Genetic structure of the abd-A gene of Drosophila

ANA BUSTURIA*, JORDI CASANOVA**, ERNESTO SÁNCHEZ-HERRERO***, ROSA GONZÁLEZ and GINES MORATA

Centro de Biología Molecular, Universidad Autónoma de Madrid, Canto Blanco, Madrid 28049, Spain.

Authors present addresses: *Zoologisches Institut der Universität Zurich, Wintherthurestrasse 190, 8057 Zurich, Switzerland.
**Neurobiology and Behaviour Center, Columbia University, 722 West 168th St., New York NY 10032.
***Genetics Department, University of Cambridge, Downing Street, Cambridge CB2 3EH

Summary

We report the embryonic and adult phenotypes of a number of mutations of the abd-A gene of the bithorax complex. Some of them result in loss of abd-A function in the whole abd-A domain and are usually lethal. These probably eliminate or inactivate abd-A protein products. Other mutations affect only part of the abd-A domain. These are viable, appear to map outside the abd-A transcription unit, and presumably alter the normal spatial regulation of abd-A products.

We propose a model of abd-A structure based on a protein-coding region and two cis-regulatory regions. Regulatory region 1, 3' to the transcription unit, contains positive and negative regulatory elements. Regulatory region 2, 5' to the transcription unit, establishes the correct level of abd-A activity in the abdominal metameres.

Key words: Drosophila, homeotic genes, bithorax complex.

Introduction

The bithorax complex (BX-C) specifies the identity of the portion of the body of Drosophila between and including parasegments 5 and 13 (Lewis, 1978; Sánchez-Herrero et al. 1985). The BX-C consists of three genes (Sánchez-Herrero et al. 1985; Tiong et al. 1985) termed Ultrabithorax (Ubx), abdominal-A (abd-A) and Abdominal-B (Abd-B) each one containing a homeobox (McGinnis et al. 1984; Scott and Wiener, 1984; Regulski et al. 1985). Of these, abd-A is responsible for the specific identity of parasegments 7 to 9 and is also necessary for the correct development of parasegments 10 to 13. Like the rest of the BX-C, abd-A has been cloned and there is a preliminary molecular characterization of its structure (Karch et al. 1985; Regulski et al. 1985; Rowe and Akam, 1988). A number of mutations of abd-A have been reported (Sánchez-Herrero et al. 1985; Tiong et al. 1985; Karch et al. 1985) but the genetic and phenotypic description of many of these mutations is still incomplete. There is not a genetic dissection of abd-A comparable to those of Ubx and Abd-B (Casanova et al. 1985a; Peifer and Bender, 1986; Casanova et al. 1986).

In this paper, we describe 19 mutations of abd-A, some of which have been defined molecularly by Karch et al. 1985. Our principal aim is to understand the functional components of abd-A. We find that some abd-A mutations eliminate or severely reduce abd-A functions and map at or close to the homeobox-containing unit. Other abd-A mutations map outside the transcription unit and alter the local distribution of abd-A products. The latter define regulatory regions of the gene.

Materials and methods

abd-A mutations used and their names

The majority of the abd-A mutations used in this work (Table 1) have already been reported, but not described, in papers by Lewis, 1978; Kuhn et al. 1981; Sánchez-Herrero et al. 1985; Tiong et al. 1985 and Karch et al. 1985. Appropriate references are given in the main text. Some mutations have been induced in our laboratory while others come from other sources, especially from Welcome Bender and Francois Karch. In naming the mutations in this paper, we have followed the suggestion of Duncan (1987) and have distinguished between mutations affecting the whole domain, labelled abd-A, and those only affecting part of the domain, which are named infraabdominal (iab) followed by a number indicating the abdominal segment where they have the most prominent effect. This nomenclature, while sensible, is not devoid of problems; mutations of the second category are often difficult to classify because they affect similarly more than one metamere. Moreover, many of them exhibit dominant gain-of-function as well as recessive loss-of-function phenotypes. In renaming some of the mutants, we have considered only the recessive phenotypes.

We also describe here some new mutations: abd-A\textsuperscript{MX3} and abd-A\textsuperscript{MX4}, which have been X-ray-induced in our laboratory,
and $iab^{-2\text{M1}}$, that appeared spontaneously in a Cy/Pm; Sb/TM2 stock.

