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Transcription activates repressed domains in the *Drosophila* bithorax complex

Welcome Bender and Daniel P. Fitzgerald

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

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SUMMARY

A series of mutations have been recovered in the bithorax complex of *D. melanogaster* that transform the first segment of the abdomen into a copy of the second or third abdominal segment. These dominant *Ultraabdominal* alleles are all associated with P element insertions which are transcribed in the first abdominal segment. The transcripts proceed past the end of the P element for up to 50 kb, extending through the regulatory regions for the second and third abdominal segments. Blocking transcription from the P element promoter reverts the mutant phenotype. Previously identified *Ultraabdominal* alleles, not associated with P elements, also show abnormal transcription of the same region.

Key words: Transcription, *Drosophila*, Bithorax, Polycomb, Ultraabdominal

INTRODUCTION

The *Drosophila* bithorax complex (BX-C) contains three homeotic genes, *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*), controlled by at least ten segmental regulatory regions (*bx*, *bxd* and *iab-2* through *iab-9*). The genes and their regulatory regions are strictly aligned on the chromosome in the order of the body segments that they affect (reviewed by Lewis, 1996). The genes and their linear order are conserved in nematodes, insects and vertebrates, although the molecular motivation for this order is still unknown.

Each regulatory region includes a variety of DNA elements which must work together to establish and maintain correctly the proper segmental patterns for the homeotic genes. Some DNA fragments, when tested in P element reporter constructs, drive segmentally limited patterns in early embryos; we call these 'initiation elements'. A few initiation elements have been well defined, with binding sites for gap and pair rule genes (Qian et al., 1993; Müller and Bienz, 1992; Shimell et al., 1994). Other DNA fragments mediate the repressive effects of the Polycomb Group of factors (the PcG) to maintain the segmental limits of the initiation elements through late embryonic and larval life. These are usually called 'Polycomb response elements' or 'PREs'. The PREs have been more difficult to define, because most Polycomb Group proteins are not DNA-binding proteins, and because PRE function is best assayed in conjunction with initiation elements. The PRE in the middle of the *bxd* region is the best studied (Chan et al., 1994; Fritsch et al., 1999; Horard et al., 2000). This PRE does not have an intrinsic segmental address; it can maintain different segmental boundaries when combined with different initiation elements (Chiang et al., 1995).

It is not known how initiation elements interact with PREs, or whether that interaction is limited in distance or orientation. It is also unclear how inappropriate influences between initiation elements in one segmental regulatory region and PREs in another are prevented. The latter problem may be solved by boundaries to the segmental regulatory regions, which block the spread of activation or repression signals. Boundary elements between domains were postulated to account for the phenotypes of several small deletions in the BX-C. Removing a putative boundary gives a dominant gain-of-function phenotype, a transformation of one segment to the character of the next more posterior segment (Mihaly et al., 1998; Barges et al., 2000). The best-studied boundaries, called *Mcp*, *Fab7* and *Fab8*, each have a PRE within or immediately adjacent to the boundary (Busturia et al., 1997; Hagstrom et al., 1997; Barges et al., 2000), although there are reasonable arguments that the *Fab-7* boundary is separable from the PRE (Mihaly et al., 1997).

We describe here a series of P element mutations in the BX-C that disturb the functions of PREs or boundaries. These give dominant gain-of-function phenotypes that mimic those of boundary deletion mutations. The P elements initiate transcripts that proceed through PREs and boundaries, and the phenotypes depend on the production of these transcripts. The accompanying paper (Hogga and Karch, 2002) suggests that transcription across a different region of the BX-C can relieve silencing, and another parallel study (Rank et al., 2002) also correlates transcription with loss of silencing, using a BX-C fragment on a transgene. These observations raise the possibility that non-coding RNAs in wild-type animals may function to activate segmental regulatory regions.

MATERIALS AND METHODS

Mutagenesis

The starting P element chromosomes were made heterozygous with a chromosome deficient for most of the BX-C [*Df(3)Ubx*^{109}] and carrying

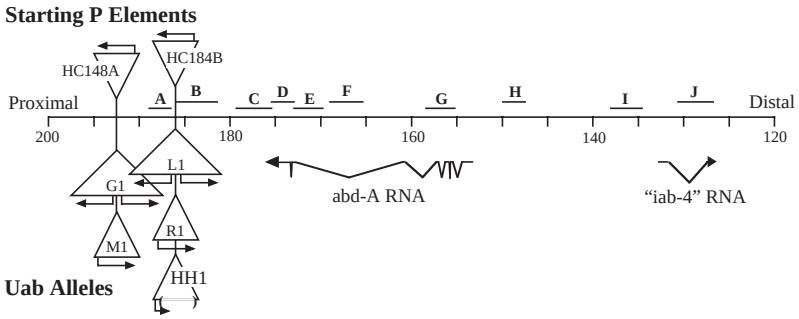

Fig. 1. Map of the central region of the bithorax complex, showing the insertions associated with the *Uab* mutations. The horizontal line diagrams the DNA sequence, marked in kb according to the published sequence coordinates (Martin et al., 1995). The horizontal bars marked A-J above the sequence line indicate restriction fragments used to prepare probes for RNA in situ hybridization. Below the sequence line, the two previously characterized transcription units in the region are shown. The two triangles at the top left indicate the P element insertions from which the *Uab* alleles were derived. The bent arrows above the triangles indicate the direction of transcription in these elements. The triangles below the sequence line indicate the insertions in various *Uab* alleles. The *G1* and *L1* alleles are head-to-head duplications of the starting elements, the *M1* and *R1* alleles are inversions of the starting elements, and the *HH1* allele is an inversion with an internal deletion.

the ry^+ 99B source of P transposase (Robertson et al., 1988). Such dysgenic males were crossed to *cn*, *ry* females, and the ry^- progeny were screened for transformations of the first abdominal tergite.

