

## Differential expression of the *Drosophila* BX-C in polytene chromosomes in cells of larval fat bodies: a cytological approach to identifying in vivo targets of the homeotic Ubx, Abd-A and Abd-B proteins

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### SUMMARY

We have analyzed the expression of homeotic Bithorax Complex proteins in the fat bodies of *Drosophila* larvae by staining with specific antibodies. We have found that these proteins are differentially expressed along the anteroposterior (AP) axis of the fat body, with patterns parallel to those previously characterized for the larval and adult epidermis. As fat body nuclei have polytene chromosomes, we were able to identify the BX-C locus and show that it assumes a strongly puffed conformation in cells actively expressing the genes of the BX-C. Immunostaining of these polytene chromosomes provided the resolution to

cytologically map binding sites of the three proteins: Ubx, Abd-A and Abd-B. The results of this work provide a system with which to study the positioning of chromatin regulatory proteins in either a repressed and/or active BX-C at the cytological level. In addition, the results of this work provide a map of homeotic target loci and thus constitute the basis for a systematic identification of genes that are direct in vivo targets of the BX-C genes.

Key words: *Drosophila*, Fat body, BX-C

### INTRODUCTION

Homeotic genes play a fundamental role in the specification of segment identity in *Drosophila*. In *Drosophila*, these genes are organized in two complexes: the Antennapedia Complex (ANT-C) (Kaufman et al., 1990) and the Bithorax Complex (BX-C) (Lewis, 1978). Many studies have shown that these genes are highly conserved phylogenetically in both their sequence and their organization (Valentine et al., 1996).

Homeotic genes have long been regarded as selector genes whose protein products bind to and regulate the transcription of downstream genes. Molecular analyses have shown that DNA binding is mediated by a domain denoted the 'homeodomain' that is shared among the homeotic proteins (Affolter et al., 1990). Although the genes of the BX-C were the first of such selector genes to be described and characterized, the numbers and types of target genes they regulate have yet to be fully characterized. Hence, a major effort since the discovery of the homeodomain has been focused on the identification of the direct targets for both the ANT-C and BX-C genes.

In several studies, immunostaining of *Drosophila* salivary gland chromosomes has been used to localize chromosomal binding sites and potential targets of several chromosomal proteins (Andrew and Scott, 1994). Although this is a powerful approach for proteins normally expressed in the salivary gland,

it has limited value for proteins that are not detectably expressed in this tissue, such as the products of the BX-C. To try to circumvent this limitation, an inducible transgene has been used to express Ubx protein in salivary glands. The binding sites of this ectopically produced protein were then characterized (Botas and Auwers, 1996). Although this strategy provides information on the potential of proteins of interest to bind specific sites, the relevance of the binding sites should be verified in tissues normally expressing the protein.

In addition to in vivo chromosomal protein localization studies, salivary gland chromosomes have also been used to reveal the presence of gene activity. Most striking has been the observation of chromosome puffs that form when specific genes are induced developmentally or through treatments such as heat shock (Ashburner, 1972). There is also evidence that repressed genes in salivary gland nuclei appear condensed or are under replicated. This is specifically the case for the BX-C genes that are severely under represented compared to other loci in salivary gland DNA (Moshkin et al., 2001), making the cytological resolution of the BX-C locus difficult in salivary chromosomes under normal circumstances.

We have investigated the potential of another tissue containing polytene chromosomes, the larval fat body, to address questions relevant to in vivo BX-C expression. We show that despite the lower degree of endoreduplication of fat body as compared to salivary gland chromosomes, fat body

cells do provide readable chromosomes. Moreover, they also provide a physiologically relevant environment for studying BX-C gene and protein expression. We also show that the resolution of our cytological studies is sufficient to document chromosomal changes associated with the activation of the BX-C locus and for the identification of chromosomal targets of the Ubx, Abd-A and Abd-B proteins.

## MATERIALS AND METHODS

### *Drosophila* strains

The *Ore-R* wild-type stock used here has been kept in our laboratory for many years. Cultures were maintained at 24°C on standard cornmeal-sucrose-yeast-agar medium.