Except in the cases described below, all these mutations complement those of $Ubx$ and $Abd-B$, hence only the $abd-A$ gene is altered. The exceptions are $abd-A^{C3}$ which contains a $bx$ mutation, $iab^{-2\text{Uab}3}$ which has a breakpoint in $bx$ and another in the $r$ region of $Abd-B$ (Karch et al. 1985; Casanova et al. 1986) and finally $iab^{-4\text{iab}3}$ which is associated with a deletion removing parts of both $abd-A$ and $Abd-B$. The presence of these mutations does not affect the $abd-A$ phenotypes reported in this paper as the tester chromosomes we have used (see Results) have normal doses of $Ubx$ and $Abd-B$.

In many instances in which it was necessary to discriminate the dominant from the recessive phenotype of the $abd-A$ mutations, we used the $T(1;3)P115$. In this rearrangement (see Casanova et al. 1985b for description), a chromosomal portion, $(Ubp115)$ carrying a normal set of BX-C genes has been translocated to the first chromosome. It can be segregated to produce flies with supernumerary copies of the BX-C.

Measure of the homeotic transformations

The assessment of homeosis in the abdominal segments is difficult because they have similar patterns. We find it, for example, very hard to distinguish the third (A3) from the fourth (A4) abdominal segment, therefore we may have overlooked transformations of one into the other. The segment A1 is clearly different from the rest because it contains in the dorsal side (tergite) bristles that are finer and shorter than those of the other segments. It also contains a characteristic cuticular arch. The posterior compartment of A1 (Kornberg, 1981) is the only posterior abdominal compartment that can be identified morphologically in the adult. On the ventral side (sternite), the A1 segment has no bristles whereas the other segments have between 15 and 20. Thus any transformation of A1 into a more posterior segment or vice versa can readily be distinguished.

Transformations involving segments A2–A4 are the most difficult to see because their tergites are very similar. Ventrally the sternite of A2 has a distinctive bristle pattern and contains a Wheeler’s organ. Also the pair of sensillae that appear in the anterior region are further apart in A2 than in other sternites. The A5 and A6 segments can be distinguished in male flies on the basis of the pigmentation of the tergite and the number of the bristles of the sternite of A6.

Cuticle preparations

Embryos were prepared for microscopic inspection following the standard method of Van der Meer (1977). Adult cuticle was digested with hot KOH, dehydrated with propanol and mounted in Canada Balsam.

$\beta$-gal staining of the adult cuticle

Abdomens were cut from freshly closed flies fixed in 0.1% glutaraldehyde (Fluka) and washed with PBS. They were then transferred to X-gal solution (0.2% 5-bromo-4-chloro-3-indoly-$\beta$-galactopyranoside in 10 mm-sodium pyrophosphate, 0.5 mm-NaCl, 1.0 mm-MgCl2, 5 mm-potassium ferricyanide, 5 mm-potassium ferrocyanide) and stained for several hours at 37°C. Stained abdomens were then dehydrated through a series of alcohols and mounted in Canada Balsam.

Results

(1) Classification of the $abd-A$ mutations used

All the mutations at the $abd-A$ locus used in this work are listed in Table 1. They have been characterized with reference to the combination $DpP10$ $DF109$ (Morata et al. 1983), which we consider as a complete lack of $abd-A$ function. This is lethal and gives a very strong $abd-A$ phenotype. In this genotype, most of $abd-A$ DNA, from +33–+36 to +86–93 (Karch et al. 1985), is deleted. Thus no $abd-A$ protein product can originate from this combination. It complements all $Abd-B$ alleles, so the $Abd-B$ gene appears to be intact. It also contains a normal $Ubx$ gene in the $DpP10$ fragment.

We have divided the mutations in two groups: (1) those that have loss-of-function phenotypes similar to that of $DpP10$ $DF109$ (hereafter referred to as $DF$), which affect the entire $abd-A$ domain and (2) those whose phenotypes affect only part of the $abd-A$ domain and/or which are associated with gain of function of $abd-A$ outside the normal domain. Those of the first group will probably eliminate or inactivate executive $abd-A$ gene products, whereas the second group will include mutations altering regulatory elements of the gene.