Southern blotting

DNA was recovered from adult flies and digested with restriction enzymes as described in Bender et al. (Bender et al., 1983b). Fragments were separated on 0.7% agarose gels, transferred to Magnacharge nylon membrane (Osmonics), and probed with radiolabeled probe prepared by random priming.

RNA in situs

RNA probes for in situ hybridization to embryos were made by in vitro transcription with digoxigenin-substituted UTP. Procedures for probe synthesis and embryo treatments have been described (Fitzgerald and Bender, 2001). The genomic DNA fragments used for the probes are defined by restriction enzyme sites (proximal end/distal end) as follows: A, *Eco*RI/*Eco*RI; B, *Eco*RI/*Hin*dIII; C, *Sal*I/*Bam*HI; D, *Bam*HI/*Sal*I; E, *Sal*I/*Eco*RI; F, *Eco*RI/*Bam*HI; G, *Eco*RI/*Eco*RI; H, *Eco*RI/*Eco*RI; I, *Hin*dIII/*Hin*dIII; and J, *Eco*RI/*Bam*HI.

Immunohistochemistry

Embryos were fixed and stained for ABD-A protein as described (Karch et al., 1990), except that the antibody was the 6A18.12 mouse monoclonal (Kellerman et al., 1990). The Uab^{HH1} chromosome was balanced over a version of TM3 with a ftz/*lacZ* transgene, and embryos were stained simultaneously for ABD-A and *lacZ*. *Uab* homozygous embryos (without *lacZ* staining) were chosen for examination. Stained embryos were dissected and flattened as described (Karch et al., 1990).

Abdominal cuticle preparation

Adults were preserved in a mixture of ethanol and glycerol (3:1). Abdomens were separated from the thorax, and split mid-dorsally with a razor blade. The abdomens were soaked in 10% KOH for 30 minutes, then rinsed in water. Internal tissues were removed manually, and the cuticles were arranged on a slide in a drop of Immumount (Shandon), and flattened with a cover slip.

RESULTS

Origins and phenotypes of new *Uab* alleles

The mutations described here were derived from P element insertions into the middle of the bithorax complex. There is a DNA fragment that causes preferential 'homing' of P elements to the neighborhood of the bithorax complex (Bender and Hudson, 2000). The fragment, which is itself from the middle of the bithorax complex, has properties of a boundary separating adjacent segmental regulatory domains, but we wished to test this notion more directly by deleting specifically this region from the bithorax complex. The collection of P insertions included two in the distal region of the *bxd* regulatory domain, adjacent to the endogenous copy of the homing fragment. Both insertions could be modified to remove nearly everything from the P element except the *lacZ* reporter fused to the P promoter. These 'trimmed down' elements, each about 5 kb in length, are illustrated in Fig. 1, with the designations HC148A and HC184B. We hoped to generate deletions of the DNA sequences flanking these insertions, by inducing imprecise excision of these P elements. Such deletions might remove a potential boundary element separating the adjacent *bxd* and *iab-2* domains. The *bxd* regulatory region controls the differentiation of the first abdominal segment (A1), or, more exactly, of parasegment 6 (PS6), and the *iab-2* region controls A2, or PS7. We expected that a boundary deletion would show a dominant phenotype, transforming the first abdominal segment into a copy of the second abdominal segment. Lewis described mutations with such a dominant phenotype, which he called *Ultraabdominal* (*Uab*) (Lewis, 1978). His alleles were associated with rearrangement breakpoints in the bithorax complex. We therefore treated the P-containing chromosomes with a source of P transposase (see Materials and Methods), and looked for *Uab* phenotypes in the progeny.

Such *Uab* flies appeared with a surprisingly high frequency (~1/50). Most showed a mild phenotype as a heterozygote, but homozygotes showed a fairly complete transformation of the dorsal first abdominal tergite towards the character of the second abdominal segment (illustrated for the R1 allele, Fig. 2). The ventral abdomens of such homozygotes showed a sternite on the first abdominal segment, where wild-type animals show none (Fig. 2).

One line, a derivative of the HC184B P element called HH1, had a particularly strong *Uab* phenotype. HH1 heterozygotes showed transformations as complete as those in homozygotes of most of the other alleles, including R1. HH1 homozygotes usually died as pharate adults. In exceptional homozygotes that did eclose, the tergite on the first abdominal segment resembled that of the second, third or fourth abdominal segments in size, pigmentation and bristle morphology (Fig. 2). The first abdominal segment had a complete sternite, which resembled that of the third or fourth abdominal segment in the number and orientation of the bristles (Fig. 2). The sternite of the