### DAPI staining of polytene chromosomes and in situ hybridization

Fat bodies of third instar larvae were dissected in physiological solution (0.7% NaCl in distilled water), transferred in a drop of ethanol-propionic acid 3:1 and incubated for 10-20 minutes. The fat bodies were then transferred in about 6 µl of fixative solution (40% acetic acid, 30% lactic acid, 30% distilled water) on a siliconized coverslip. After 3-5 minutes, a very clean, dust-free slide was lowered onto the coverslip, and pressed very lightly with a finger. Then, the sandwich was reversed and squashed very gently for 1-2 minutes between two sheets of blotting paper. After squashing, the slide was immersed in liquid nitrogen for about 20 seconds and, after removal of the coverslip by a razor blade, the slide was immediately immersed in PBS at room temperature and stained with 0.05 µg/ml of DAPI dissolved in 2×SSC for 4 minutes. The in situ hybridization assays were performed according to Pimpinelli et al. (Pimpinelli et al., 2000).

### Antibodies and probes

The antibodies used for immunostaining experiments were: mouse anti-Ubx monoclonal FP3.38 antibody (White and Wilcox, 1984); rat anti-Abd-A polyclonal antibody (Macias et al., 1990); mouse anti-Abd-A monoclonal Dmabd-A.1 antibody (Kellerman et al., 1990); mouse anti-Abd-B monoclonal antibody (Celniker et al., 1989); rabbit anti-Polycomb polyclonal antibody affinity purified obtained by R. Paro; and rabbit anti-Trithorax polyclonal antibody obtained by P. Harte.

### Immunostaining of whole fat bodies

Whole fat bodies of third instar larvae were dissected in 0.7% NaCl, 1% Triton solution on a siliconized slide and incubated for 2 min in fixative 1 (3% formaldehyde, 1% Triton in PBS) and then in fixative 2 (45% acetic acid, 3% formaldehyde, 1% Triton) for 8 minutes. After blockage for 30 minutes in PBS containing 1% nonfat dry milk and 1% Triton at room temperature, the tissues were incubated with primary antibody diluted in PBS/BSA 1% for 1 hour at room temperature in a humid chamber. After three washes (5 minutes each) in PBS, the tissues were incubated with secondary antibody diluted in PBS/BSA 1% for 1 hour at room temperature, washed in PBS for three times (5 minutes each) in PBS and stained with 0.05 µg/ml of DAPI dissolved in 2×SSC for 4 minutes.

### Immunostaining of polytene chromosomes

For immunostaining of polytene chromosomes of fat bodies with antibodies against Polycomb and Trithorax, the chromosomes were fixed as for DAPI staining. For immunostaining with antibodies against Ubx, Abd-A and Abd-B, the chromosomes were fixed according to James et al. (James et al., 1989). In both cases, the fixed preparations were stained with 0.05 µg/ml of DAPI dissolved in 2×SSC for 4 minutes, washed in PBS and examined under the fluorescence microscope to select satisfactory preparations (slides

were examined in PBS). The selected slides were immersed into PBS containing 1% Triton X 100 and left for 20 minutes at room temperature, then transferred in PBS containing 1% nonfat dry milk for 30 minutes at room temperature and then incubated with primary antibody (diluted in PBS/BSA 1%) for 1 hour at room temperature and overnight at 4°C in a humid chamber. After incubation with the primary antibody, the slides were washed three times (5 minutes each) with PBS containing 0.5% non fat dry milk and incubated with secondary antibody (diluted in PBS/BSA 1%) for 1 hour at room temperature. The slides were then washed three times (5 minutes each) in PBS at 4°C. and stained with DAPI for 4 minutes. After washing in PBS for about 2 minutes, the slides were finally mounted in anti-fading medium and sealed with nail polish or rubber cement. Owing to the difficulty in obtaining very good pictures of entire polytene chromosomes, the mapping of the homeotic proteins has been carried out by examining about 50 squashed polytene nuclei for each staining.

### Southern blot analyses

DNA samples were extracted from different tissues of *Drosophila* larvae and adults. The samples were then used for Southern blot hybridization signals detection according to Moshkin et al. (Moshkin et al., 2001).