(2) Mutations eliminating or reducing $abd-A$ function in the entire $abd-A$ domain

Except those involving $abd-A^{M3}$, all the homozygotic or trans-heterozygotic combinations of these mutations were zygotic lethal. We have examined the hemizygous embryonic phenotype of all them. Those of $DF$ and $abd-A^{P10}$ (formerly $T(2;3)P10$) have already been described (Morata et al. 1983).

Except in the cases of $abd-A^{P10}$, $abd-A^{C26}$ and $abd-A^{M2}$, the embryonic phenotypes were very similar and like that of $DF$; abdominal parasegments 7–9 are completely transformed into parasegment 6 and parasegments 10–13 also resemble parasegment 6 but the transformation is not complete (Sánchez-Herrero et al. 1985). Of the exceptions, $abd-A^{P10}$ and $abd-A^{C26}$ show

<table>
<thead>
<tr>
<th>Affecting the entire domain</th>
<th>Affecting part of the domain</th>
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<tbody>
<tr>
<td>$abd-A^{M1}$</td>
<td>$iab^{-2\text{K}}$</td>
</tr>
<tr>
<td>$abd-A^{M2}$</td>
<td>$iab^{-2\text{M}1}$</td>
</tr>
<tr>
<td>$abd-A^{M2a}$ ($iab^{-2\text{M2a}}$)</td>
<td>$iab^{-2\text{M}1}$</td>
</tr>
<tr>
<td>$abd-A^{M2k}$</td>
<td>$iab^{-2\text{M}1}$</td>
</tr>
<tr>
<td>$abd-A^{M3}$</td>
<td>$iab^{-2\text{M}1}$</td>
</tr>
<tr>
<td>$abd-A^{P10}$ ($TP10$)</td>
<td>$iab^{-2\text{M}1}$</td>
</tr>
<tr>
<td>$abd-A^{CA6}$ ($iab^{-2\text{CA6}}$)</td>
<td>$iab^{-2\text{M}1}$</td>
</tr>
<tr>
<td>$abd-A^{CA6}$ ($iab^{-2\text{CA6}}$)</td>
<td>$iab^{-2\text{M}1}$</td>
</tr>
<tr>
<td>$abd-A^{M3}$</td>
<td>$iab^{-2\text{M}1}$</td>
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<td>nd</td>
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* These breaks are outside the $abd-A$ gene.

Position of the mutations are given in kilobases as in Karch et al. 1985 and Weiffenbach and Bender (personal communication). In the cases we are changing the name of the mutation, the old name is in parenthesis.
less extreme phenotypes, indicating that *abd-A* is not wholly inactive. This is supported by the finding that epidermal cell clones hemizygous for *abd-A*<sup>P10</sup> show partial adult transformations whereas in the cases of *Df* or *abd-A*<sup>M1</sup> adult transformations are more extreme (Morata et al. 1983; Sánchez-Herrero et al. 1985). Nevertheless, embryos hemizygous for either *abd-A*<sup>P10</sup> or *abd-A*<sup>C26</sup> have all abdominal segments (except A1) affected and this comprehensive phenotype is probably responsible for the lethality.

The hemizygous viable mutation *abd-A*<sup>M</sup> shows a very weak embryonic phenotype; it can only be detected as some reduction in the width of the denticle belt of A2–A4 segments. This may be an indication of transformation towards A1.

The adult phenotype of *abd-A*<sup>M</sup> in combinations with the other mutations of this class is characterized by a general effect on segments A2–A6; the number of bristles in the sternites of all these segments is reduced, although the effect on A2 is more marked in the tergites where we observe the presence of some fine, short bristles and often a lateral cuticular arch. These are characteristic pattern elements of A1. In addition, we have observed in the naked territory between tergites some tubular structures which resemble the posterior compartment of T3p. By *en*-βgal staining (Busturia and Morata, 1988), we have shown that these structures belong to the posterior compartment. These results suggest that in *abd-A*<sup>M</sup>/Df flies there is a weak transformation of parasegments 7, 8 and 9 towards parasegment 6 (T3p–Al).

The combinations of *abd-A*<sup>M</sup> with *abd-A*<sup>P10</sup> *abd-A*<sup>C26</sup>, *abd-A*<sup>MX1</sup>, *abd-A*<sup>MX2</sup> and *abd-A*<sup>MX3</sup> present a weaker phenotype than the rest. This was expected for *abd-A*<sup>P10</sup> and *abd-A*<sup>C26</sup> but not for *abd-A*<sup>MX1</sup> *abd-A*<sup>MX2</sup> and *abd-A*<sup>MX3</sup> which exhibit strong embryonic phenotypes. It suggests that the latter mutations may not completely inactivate *abd-A*.