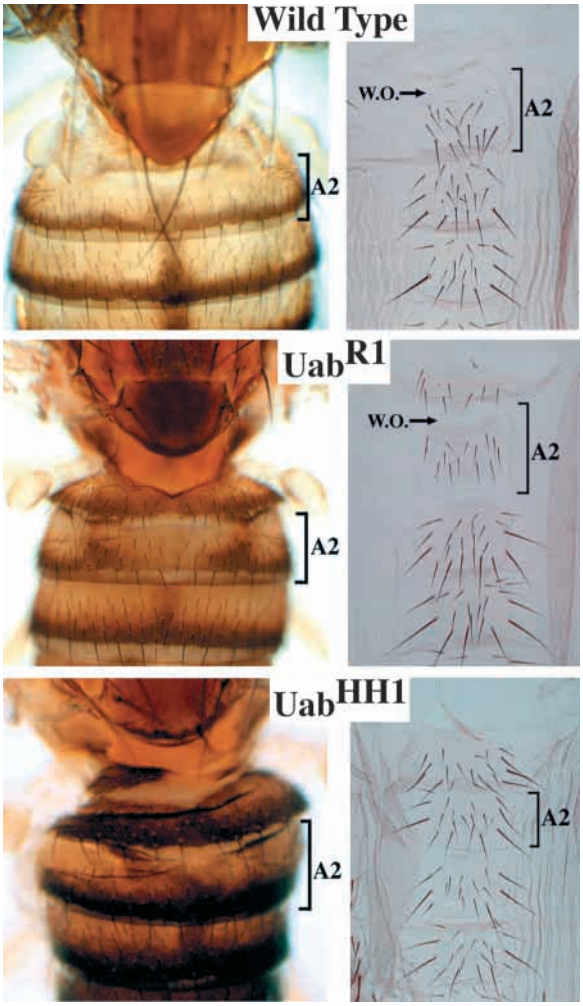

Fig. 2. Segmental transformations of two *Uab* alleles. For each genotype, a dorsal view of the abdominal tergites is shown on the left, and a close-up of the ventral sternites is on the right. The cuticle of the second abdominal segment (A2) is marked with a bracket in each panel. All panels show female cuticles, except that the dorsal picture of Uab^{HH1} shows a male. Wild type: the tergite of the first abdominal segment is narrower than in the second abdominal segment, and it lacks the band of pigment and large bristles at the posterior margin. On the ventral side, the first abdominal segment lacks a sternite. The sternite of the second abdominal segment has fewer bristles than that of the third abdominal segment, and these bristles all point directly posterior. The wild-type second abdominal sternite also includes a clear anterior patch, called the Wheeler's organ (marked W.O.). Uab^{R1} homozygote: the A1 tergite is enlarged, and has pigmentation and bristles like that of the A2 segment. There is a clear sternite in the A1 segment, although it is not completely transformed to a more posterior type. Uab^{HH1} homozygote. These animals rarely eclose as adults. The A1 tergite appears fully transformed to the character of the A2 or A3 segment. This individual also shows an extra half tergite anterior to A1. This tergite also has pigment and large bristles typical of the A2 tergite. The A1 sternite resembles that of A3. Note that the A2 sternite also resembles that of A3, as judged by bristle number and orientation, and by the lack of a Wheeler's organ.

second abdominal segment was also transformed to the character of a more posterior segment. The HH1 homozygotes also show a thin band of cuticle between the thorax and abdomen. In rare individuals, this is expanded on one side into a recognizable half tergite (Fig. 2), similar to the normal tergites on the second through fourth abdominal segments, as judged by bristle morphology. This 'extra' abdominal-like segment is most probably derived from the third thoracic segment, which normally does not contribute to dorsal cuticle in the adult. Homozygotes also had enlarged or missing halteres, they typically were not able to flatten their wing blades, and they often had malformed third legs. If the *Uab* mutation affects PS6, the transformation should include the posterior third thoracic segment. This would explain the emergence of the extra abdominal segment, as well as the haltere and leg phenotypes.

Molecular characterization of the new *Uab* alleles

When the *Uab* chromosomes were analyzed by Southern blotting, none had deletions adjacent to the P elements, and all retained P element sequences. The Southern blots revealed a variety of different rearrangements of the starting P elements. Several independent lines appeared to have generated inverted duplications of the starting P element, represented by the G1 and L1 *Uab* alleles (Fig. 1). Several additional independent lines appeared to be precise inversions of the starting P element, as in the M1 and R1 alleles (Fig. 1). Others had more complicated rearrangements, and were not analyzed further.

The Uab^{HH1} line, with the particularly strong *Uab* phenotype, appeared to retain a small P element derivative. This was recovered by PCR and sequenced. The element was 690 bp in length, which included 656 bp from the 5′ end of HC184B, and 15 bp from the 3′ end, separated by 19 bp of AT-rich sequence with no obvious homology to the starting element. The element retained the P element promoter at the 5′ end, and the first 65 bases of the β-galactosidase sequence. This deleted P was inverted relative to HC184B, so that the P promoter pointed toward the distal end of the chromosome arm (Fig. 1).

Ectopic RNA transcripts on the *Uab* chromosomes

A comparison of all the P insertions suggests that the *Uab* phenotypes are not caused by disruption of endogenous BX-C sequences or by juxtaposition of particular P sequences with BX-C sequences. However, all the *Uab* chromosomes had inversions of the P elements, resulting in distally-directed promoters. Therefore, we looked for RNA products, downstream of the P elements, coming from these promoters. The intact P elements (such as the M1 and R1 alleles) have two poly(A) addition sites 3′ to the *lacZ*-coding region, one derived from the *Hsp70* gene (Mlodzik and Hiromi, 1992), and the other from the 3′ end of the P element. The latter poly(A) site is known to be only ~50% efficient (Laski et al., 1986); the efficiency of the *Hsp70* poly(A) site is not known. The strongest allele, Uab^{HH1} lacks both these terminators.