## RESULTS AND DISCUSSION

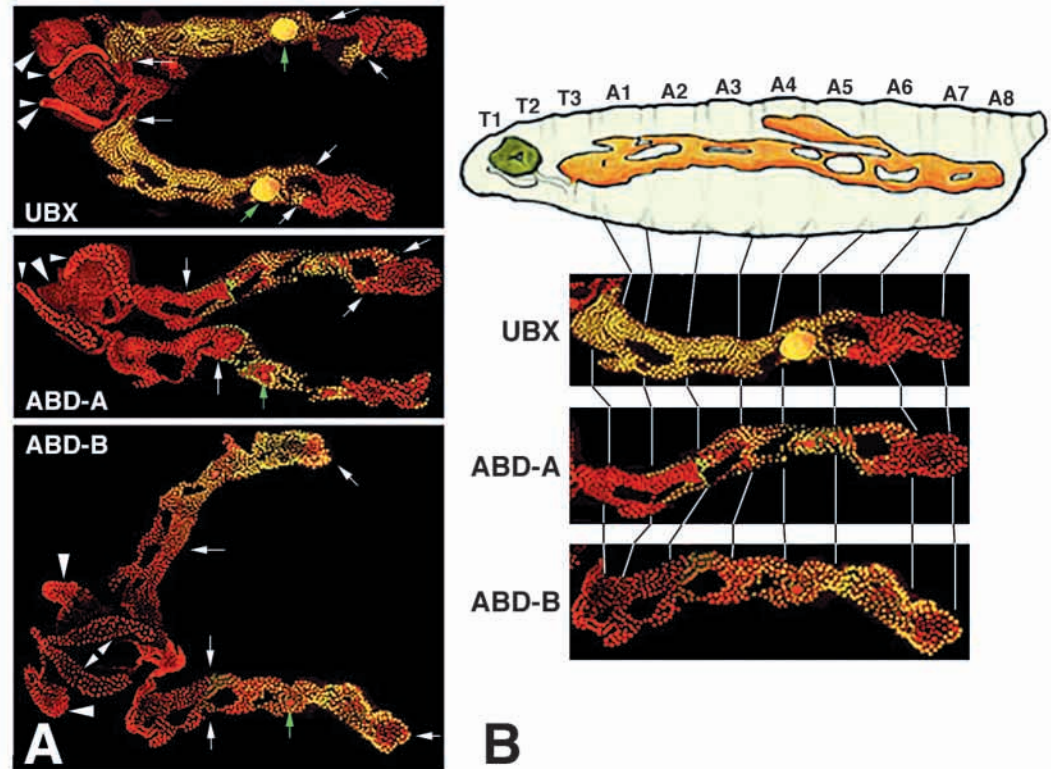
### The Ubx, Abd-A and Abd-B proteins are differentially expressed along the fat body

The larval fat bodies are mesodermal in origin and as noted are comprised of cells with polytene chromosomes. They are symmetrically positioned between the gut and the muscles of the body wall and extend longitudinally along the right and left sides of the larva (Bate and Martinez-Arias, 1993). The most anterior part of this tissue is associated with the salivary glands, while its posterior-most extension is to the eighth abdominal segment. Previous data have strongly suggested that the fat bodies are likely to be segmentally specified, and, in fact, they display homeotic transformations in homozygous Ubx mutant larvae (Ritzki and Ritzki, 1978).

In order to characterize the anterior to posterior expression patterns of the BX-C genes along the length of the fat body, we stained whole tissues with antibodies directed against the Ubx, Abd-A and Abd-B proteins. The patterns of expression of these proteins are shown in Fig. 1A and diagrammed in Fig. 1B. We found that Ubx is intensely expressed in a contiguous region, with an anterior limit distal to, but near, the anterior crossbridge in the third thoracic segment (T3). The domain includes the gonad, and the posterior limit falls in a region corresponding approximately to segments A6/A7. The Abd-A protein is expressed anteriorly in a longitudinal line of cells in a region corresponding to the A2 segment. From that point posteriorly it is accumulated in almost all of the cells in a region that is co-extensive with abdominal segments A3-A7. Finally, the Abd-B protein is expressed to the posterior end of the fat body with an anterior limit in the middle of A4.