Some of these mutations have been localized on the DNA by Karch et al. 1985 (see also Duncan, 1987) as shown in Table 1. They define a DNA interval from +35 kb to +57 kb, which includes the homeobox-containing transcription unit (Karch et al. 1985; Rowe and Akam, 1988). Therefore, these mutations eliminate or inactivate *abd-A* protein products. The cases of *abd-A*<sup>P10</sup>, *abd-A*<sup>C26</sup> and *abd-A*<sup>MX2</sup> do not conform to this rule, for their moderate phenotypes indicate that there is *abd-A* product left. However, the first two mutations map around +35 kb, very close to the 3'end of the transcription unit, whereas *abd-A*<sup>MX2</sup> is located around +57 kb, very close to the 5'end. In these cases, we suspect the lesions may lie just outside the coding unit or, if inside, there still is some functional protein produced.

(3) Mutations affecting part of the *abd-A* domain

As we indicated above these mutations are likely to alter regulatory elements of the gene. We describe them in the order they are listed in Table 1 according to the adult abdominal segment where they have the most prominent effect, starting from A1–A2.

**Infraabdominal-2K (iab-2K)**

It is caused by a gypsy transposon inserted at +27.5 kb (Karch et al. 1985). The adult phenotype of *iab-2K* was first described by Kuhn et al. 1981 and consists of a partial transformation of the second abdominal segment (A2) towards A1. The strongest phenotype appears in hemizygous combinations or in combinations with some of the lethal *abd-A* alleles described above. Of the *abd-A* domain, only the anterior compartment of A2 is affected by the mutation; the bristles of the dorsal side (tergite) become smaller and finer, resembling those of A1 (Fig. 1B). The number of bristles on the ventral side (sternite) is reduced as the A2 sternite is transformed towards the A1, which has no bristles. For example, in the A2 sternite, there are 15±2 bristles in the wild type and 2±1 in *iab-2K*/*abd-A*<sup>M1</sup>. In addition the Wheeler's organ, a characteristic structure of A2, is nearly always absent. The homozygous *iab-2K* phenotype is similar but weaker.

However, we observed that *iab-2K*/+ and *iab-2K*/ *iab-2K* flies frequently present in the A1 tergite some bristles that are longer and thicker than those of A1, suggesting a partial transformation of A1 towards A2 (or other more posterior tergite). Since the A2 pattern is established by *abd-A* activity, which is not normally active in A1 (part of the *Ubx* domain), this observation suggests that in *iab-2K*, together with a loss of function in A2, there is some ectopic activity of *abd-A* in A1.

This gain-of-function phenotype of *iab-2K* flies is dramatically enhanced if they are also made homozygous for *su(Hw)*, which suppresses most of the mutations caused by gypsy transposons (Modolell et al. 1983). In the doubly mutant flies, the tergite of A2 is entirely normal but that of A1 is strongly transformed towards a more posterior segment (Fig. 1C). This transformation affects preferentially the tergite. In addition, we observed the presence of abdominal tissue anterior to A1. We have tested the segmental provenance of this tissue by generating *en*-βgal/+; *su(Hw)*<sup>iab-2K*/su(Hw)*<sup>2K*/<sup>iab-2K* flies in which the adult posterior compartments can be labelled by β-gal activity (Busturia and Morata, 1988). We find that the abdominal tissue anterior to A1 is located anterior to the T3p stripe and therefore is originated in the anterior compartment of the third thoracic segment.

Flies homozygous for *su(Hw)*<sup>2K</sup> but heterozygous for *iab-2K* also show a clear, though milder, transformation of A1 (Fig. 1D), indicating that in the absence of the *su(Hw)* product, *iab-2K* behaves as a dominant, gain-of-function mutation. Flies homozygous for *su(Hw)*<sup>2K</sup> alone do not show this effect. All the genotypes generated for the study of *iab-2K* and two other *iab-2* mutations are presented in Table 2. These observations indicate that the recessive (A2 towards A1) transformation of *iab-2K* flies is mostly or entirely due to the activity of the gypsy transposon, which requires the normal products of *su(Hw)* (Parkhurst and Corces, 1986; Mazo et al. 1989).
This gypsy activity probably interferes with the \textit{abd-A} transcription unit in A2. Once the gypsy interference is removed by eliminating the $su(Hw)^+$, the $iab-2^K$ breakpoint reveals its genuine, dominant gain-of-function phenotype.