Distally directed transcripts from the *Uab* P elements were easy to detect. To probe for RNA products, we subcloned a variety of fragments from the region, as shown in Fig. 1. The fragments were used to prepare strand-specific RNA probes. These were hybridized to embryos from the various *Uab* strains. We first looked for transcripts 3′ to the *Uab* P elements, using a probe made from fragment B to detect distally-directed transcripts. Wild-type embryos showed no detectable RNA

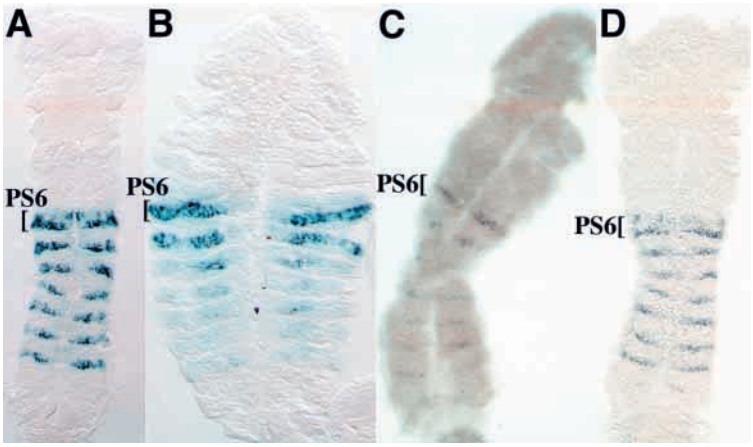

Fig. 3. RNA in situ hybridization to *Uab* embryos. (A) An embryo from Uab^{HH1}/*MKRS* parents, hybridized with a probe from fragment B to detect distally oriented transcripts. The embryo is at stage 11 (~6 hours old), and is dissected to show the whole epidermis in one plane. The anterior end is at the top; the anterior limit of RNA expression is in PS6, as marked. (B) Same as A, except the embryo is stage 13 (~10 hours old). (C) Same as A, except that the probe is from fragment I, again detecting distally oriented transcripts. The weak signals represent rare transcripts that have extended 50 kb from the Uab^{HH1} insertion. (D) Same as A, except that the embryo was from Uab^{R1}/*MKRS* parents. There is less readthrough transcription than in Uab^{HH1}, presumably because of the intact poly(A) addition signals in the Uab^{R1} insertion. Wild-type embryos show no distally directed RNA with probes from either the B or the I fragments.

with this probe. In Uab^{HH1} embryos, weak staining was detectable as early as embryonic stage 5 (cellular blastoderm) in a narrow band from 25% to 35% egg length (from the posterior pole). During gastrulation, the zone of RNA expression spread forward to the anterior edge of PS6. By stage 10 (extended germ band), the RNA signal was at its highest level. Hybridization was widespread throughout the epidermis of PS6, with more narrow bands of hybridization in PS7-12 (Fig. 3A). The RNA signal gradually decreased in later embryonic stages, but it always showed a sharp boundary at the anterior edge of PS6 (Fig. 3B). Embryos from the Uab^{R1} and Uab^{M1} strains showed a very similar pattern with the same probe, but the signal intensities were much reduced (Fig. 3D). The G1, M1, L1 and R1 alleles all have intact *lacZ*-coding regions; all make β-galactosidase in PS6-12 in a pattern similar to that of the RNA detected by probe B.

The transcripts from Uab^{HH1} were surprisingly long. Probes from fragments C through I (Fig. 1) all detected distally directed RNAs in the same pattern as that seen with probe B, although the signal intensity declined with the more distal probes. Wild-type embryos showed no distally directed transcripts with probes from fragments A-I. Fig. 3C shows Uab^{HH1} embryos with probe I; the signal is quite weak but still clearly in the same pattern as probe B (Fig. 3A). Probe J detects the 'iab-4' RNA (Cumberledge et al., 1990); this transcription unit most probably lies within the *iab-3* segmental domain (Bender and Hudson, 2000). The signal from probe J begins in PS8 in both wild-type and Uab^{HH1} embryos; there is no signal in PS6 like that seen with probes B-I. In summary, the Uab^{HH1} P element drives an RNA product that extends for about 50 kb, although the majority of transcripts must terminate at shorter distances. The Uab^{R1} P element makes a similar transcript that is less abundant, presumably because of partial termination within the P element.

RNA probes were also used to detect proximally directed transcripts. A probe from fragment A detected a proximally directed transcript from the HC184B starting P element, but nothing from wild type, Uab^{R1} or Uab^{HH1}. Three other proximally directed RNAs were detected in both wild-type and Uab^{HH1} embryos. The first was the *abd-A* transcript, revealed by probes from fragments C-G. The second RNA, detected with the fragment I probe, was seen only in blastoderm and early gastrulating embryos in a zone from ~10% to 40% egg length (from the posterior pole). A similar pattern of RNA expression was discovered (Sánchez-Herrero and Akam, 1989), using a double-stranded probe from the same region. The third RNA appeared to be the 'PS13-15' RNA (Sánchez-Herrero and Akam, 1989). It was seen most strongly with the fragment J probe, beginning in early elongated germ band embryos, widespread in the epidermis of PS13 and PS14. After germ band shortening, the RNA faded in the epidermis but persisted in the posterior CNS. The same pattern was seen with probes from fragments I and H, although weaker. With probes from fragments C-G, we did not detect this posterior RNA in addition to the *abd-A* pattern, but a probe from fragment B did show faint staining in the same pattern in the posterior CNS of late embryos. This RNA product could represent a read-through product initiated at one or more of the *Abd-B* promoters. Alternatively, it might come from an 'iab-8 promoter' (Zhou et al., 1999). In either case, transcripts extending through fragment B would measure at least 125 kb.