It is interesting to note that although Ubx is detected in all the nuclei of its domain, Abd-A and Abd-B are only expressed in subsets of nuclei in their respective domains. However, in the region corresponding to segments A4-A6 all of the proteins are co-expressed in most nuclei. These observations demonstrate that the protein products of the BX-C are

**Fig. 1.** Expression patterns of the Ubx, Abd-A and Abd-B proteins on the AP axis of the fat body. (A) Patterns produced by antibodies directed against the BX-C encoded proteins on the AP axis of *Drosophila* larval fat body. Green arrows indicate the larval gonads. (Top) The Ubx pattern in the fat body of a third instar male larva. The expression of the Ubx protein starts from the anterior bridge (white arrows) and ends in a region situated posterior to the gonad (white arrows) while the anterior of the fat body (large arrowheads) and the salivary glands (small arrowheads) appear devoid of signal. (Middle) Expression pattern of Abd-A in the fat body of a third instar female larva. This protein is accumulated more posteriorly than Ubx (arrows) but anteriorly partially overlaps the Ubx domain. Note that Abd-A is not expressed in all nuclei within its domain. (Bottom) The expression pattern of Abd-B in the fat body of a third instar female larva. This protein is the most posteriorly expressed of the three (arrows). However, its anterior limit of expression does partially overlap with the expression of the other two proteins. As with Abd-A, the protein is not expressed in all the nuclei of its domain. Note that the same immunopatterns were observed in both male and female fat bodies. However, the male gonads showed a low level of immunostaining with all the antibodies, while the female gonads lack any staining. At present, it is not possible to determine if this difference is due to a sex specific function of the homeotic proteins in the gonadal mesoderm or to the difference in size of the gonads in the two sexes. (B) The topological relationship of the Ubx, Abd-A and Abd-B staining patterns with the cuticular ectodermal segments (top). The most anterior expression of the Ubx corresponds approximately to T3 while the posterior border is approximately situated in the A6 segment. The Abd-A protein is expressed in a region co-extensive with segments A3-A7. At its anterior limit it is expressed along two external lines of cells in a region corresponding to segment A2. The Abd-B protein is expressed from a region corresponding to the middle of A4 to the posterior end of the fat body. Note that while Ubx seems to be expressed homogeneously along its domain, Abd-A and Abd-B are expressed only in subsets of cells within their domains of accumulation. Another point of interest is that the immunopatterns seem to suggest that the three proteins could be simultaneously expressed in most nuclei in a region corresponding to segments A4-A6. This point deserves to be better analyzed by tripe labelling when appropriate primary antibodies become available.