We have studied the embryonic phenotypes of $iab$-
Fig. 1. Dorsal aspect of the first three abdominal segments in mutants affecting A1 and A2 development. (A) WT. Note that the bristles in A1 are shorter and finer than those of A2 and A3. There is also a characteristic cuticular arch (arrow). The posterior compartment of A1 (Kornberg, 1981) is a depigmented region (dotted line) devoid of bristles and is connected smoothly with A2a. In contrast, the posterior compartments of all other abdominal segments are not normally visible as they form a fold located under the anterior compartment. (B) iab-2K/Df. The A2 segment is partially transformed into A1 as indicated by the small size of the bristles. The transformation appears to be compartmental as A1p and A2p are not affected. Note in A1a (arrows) some bristles are longer than normal indicating a slight transformation towards a more posterior segment. (C) su(Hw)2 iab-2K/su(Hw)2 iab-2K. Almost complete transformation of A1 into A2. The transformation affects A1a as indicated by the size of the bristles and the disappearance of the cuticular arch. A1p is also transformed, generating a discontinuity between A1 and A2. (D) su(Hw)2 iab-2K/su(Hw)2. This transformation is similar to but weaker than that of C. Both A1a and A1p are affected. (E) iab-2M1 iab-4M1. Reciprocal partial transformation of A1 towards A2 and of A2 towards A1. As a result A1 and A2 look quite similar (compare with WT). The transformation affects both A1a and A1p. Note the presence of the characteristic cuticular arch (arrows) in the two segments. (F) iab-2M1/Df. Partial transformation of A2a towards A1a. The existence of the discontinuity between A2 and A3 suggests that A2p is not transformed. Alp also remains normal.

2K/Df and of su(Hw)2 iab-2K/su(Hw)2 iab-2K and found in both cases a normal pattern in the larval epidermis. Thus the phenotypes associated with iab-2K appear to be restricted to imaginal cells.

Infraabdominal-2S3 (iab-2S3)

It is associated with a breakpoint in the position +26 kb (Karch et al., 1985), very close to that of the gypsy insert of iab-2K (+27.5 kb). It also shows a transformation of A2 towards A1, but is weaker than that of iab-2K. A mild transformation can be observed in hemizygous flies as some reduction in the number of bristles in A2 sternite.

Given the proximity to the iab-2K lesion, we checked for a possible gain-of-function phenotype in A1 and found that indeed the A1 tergite shows some large bristles indicative of transformation towards a more posterior segment. This phenotype can be observed in heterozygous iab-2S3+ flies but it is enhanced in homozygotes. It can also be observed in flies carrying supernumerary doses of the BX-C, indicating that the transformation of A1 is a genuine gain-of-function phenotype.

Infraabdominal-2Lab1 (iab-2Lab1)

This mutation shows a dominant and a recessive phenotype. Hemizygous iab-2Lab1/Df adults show partial transformations of A1 into A2 (described by Davis and Kiger, 1977 and Lewis, 1978) and of A2 into A1. As a result segments A1 and A2 look very much alike (Fig. 1E); other abdominal segments are not affected. The same phenotype appears in iab-2Lab1/abr-AM1. In iab-2Lab1/+ or DpP115; iab-2Lab1/Df adults, which have one normal abd-A gene, the transformation of A1 remains unaltered whereas that of A2 is completely suppressed. The transformation of A1 is also unaffected by supernumerary copies of abd-A.

All these observations indicate that iab-2Lab1 produces adventitious activity of abd-A in A1, responsible for the dominant phenotype, and a reduction of the normal abd-A function in A2.

The dominant transformation of A1 seen in adults is not detected in iab-2Lab1/+ larvae. Even homozygous iab-2Lab1 embryos containing an extra dose of the BX-C (necessary to cover the recessive bxd, abd-A and Abd-B phenotypes) display a normal segment pattern.