Suppression of the *Uab* phenotype in P cytotype

If the *Uab* phenotypes are due to RNAs initiated at the P element promoter, then P cytotype should revert the phenotype. P cytotype is conferred by strains with multiple P element insertions, some of which produce a truncated 66 kDa form of the P transposase, which is a repressor of P activity (Misra and Rio, 1990). Crosses to such strains repress P/*lacZ* fusion genes, regardless of the direction of the cross (Lemaitre and Coen, 1991). P cytotype did indeed revert the *Uab* phenotype. *UabHH1/MKRS* flies were crossed to flies of the Harwich, Π2, and MR-h12 strains. Uab^{HH1}/Harwich heterozygotes appeared virtually wild type (Fig. 4), and the phenotype was the same with the Harwich chromosomes derived from either the maternal or paternal lineage. Similar crosses to Π2 gave partial suppression of the *Uab* phenotype, although our MR-h12 stock gave no suppression. Uab^{HH1}/Harwich males were crossed to Uab^{HH1}/*MKRS* females to recover Uab^{HH1} homozygotes with a ~25% contribution of Harwich chromosomes. These homozygotes were healthy, and had variable *Uab* phenotypes; some flies were near wild type. These homozygotes all retained the Uab^{HH1} P element insertion, as determined by Southern blots. The repression of P transcription was confirmed by RNA in situ hybridization. Uab^{HH1}/Harwich embryos were stained for distally directed RNA products homologous to probe B. Weak staining was seen in blastoderm embryos, but transcripts in older embryos were nearly completely suppressed.

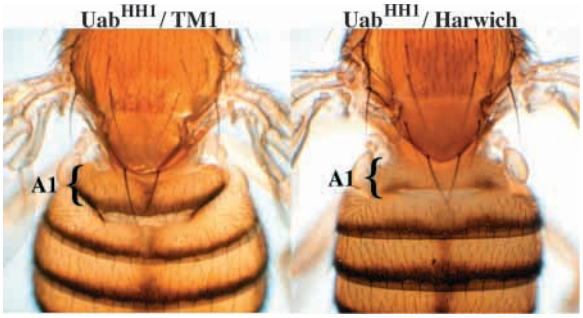

Fig. 4. Suppression of the *Uab* transformation by P cytotype. Uab^{HH1}/TM1 female. The transformation of the A1 tergite is dramatic, but not as complete as in the homozygote, as shown in Fig. 2. Uab^{HH1}/Harwich female. The A1 tergite is restored to the wild-type character by the presence of the Harwich chromosomes, which confer the P cytotype.

Ectopic transcripts in *Uab^{1}* and *Uab^{5}*

Since our P element *Uab* alleles are associated with transcription of the *iab-2* region, we checked the preexisting *Uab* rearrangement alleles for similar RNA products. *Uab^{1}* is associated with an inversion within the bithorax complex between the *bxd* (PS6) and *iab-8* (PS13) regulatory regions (Fig. 5A; between positions 225 kb and 22 kb in SEQ89E coordinates) (Karch et al., 1985). *Uab^{1}* homozygous or heterozygous embryos show RNA products with probe B starting in PS6, primarily in the epidermis (Fig. 5B). The staining is weaker in PS7-12, but strong again in PS14. The inversion juxtaposes the *iab-2* region of probe B with the *iab-9* region. It seems likely that a promoter in the *iab-9* region [most likely the 'class C' promoter of *Abd-B* (Zavortink and Sakonju, 1989)] is influenced by both *bxd* and *iab-9* regulatory sequences. Such a transcript would extend at least 40kb to reach the position of fragment B.

Uab^{5} is associated with a translocation to the X chromosome (Lewis, 1978) which breaks in the bithorax complex at a site nearly coincident with the HC184B insertion site (Fig. 5A) (B. Weiffenbach and W. B., unpublished). Probe B detects RNA in *Uab^{5}* heterozygous embryos after stage 13 in the CNS and lateral epidermal cells in all segments (Fig. 5C). The posterior transformation in *Uab^{5}* is limited to the first abdominal segment (PS6). It is possible that ectopic expression of ABD-A in PS5 and in more anterior segments has little effect because of high levels of the *Antennapedia* protein in those segments.

ABD-A expression in *Uab^{HH1}*

In wild-type flies, the identities of the second through fourth abdominal segments (PS7-9) are specified by the *abd-A* gene product. A posterior transformation of the first abdominal segment (PS6) in *Uab* flies is expected to be due to anterior misexpression of ABD-A. We found such ectopic ABD-A in *Uab^{HH1}* embryos, but it was surprisingly subtle and late in onset. *Uab^{HH1}* homozygotes looked like wild type in ABD-A patterns prior to stage 13 (germ band retraction). About half of the older homozygous embryos showed one or a few cells of the central nervous system in PS6 expressing ABD-A, although there was no apparent misexpression in the epidermis (Fig. 6). Fig. 6 also shows reduced ABD-A expression in the epidermis of PS7. This may be due to antisense effects of the *Uab^{HH1}* transcripts (see below). Embryos carrying the *Uab* breakpoint alleles have near normal patterns of ABD-A expression (Karch et al., 1990).