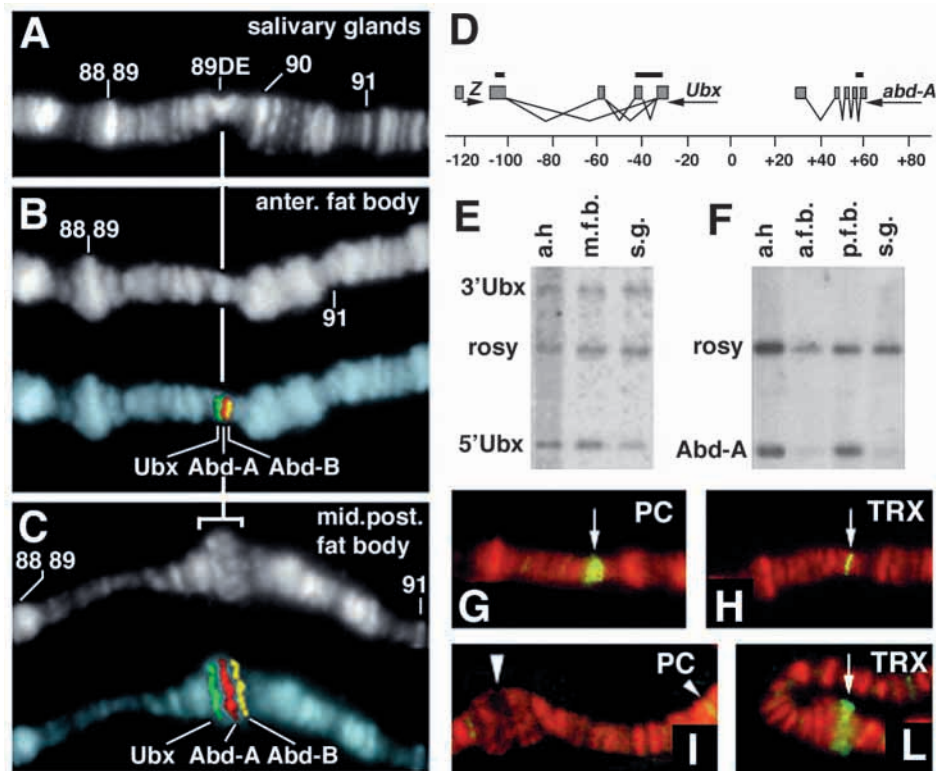
differentially expressed along the AP axis of the fat body in a manner reminiscent of their accumulation patterns in the epidermis. However, the similarity of expression patterns of the proteins between the two tissues is more evident at their anterior limits than in their posterior extent. Perhaps the most striking result is the overlap of the three proteins in the region around the gonads. It will be interesting to determine if this overlap of domains has some operational significance, or if it is functionally irrelevant as is suggested by studies of the larval epidermal cells which give rise to the cuticle (Gonzales-Reyes et al., 1990).

#### The active BX-C genes form visible puffs on polytene chromosomes of fat body

To determine if the expression of BX-C encoded proteins is correlated with cytologically visible changes at the BX-C locus, we compared the appearance of the region corresponding to the 89DE salivary gland chromosome interval in fat body cells derived from different regions of the fat body. Cells from various segments along the anterior

posterior axis of the fat body were isolated and polytene chromosomes prepared for DAPI staining. As shown in Fig. 2B, we found that in chromosomes from anterior fat body cells the BX-C locus appears highly condensed as revealed by its intense DAPI bright fluorescent. This appearance is similar to the BX-C in polytene chromosomes in salivary glands (Fig. 2A). However, in chromosomes taken from fat body cells extracted from the mid-posterior part of the larvae, the 89DE region clearly appears puffed (Fig. 2C). In situ hybridization with cDNA probes derived from the *Ubx*, *abd-A* and *Abd-B* genes clearly shows that although the signals appear tightly closed in anterior fat body nuclei (Fig. 2B), in chromosomes from the mid-posterior fat body, the signals appear more distantly positioned relative to each other and show that the puffed region corresponds to the BX-C (Fig. 2C). These results clearly demonstrate that the activity of BX-C genes is accompanied by visible changes at the chromosomal and locus level. We investigated the extent to which this change in visible appearance might reflect changes at the physical level. It has been shown that in