Infraabdominal-2M1 (iab-2M1)

It appeared spontaneously in our laboratory. In hemizygous adults, the A2 segment is transformed towards A1 with an expressivity similar to that of iab-2K/Df (Fig. 1F). However, unlike iab-2K, it does not have any effect on the A1 segment.

Trans combinations with lethal abd-A alleles like abd-A1, abd-A2 and abd-A3 and abd-A.C51 present similar phenotypes. There is no effect on A3 or any other segment, thus this allele has the features of a genuine iab-2 mutation of the type predicted by Lewis, 1978.

We have examined the embryonic phenotype in hemizygous condition and found it like the wild type.

Infraabdominal-3277 (iab-3277)

This mutation has been described by Karch et al., 1985. It is an X-ray-induced partial revertant of Mcp and contains a breakpoint in +64 kb.

Adults of genotype iab-3277/Df present a partial transformation of A1 towards A2 and of A3–A5 towards A2. These transformations are visualized by the appearance of Wheeler’s organs, more or less defective, in all these segments. Although transformations of posterior compartments of abdominal segments are normally difficult to assess given the lack of

Table 2. Transformations caused by iab-2 mutations in A1 and A2 segments

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Segment identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>wildtype</td>
<td>A1</td>
</tr>
<tr>
<td>iab-2K/+</td>
<td>A1 → A2(s)</td>
</tr>
<tr>
<td>iab-2K/iab-2K</td>
<td>A1 → A2(s)</td>
</tr>
<tr>
<td>su(Hw)2 iab-2K</td>
<td>A1 → A2(s)</td>
</tr>
<tr>
<td>su(Hw)2 iab-2K</td>
<td>A1 → A2(s)</td>
</tr>
<tr>
<td>su(Hw)2 su(Hw)2</td>
<td>A1 → A2(s)</td>
</tr>
<tr>
<td>iab-2S3/+</td>
<td>A1 → A2(s)</td>
</tr>
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<td>iab-2S3/iab-2S3</td>
<td>A1 → A2(s)</td>
</tr>
<tr>
<td>iab-2S3/Df</td>
<td>A1 → A2(s)</td>
</tr>
<tr>
<td>DPP115 iab-2S3/Df</td>
<td>A1 → A2(s)</td>
</tr>
<tr>
<td>iab-2Lab1/+</td>
<td>A1 → A2(s)</td>
</tr>
<tr>
<td>iab-2Lab1/Df</td>
<td>A1 → A2(s)</td>
</tr>
</tbody>
</table>

(s) Indicates a slight transformation, usually only discernible under the compound microscope.
morphological landmarks, in good preparations we have observed that the posterior compartments of A2–A5 of these flies are transformed into A1p (Fig. 2A). Thus the anterior abdomen of iab-3277/Df adults appear to be formed by A1p-A2a units, corresponding to parasegment 7. The same set of transformations is observed in iab-3277/abd-AM1 and in homozygous iab-3277 adult flies. The iab-3277 mutation is therefore associated with a gain of function of abd-A activity in A1 and a relative loss in A3–A5. We have discriminated these two phenotypic components by generating adults with one or more copies of the normal abd-A gene; the genotypes iab-3277+/+, DpP115; iab-3277/+ and DpP115; iab-3277/iab-3277 show suppression of the transformations of A3–A5 but A1 remains partially transformed towards A2.

Infraabdominal-3VaM (iab-3VaM)

This mutation maps at +63 (Karch et al. 1985) and also has a dominant (Lewis, 1978) and a recessive phenotype. iab-3VaM/Df adults show a phenotype similar to that of iab-3277/Df; A1 is transformed towards A2 and A3–A5 become like A2. The posterior compartments are also transformed into A1p, although not as clearly as in iab-3277/Df. The transformation of A1 is stronger than in iab-3277 and in addition there is frequently a group of abdominal-like bristles located anterior to A1. By homology with the su/(Hw)2 iab-2k/su(Hw)2 iab-2k phenotype, we interpret these bristles as originated in T3a, thus suggesting that the iab-3VaM phenotype extends to the thorax. We have distinguished the dominant and recessive phenotypic components by adding extra copies of abd-A. DpP115; iab-3VaM/Df, iab-3VaM/+ and DpP115; iab-3VaM/+ have A3–A5 segments wild type but the transformations of T3 and A1 remain as in iab-3VaM/Df.