We also examined third instar larvae homozygous for the *Uab^{HH1}* mutation, looking for evidence of segmental

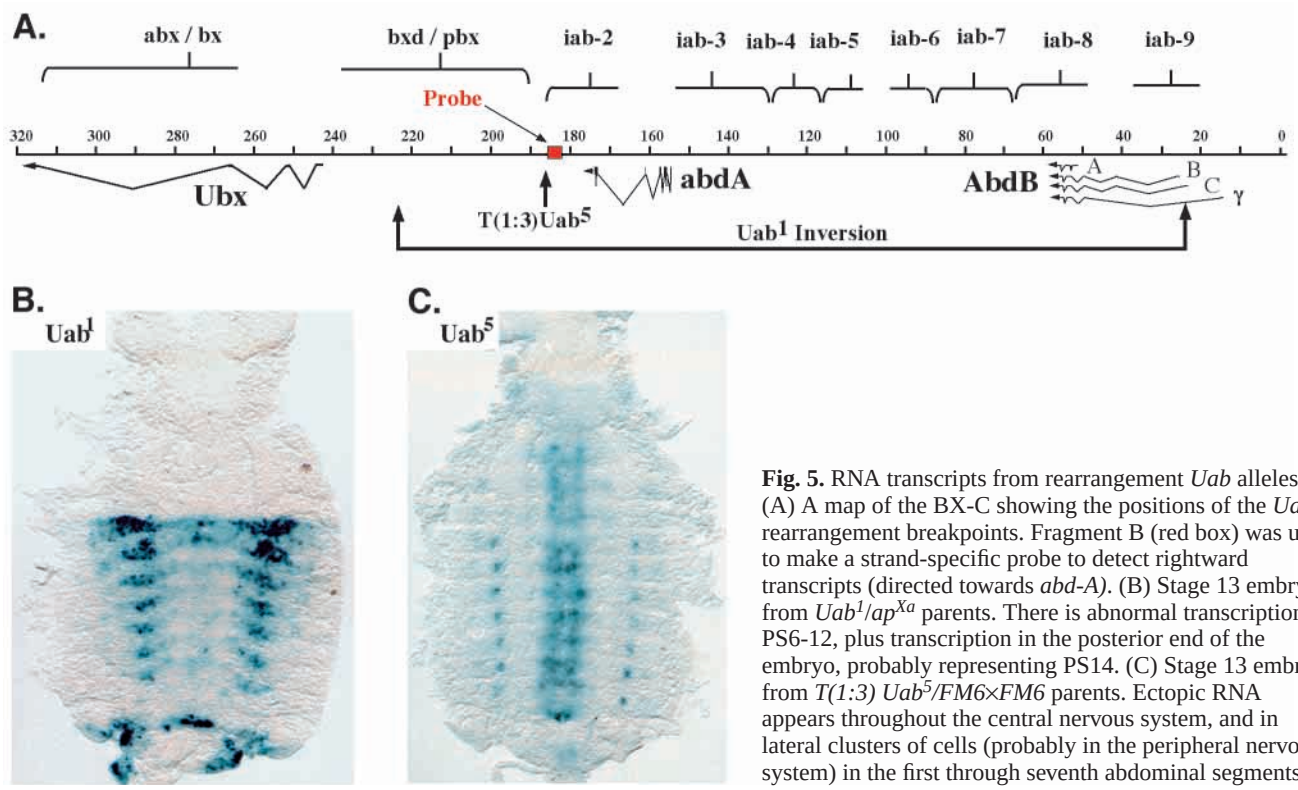

Fig. 5. RNA transcripts from rearrangement *Uab* alleles. (A) A map of the BX-C showing the positions of the *Uab* rearrangement breakpoints. Fragment B (red box) was used to make a strand-specific probe to detect rightward transcripts (directed towards *abd-A)*. (B) Stage 13 embryo from *Uab^{1}/ap^{Xa}* parents. There is abnormal transcription in PS6-12, plus transcription in the posterior end of the embryo, probably representing PS14. (C) Stage 13 embryo from *T(1:3) Uab^{5}/FM6×FM6* parents. Ectopic RNA appears throughout the central nervous system, and in lateral clusters of cells (probably in the peripheral nervous system) in the first through seventh abdominal segments.

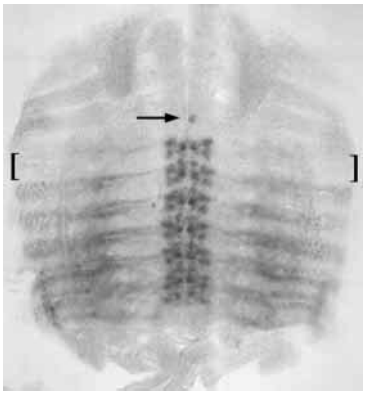

Fig. 6. *Uab*^{HH1} homozygous embryo at stage 14 (~11 hours) stained with antibody to ABD-A protein. The arrow marks a cell in the ventral nerve chord in PS6 which is expressing ABD-A. At most only a few cells in PS6 show ABD-A misexpression by late embryogenesis. ABD-A is reduced in the epidermis of PS7 (flanked by brackets), along the border between the first and second abdominal segments.

transformations. The denticle bands on the first abdominal segment of such larvae usually showed a subtle transformation towards the character of the wild-type second abdominal segment (Fig. 7). These larvae also showed a subtle transformation of the second abdominal denticle belt towards the first (Fig. 7). Again, this may be due to antisense effects (see below). In any case, the transformation of the larval epidermis in the first abdominal segment appeared much less severe than that of the adult (Fig. 2). It seems most likely that ABD-A misexpression in PS6 is most pronounced in the pupa, when the adult structures are forming.