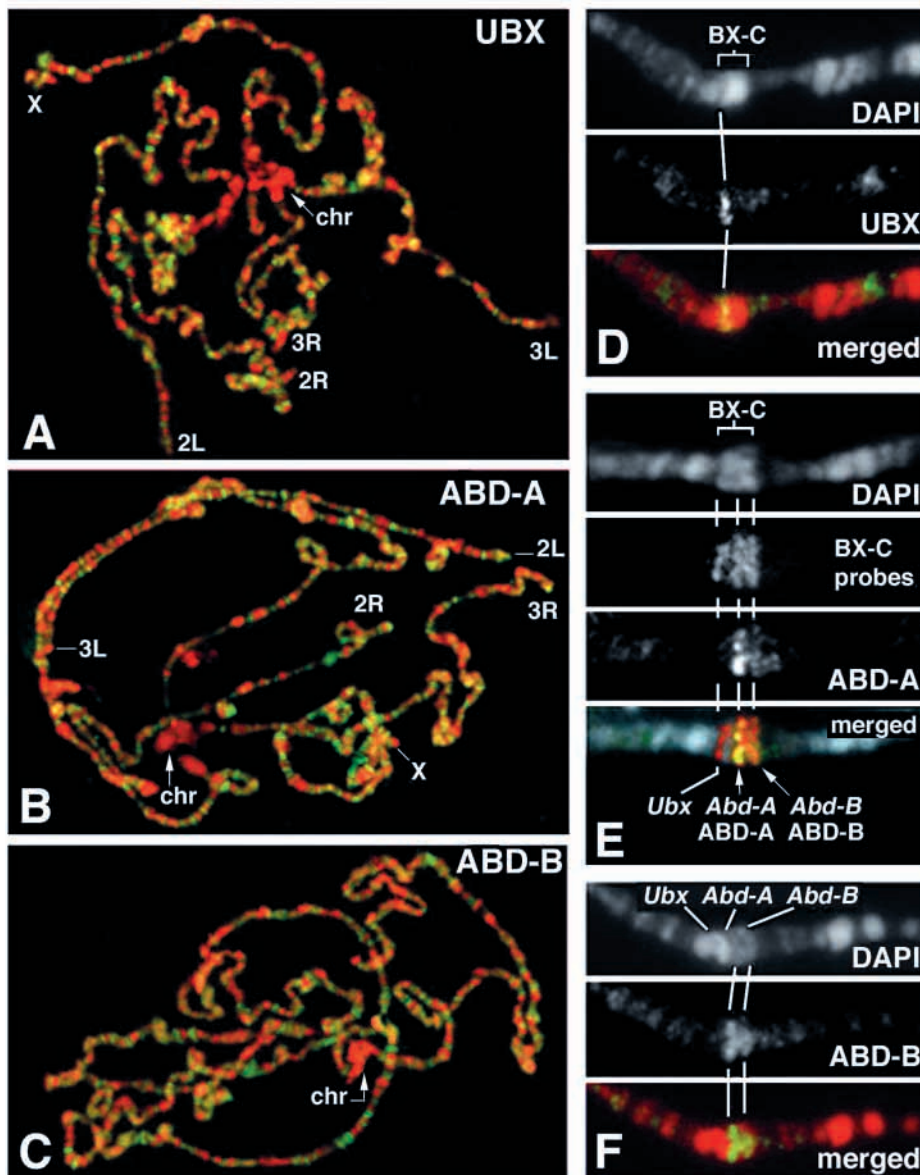
**Fig. 2.** The expression of the BX-C genes in the fat body is related to cytologically visible changes of the 89DE BX-C region of the polytene chromosomes. (A) The 89DE region appears strongly condensed in polytene chromosomes of the salivary glands. (B) The 89DE region also appears condensed in polytene chromosomes in cells from the anterior parts of the fat body (big arrows in Fig. 1A). In situ hybridization with probes corresponding to the *Ubx*, *abd-A* and *Abd-B* genes clearly shows that these genes are tightly physically linked. (C) The 89DE region in polytene chromosomes of mid-posterior fat body cells appear strongly puffed and, by in situ hybridization with the same probe as in B, the three genes appear more physically distinct. (D-F) The BX-C genes that are active in fat body cells are also amplified relative to cells in which they are quiescent. (D) The structure of the *Ubx* and *abd-A* genes. The gray boxes indicate the exons. The black blocks above indicate the probes used to determine the degree of amplification of the corresponding genomic sequences. (E) The panel shows a Southern blot of genomic DNA from adult heads (a.h.), mid fat body (m.f.b.) and salivary glands (s.g.) probed with a DNA fragment corresponding to the *rosy* gene and two other fragments corresponding to the 5' and 3' regions of the *Ubx* gene. As has been shown (Moshkin et al., 2001), a comparison of the two *Ubx* signals with the *rosy* signal between adult heads and the salivary glands demonstrates that the 3' end of the *Ubx* gene is amplified while the 5' end is under replicated. We observed the same pattern in anterior fat body where the *Ubx* gene is repressed (not shown). However, in mid fat body, where the *Ubx* gene is active, both the 3' and 5' ends appear amplified. (F) The panel shows a Southern blot of genomic DNA from adult heads (a.h.), anterior fat body (a.f.b.), posterior fat body (p.f.b.) and salivary glands (s.g.) probed with a DNA fragment corresponding to *rosy* and a fragment corresponding to a region of *abd-A*. Again *abd-A* appears under replicated in salivary glands and anterior fat body where this gene is repressed, while it appears amplified in posterior fat body where *abd-A* is expressed. (G-L) Patterns of localization of the Polycomb (Pc) and Trithorax (Trx) proteins in the 89DE region of polytene chromosomes from salivary glands and mid-posterior fat body. (G,H) Staining of polytene chromosomes from salivary glands with Pc and Trx antibodies respectively. As has been shown, Pc is strongly accumulated on the BX-C locus (arrow). Trx is also located in the same region (arrow) although the fluorescence intensity suggests that is accumulated at much lower levels than Pc. (I,L) Staining of polytene chromosomes from mid posterior fat body with Pc (I) and Trx (L) antibodies. Pc is not detectable on strongly puffed 89DE region (large arrowhead) where the BX-C genes are active, while the same region shows a strong accumulation of Trx (arrow). Small arrowhead indicates a PC signal outside the 89DE region.