Infraabdominal-4302 (iab-4302)

Karch et al. (1985), described the hemizygous phenotype and also the molecular lesion, a breakpoint at +86 kb. The segment A4 is transformed into A3 and A2 is transformed A3 as revealed by the loss or abnormal development of the Wheeler’s organ and the alteration of the bristle pattern of the A2 sternite. We have confirmed their observations for iab-4302/Df adults although the pigmentation pattern of A4 is variable and its transformation sometimes difficult to assess. We find the same phenotype in iab-4302/abd-AM1 and in iab-4302/abd-AM2 flies. Homozygous iab-4302 adults also show a similar phenotype but, in addition, the A1 segment, particularly the posterior compartment, is transformed towards a more posterior segment. The same phenotype is seen in A1 of DpP115; iab-4302/iab-4302 flies (Fig. 2B).

Infraabdominal-4MX4 (iab-4MX4)

It has recently been X-ray induced in our laboratory. It has some features in common with iab-4302 so we have labelled it as iab-4. iab-4MX4/Df adults show a transformation of A1, especially Alp, towards a more posterior segment. The sternite of A2 frequently has the Wheeler’s organ abnormal or absent. The pigment pattern of the A4 tergite is modified and resembles A3. The hemitergites do not fuse well and leave a cleft in the medial region.

The effects seen in A1 and A2 are not recessive as they appear in heterozygous iab-4MX4+/+ and in DpP115; iab-4MX4/Df adults. This indicates that iab-4MX4 produces some gain of function of abd-A in A1 and A2, together with a relative loss in A4. Unfortunately, the abd-A-MX4 chromosome is homozygous lethal, most probably due to a second site mutation, so we did not
study the effect of two doses of the gain-of-function phenotype of the mutation.

**Infraabdominal-4, 5^{DB} (iab-4, 5^{DB})**

This mutation is caused by a DNA deletion from +83 to +113 (Karch et al. 1985). The deletion extends into Abd-B as it removes a piece of DNA where several Abd-B mutations map (Karch et al. 1985). However, the tester abd-A mutant chromosomes we have used contain Abd-B^{+} so the Abd-B phenotypic component should have been eliminated.

Flies of the genotype iab-4, 5^{DB}/Df or iab-4, 5^{DB}/abd-A^{M1} show A1 slightly modified towards a more posterior segment; also the Wheeler’s organ is sometimes abnormal suggesting a transformation of A2. In males, the A5 tergite is depigmented resembling that of A4. However, iab-4, 5^{DB}/+ males also present a partially depigmented A5 tergite suggesting that the A5 depigmentation may be in part due the haplo insufficiency of the Abd-B fragment missing in the iab-4, 5^{DB} chromosome. The transformations in A1 and A2 are stronger in homozygous flies but are not affected by supernumerary doses of abd-A^{+} indicating that the effects seen in A1 and A2 are due to gain of function of abd-A.

(4) Complementation studies

The abd-A locus is defined as a single complementation group (Sánchez-Herrero et al. 1985). The abd-A mutations do not complement among themselves and also fail to complement the iab alleles.

Complementation analyses among the iab alleles are complicated by the fact that, as we show above, many of them exhibit a combination of gain- and loss-of-function phenotypes. For complementation we have only considered the latter.

**Trans-heterozygous combinations of iab-2 alleles**

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Fig. 3. Model of abd-A structure. The arrows point to the approximate positions of the lesions associated with the mutations regardless of whether they are breakpoints, insertions or deletions. The gene contains one transcription unit encoding all the executive products of the gene, and two regulatory regions connected with the spatial distribution of the gene products. Regulatory region 1 contains some control elements to prevent abd-A expression anterior to its normal domain and may also contain other elements to establish the appropriate level of abd-A product in A2. Regulatory region 2 appears to be connected with the general expression of abd-A in the abdomen since mutations in this region lead to a general alteration in the pattern of abd-A function (see text for details) affecting several segments. "hb" indicates the approximate position of the homeobox. The positions of the mutations abd-A^{52} and iab-4^{27}, which have not been studied in this work, have been included as their phenotypes (Karch et al. 1985) match those of mutations in the same region.

Two lines of argument. First, in iab mutants, part of the abd-A domain develops normally while other parts show loss of abd-A function, i.e. abd-2^{M1} transforms A2 but A3 and A4 are normal; in iab-3^{277}, A2 is normal but A3 and A4 are transformed. This is expected if the distribution of abd-A products is abnormal. The second argument is that many of these mutations exhibit a gain-of-function phenotype in A1, outside the abd-A domain. This again suggests an abnormal distribution of abd-A products.