DISCUSSION

Effects of the *Uab* transcripts

The *Uab* alleles we have examined all show ectopic transcription of the *iab-2* regulatory region, which normally controls the differentiation of the cells in the second abdominal segment (PS7). The ectopic transcripts are abundant in the cells of the first abdominal segment (PS6). The P elements HC148A and HC184B, from which the *Uab* alleles were derived, respond to the PS6 regulatory information. They express β-galactosidase in PS6-12 (Bender and Hudson, 2000), but they do not give *Uab* phenotypes. The P elements in the *Uab*^{M1} and *Uab*^{R1} alleles also express β-galactosidase in the same PS6-12 pattern, but they show the dominant phenotype. Because the *Uab* P elements are flipped in orientation relative to their precursor elements, the RNA transcripts extend distally through the *bxd/iab-2* boundary and across the *iab-2* regulatory region. The *Uab*^{1} inversion can be understood in much the same way; the juxtaposition of *bxd* regulatory sequences next to an *Abd-B* promoter drives that promoter in PS6. Again, the resulting transcript extends into the PS7 regulatory region. The rearrangement in *Uab*^{5} fuses an X chromosome transcription unit with the PS7 regulatory region, so that the latter is transcribed in all segments.

Fig. 8 illustrates a simple model for the transcription effect. The *iab-2* regulatory region is normally repressed in PS6 and in more anterior parasegments, and is active in PS7 and more posterior parasegments. Transcription across the *iab-2* region in the cells of PS6 switches the region from repressed to active, so that the developmental program for the second abdominal segment (PS7) is expressed in the first abdominal segment (PS6). The strongest *Uab* mutation, *Uab*^{HH1}, drives transcription farther, through the *iab-3* regulatory region, so that it, too, becomes active in PS6. The ectopic transcripts are also present in PS7 through PS12, and so the *iab-3* region is also active in PS7. Thus in *Uab*^{HH1} animals, both the first and the second abdominal segments (PS6 and PS7) assume the character of the third (PS8). In PS8-PS12, the transcripts have no apparent effect, because the transcribed regions are already active, according to the normal functioning of the BX-C.

Transcription might change the chromosome in several ways. The RNA polymerase II complex involved in elongation includes a histone acetyltransferase (Wittschieben et al., 1999) that could modify nucleosomes across the transcribed region. The act of transcription might remove bound complexes [such as the Polycomb complex (Shao et al., 1999)] or prevent their spread along the chromosome. This mechanism was suggested by Sandell et al., who showed that transcription of yeast telomeres relieved the telomere position effect (Sandell et al., 1994). Transcription might also allow transient access to DNA sequences near the RNA polymerase which might otherwise be covered with nucleosomes or packaged in a 'higher order' structure. A further possibility is that the ectopic RNA product has a function in activation.

It is not clear what site or function is affected by the ectopic transcripts. The boundary between the *bxd* and *iab-2* regulatory regions most likely lies just distal to the *Uab*^{HH1} insertion site (Bender and Hudson, 2000) (M. McLaughlin and W. B., unpublished); perhaps the ectopic transcription disrupts this boundary. Alternatively, the *iab-2* region includes at least one PRE (Chiang et al., 1995; Shimell et al., 2000); perhaps transcription across this site relieves the repression imposed by the Polycomb Group. Unfortunately, there is no clear indication from the available BX-C mutations what phenotype to expect from the loss of a PRE.

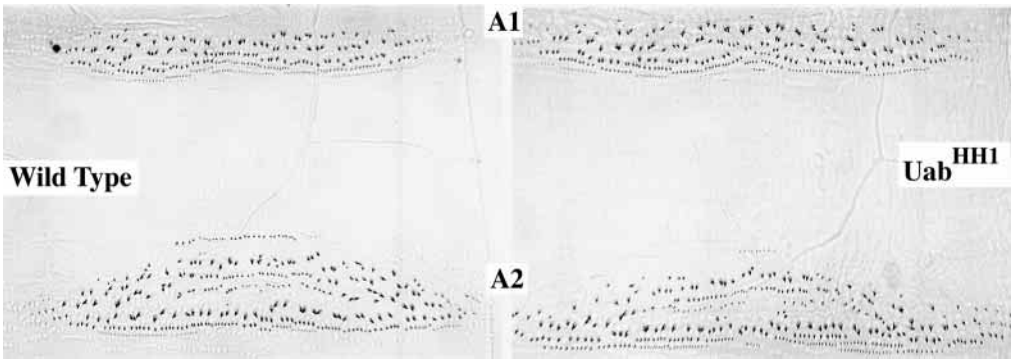

Fig. 7. Ventral cuticles of third instar larvae. The denticle bands from the first and second abdominal segments are shown, with a wild-type larva on the left and a *Uab*^{HH1} homozygous larva on the right. The segmental transformations are subtle. In the mutant, the A1 denticle band has slightly more and larger denticles than wild type, while the A2 denticle band has slightly fewer denticles.