polytene chromosomes of salivary glands the BX-C region is under replicated and that the under replication seems to exclude the proximal and distal parts of the complex corresponding to the 3' and 5' ends of the *Ubx* and *Abd-B* genes, respectively (Moshkin et al., 2001). We tested whether the observed chromatin changes related to the expression of the homeotic gene in fat bodies also includes changes in their level of endoreduplication. To this end, we performed Southern blot experiments, as described previously (Moshkin et al., 2001), on genomic DNA extracted from salivary glands, fat bodies and diploid cells from adult heads. These blots were hybridized with specific probes from the 3' and 5' ends of *Ubx*, from an exon of *abd-A* and from the *rosy* gene as a control. As shown in Fig. 2D-F, we found that in the anterior fat body, where the genes are repressed, there is under replication of BX-C sequences similar to the polytene chromosomes of salivary glands. By contrast, the same sequences appear amplified relative to controls in fat body cells where they are actively expressed.

### The BX-C puffed region on polytene chromosomes of fat body is enriched for the Trithorax protein and lack the Polycomb protein

The maintenance of the repressive or active states of the homeotic genes depends on the function of two groups of proteins, the Polycomb-Group (Pc-G) (for reviews, see Paro, 1990; Pirrotta, 1997) and the trithorax-Group (trx-G) (for a review, see Kennison, 1993), respectively. Staining using Pc-G- or trx-G-specific antibodies has revealed that the Pc protein is strongly accumulated on the BX-C region (Zink and Paro, 1989), while the Trx protein is only faintly detected on polytene chromosomes of larval salivary glands (Chinwalla et al., 1995). To assess possible visible changes at chromosomal level in the localization patterns of Pc and Trx proteins in fat body cells, we immunostained polytene chromosomes from the anterior and mid-posterior parts of larval fat bodies. As shown in Fig. 2G-L, we found that in chromosomes from the anterior of the fat body, where the homeotic genes are repressed, Pc appears to be strongly accumulated on the BX-C locus (Fig. 2G), whereas only a weak signal is produced by staining with the Trx antibody in



**Fig. 3.** Patterns of localization of Ubx, Abd-A and Abd-B on polytene chromosomes of the fat body. (A-C) The patterns after staining with Ubx (A), Abd-A (B) and Abd-B (C) antibodies. All the antibodies give numerous signals along the length of all of the chromosomes with the exception of the chromocenter. (D) Enlarged picture of the BX-C region showing that Ubx is located in the proximal part of the region corresponding to the *Ubx* locus. (E) Enlarged picture of BX-C region sequentially stained with Abd-A antibodies and in situ hybridized with probes derived from *Ubx*, *abd-A* and *abd-B* cDNAs. The merged figure clearly shows that the Abd-A signal overlaps with both the *abd-A* and *abd-B* genes, while the *Ubx* gene appears devoid of signal. (F) Enlarged picture of the BX-C region showing the Abd-B staining pattern. Similar to the Abd-A protein, we observed that Abd-B is located on the *abd-A* and *abd-B* genes but is excluded from *Ubx*. X, X chromosome; 2L and 2R, left and right arms of the second chromosome; 3L and 3R, left and right arms of the third chromosome; chr, chromocenter.

