types of regulatory elements within iab-5,7:** A series of regulatory interactions within the iab-5,7 region appears to be necessary to initiate appropriate Abd-B expression during embryogenesis. The initial interaction, which may be either positive or negative, is between discrete sequences within the region and trans-regulatory molecules which confer positional information (SIMON et al. 1990; QIAN, CAPOVILLA and PIRROTTA 1991; ZHANG et al. 1991). This step is probably effected by the products of the gap genes and pair-rule genes (WHITE and LEHMANN 1986; INGHAM and MARTINEZ-ARIAS 1986; MARTINEZ-ARIAS and WHITE 1987; IRISH, MARTINEZ-ARIAS and AKAM 1989; REINITZ and LEVINE 1990; QIAN, CAPOVILLA and PIRROTTA 1991; ZHANG et al. 1991). Our results suggest that such position-responsive elements are in close proximity to (or may also serve as) activating elements. Once positional information has been transmitted to the activating elements, adjacent cell-type-specific elements are activated in appropriate parasegments. Analysis of the Corset mutation lends strong support to the idea that the cell type-specific elements are not themselves responsive to the position-specific regulatory information provided by the early trans-regulatory molecules (PEIFFER, KARCH and BENDER 1987; SIMON et al. 1990); rather, they interact somehow with the adjacent BX-C elements that receive that positional information.

Analysis of Corset has also led to the hypothesis that many of the the cell type-specific elements within this region of the BX-C are reiterated and interchangeable. The gradient of Abd-B gene expression normally observed in the posterior abdomen may be achieved by simply activating more and more copies of reiterated regulatory elements in successive segments.

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**LITERATURE CITED**


Regulation of the Abdominal-B Gene


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