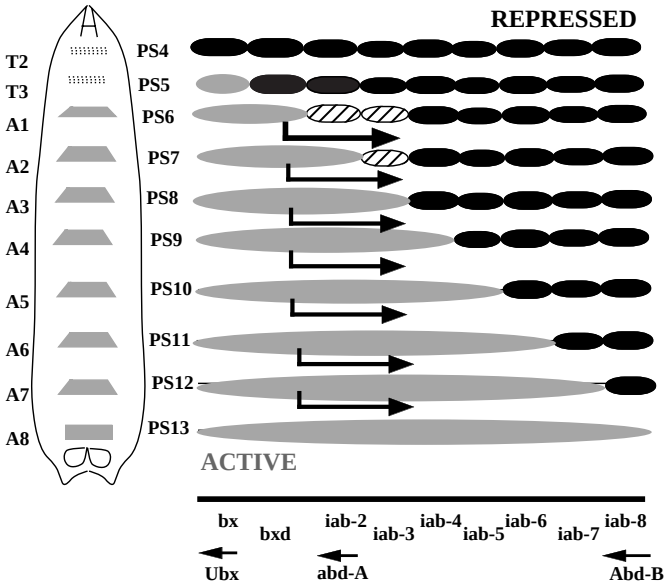

Fig. 8. Model for the segmental transformations of *Uab*^{HH1}. The ventral cuticle of a wild-type larva is illustrated on the left, with the positions of segments and parasegments marked. A map of the BX-C is drawn along the bottom, showing the nine regulatory regions responsible for the development of parasegments 5-13. The segmental domains of the BX-C have different structures in different parasegments, progressing from all repressed (black ovals) in PS4 to all active (gray ellipses) in PS13. In *Uab*^{HH1}, new transcripts (arrows) appear in PS6-PS12, and these transcripts span the *iab-2* and *iab-3* regions. Transcription in the mutant animals switches these regulatory regions to the active state (hatched ovals), and their activity transforms the morphology of PS6 and PS7 to that of PS8.

The ectopic RNAs appear not to affect the segmental regulation of the complex early in embryonic development, although the transcripts are abundant in PS6 from stage 10 (elongated germ band) onwards. Misexpression of ABD-A in PS6, which is presumably necessary for the observed segmental transformations, is not seen in embryos except in occasional cells in the central nervous system. Perhaps there is a critical time later in development when ectopic RNA matters, such as the time of abdominal histoblast proliferation in the pupa. Alternatively, continuous transcription might activate *abd-A* stochastically, so that over time the majority of PS6 cells switch to the active state.

The RNA transcripts from *Uab*^{HH1} are antisense to the normal transcripts of the *abd-A* gene in PS7-12, and one might expect *abd-A* expression to be blocked. Indeed, the level of ABD-A protein in *Uab*^{HH1} embryos is reduced in the PS7 epidermis (Fig. 6) relative to wild type (Karch et al., 1990). ABD-A expression appears normal in the developing central nervous system, and in the epidermis of PS8-PS12, presumably because the *Uab*^{HH1} transcripts in older embryos are primarily in the epidermis of PS6 and PS7 (Fig. 3B). In *Uab*^{HH1} larvae, there is also evidence of loss of *abd-A* function in PS7; the second abdominal setal belt is weakly transformed towards the first (Fig. 7). The *Uab*^{HH1} adults don't show anterior transformation (loss of *abd-A* function) in PS7 (Fig. 2), but any such effect would be masked by the strong posterior transformation (gain of *abd-A* function).

Other readthrough mutations

The discovery of ectopic transcripts in a variety of *Uab* alleles prompts a reconsideration of other mutant classes. Mutations in the vestigial locus have recently been reported (Hodgetts and O'Keefe, 2001) that are due to transcription from P elements, but these are recessive, loss-of-function alleles. It is possible that loss-of-function P alleles in the BX-C and elsewhere would be reverted by P cytotype; it has seldom been checked. Other mobile element alleles could also do their damage with readthrough transcripts.

It seems surprising that there are not more gain-of-function alleles in the BX-C or elsewhere due to readthrough from P elements. However, most P element transposons contain selectable marker genes downstream of the P promoter; perhaps these sequences help to terminate transcripts initiated at the P promoter. It is also likely that strong gain-of-function mutations would be dominant lethals. There are a variety of gain-of-function mutations in the BX-C associated with rearrangements, which could mediate their effects by non-coding readthrough transcription from the juxtaposed DNA. *Contrabithorax* alleles, like *Cbx*^{3} and *Cbx*^{Txt} (Bender et al., 1983a), are good candidates.

Role of transcripts in wild type

The dramatic effects of ectopic transcription hint at a function for non-coding transcripts in the wild type BX-C. Non-coding transcripts have been documented in the human β-globin locus (Ashe et al., 1997; Plant et al., 2001), and such transcription has been correlated with changes in DNaseI sensitivity (Gribnau et al., 2000). Several non-coding transcripts have been described in the BX-C, most notably in the *bxd* and *iab-3* regions (Lipshitz et al., 1987; Cumberledge et al., 1990). These RNA products appear in blastoderm embryos, at or before the onset of segment-specific expression of the homeotic proteins. Other early RNAs, not associated with BX-C protein products, have been detected by RNA in situs in early embryos (Sánchez-Herrero and Akam, 1989). The proximally directed RNA detected by probe I (see above) represents one such transcript.

There is, so far, no evidence for a function of these RNAs. A deletion (*pbx*^{1}) that removes the promoter for the *bxd* RNA has no effect on the embryonic expression pattern of UBX (W. B., unpublished), although the UBX pattern in imaginal discs is changed (White and Wilcox, 1985). The latter effect of *pbx*^{1} may well be due to loss of imaginal disc enhancers, but the *bxd* RNA could matter for the development of the adult, just as our ectopic RNA does. A difference between embryos and larvae has been reported (Poux et al., 2001) in their requirements for Polycomb Group repression. Perhaps the later mode of Polycomb Group repression is sensitive to and regulated by non-coding transcripts.

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