One important question is whether there exist within the iab regions individual elements responsible for abd-A expression in specific metameres, as proposed by recent models (Casanova et al. 1987; Peifer et al. 1987), i.e. whether there exist iab-2, iab-3, iab-4 etc. as distinct genetic elements. The recessive phenotype of iab-2 mutants suggests a metameric specific control (it is not clear whether compartmental, segmental or parasegmental). Also the observation that iab-4 mutations complement the recessive phenotype of iab-3 alleles suggests that they affect distinct regulatory regions, perhaps metameric specific, at the 3' side of the gene. However, the iab-3 mutations show in parasegments 8 and 9 a similar transformation to parasegment 7. This would not be expected if there are distinct elements for parasegment 8 (iab-3) and 9 (iab-4). Besides we have shown that iab-3 and iab-4 mutations give rise to ectopic expression in A1 and A2 (or parasegments 6 and 7) and this is not easily compatible with models based on metameric specific regulatory elements. The simplest view about the iab-3 and iab-4 mutations is that they cause a general misregulation of abd-A expression. This results in an abnormal distribution of abd-A products, which now may appear inappropriately in the Ubx domain or at abnormally high levels in parts of the abd-A domain (A1p and A2a). This phenomenon would produce a gain-of-function, usually dominant, phenotype. Other parts of the abd-A domain (A2p, A3 and A4) would have a reduced level of product and consequently a loss-of-function phenotype. This interpretation has the advantage that offers a single explanation for a complex mutant syndrome.

One peculiar feature of many of these mutations is that the dominant transformations are restricted to adult structures; larval segments appear normal. This might be interpreted as indicating differential regulation for larval and adult cells. However, another possibility is that any dominant transformation affecting larval pattern may result in lethality. This phenomenon would result in the selection of mutations having little or no effect on larval patterns.

A model of abd-A structure

Our genetic results, together with the available information about the molecular mapping of abd-A mutations (Karch et al. 1985) suggest a model of abd-A structure outlined in Fig. 3. The model we propose is of
necessity very general at this stage and will have to be refined in the light of subsequent molecular and genetic information.

All the protein products responsible for 
\textit{abd-A} functions are encoded in the homeobox-containing transcription unit which is flanked by two \textit{cis}-acting regulatory regions, 1 and 2.

The transcription unit is responsible for all the executive products of the gene. Therefore mutations that impede its function present a null phenotype equivalent to the deletion of the entire gene. Little is still known about the different components of this unit, number, size and disposition of exons and introns etc.

Regulatory region 1 is located 3' to the transcription unit and is defined by the \textit{tab-2\textsuperscript{R}} and \textit{tab-3\textsuperscript{R}} breakpoints. This part of the gene is connected with the repression of \textit{abd-A} activity anterior to its domain, that is, it acts as a negative regulatory element. Another BX-C gene, \textit{Ubx} appears to have similar components localized in a homologous position, defined by the mutations \textit{Cbx\textsuperscript{R}} and \textit{Cbx\textsuperscript{TMT}} (Bender et al. 1983).

However, both \textit{tab-2\textsuperscript{R}} (in the presence of \textit{su(Hw)}\textsuperscript{+}) and \textit{tab-3\textsuperscript{R}} show a reduction of \textit{abd-A} function in A2, suggesting they also interfere with another function necessary to promote \textit{abd-A} activity in A2 and that should also be located 3' to the transcription unit. The eventual localization of the DNA lesions of \textit{tab-2\textsuperscript{R}} and \textit{tab-2\textsuperscript{Uab}} will help to further define these regions.

Regulatory region 2 extends for about 25 kb and is defined by the \textit{tab-3} and \textit{tab-4} mutations which provoke abnormal levels of \textit{abd-A} function in A1–A4 segments. Unlike regulatory region 1, whose role appears to be restricted to the A2 segment and part of the \textit{Ubx} domain, the role of regulatory region 2 appears to be more general. It is connected with the general regulation of \textit{abd-A} products in the abdomen. It is still an open question whether there are distinct components within it. Further genetic and molecular analysis of this region is still required.

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References


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