cells from the same region (Fig. 2H). In chromosomes from the mid-posterior part of the fat body, where the homeotic genes are active, we can detect no Pc (Fig. 2I) but see clear evidence of Trx accumulation (Fig. 2L), thus confirming that these two proteins are differentially present in repressed or active chromatin domains. We are currently analyzing the binding patterns of several other Pc-G and trx-G proteins to obtain a general picture, at the cytological level, of their accumulation on the BX-C locus in relation to its differential gene activity.

#### The BX-C proteins bind multiple mappable sites on polytene chromosomes of fat body

Given the relatively high resolution of localization we were able to obtain for the Pc and Trx proteins, we next examined whether the binding sites for Ubx, Abd-A and Abd-B were discernable in the fat body polytene chromosomes thereby providing evidence for homeotic gene targets in this tissue. To this end, we individually stained squashed polytene nuclei from different segments of the fat bodies with antibodies

specific for the three proteins (James et al., 1989). As shown in Fig. 3, the staining patterns reveal that Ubx (Fig. 3A), Abd-A (Fig. 3B) and Abd-B (Fig. 3C) can be found at many sites on the chromosomes. The results of a detailed analysis of the staining patterns/binding sites of the three homeotic proteins are reported in Table 1. Interestingly, some of the Ubx binding sites correspond to loci known as targets of homeotic proteins (Botas and Awers, 1996; Graba et al., 1997) while other known target genes map in regions that lack any signal. This suggests the possibility that the technique is revealing tissue specific regulation of the expression of different sets of genes. An inspection of the staining patterns produced by the Ubx, Abd-A and Abd-B antibodies reveals that these proteins appear to share several targets. Particularly intriguing is the pattern produced by these proteins along the BX-C itself. In detail, the antibody against the Ubx protein produces a unique signal correlated with the *Ubx* locus (Fig. 3D). The antibodies against the Abd-A (Fig. 3E) and Abd-B (Fig. 3F) proteins produce overlapping signals where the *abd-A* and *abd-B* genes are located. The absence of Abd-A and Abd-B staining at the *Ubx* locus suggests that these proteins are not involved in regulating *Ubx* and provides an explanation for the distribution of Ubx expressing cells along the AP axis of the fat body (see Fig. 1). The presence of Ubx on *Ubx* does, however, suggest the possibility of positive autoregulation at that locus. Moreover, the binding patterns of Abd-A and Abd-B suggest that these proteins may also cooperate in regulating themselves as well as each other.

**Table 1. Binding sites of Ubx, Abd-A and Abd-B on polytene chromosomes of the fat body**

X*	Ubx	Abd-A	Abd-B	2L*	Ubx	Abd-A	Abd-B	2R*	Ubx	Abd-A	Abd-B	3L*	Ubx	Abd-A	Abd-B	3R*	Ubx	Abd-A	Abd-B
1	-	+	-	21B1	-	+	+	41B	+	-	-	<b>61A</b>	+	+	+	82D	+	-	-
2	-	+	+	21C	-	-	+	41C	+	-	-	61B	-	+	+	82E	+	-	-
3	-	+	+	21E	-	+	-	41D	+	-	-	61C	+	+	-	82F	+	-	-
3E	-	+	-	21F	+	-	-	41F	+	-	-	62E	+	-	+	83C	+	-	-
4	-	-	+	22A	-	+	-	42A	+	-	+	63A	+	-	-	83F	-	-	+
4C	-	+	-	<b>22C</b>	+	+	+	42B	-	-	+	63C	-	+	-	84A	+	-	-
4D	-	-	+	22F	+	-	+	42D	-	+	-	63D	-	-	+	84B	+	-	-
5A	-	+	+	23A	-	-	+	42E	+	-	-	64A	+	-	-	84D	+	-	+
5B/C	-	-	+	23C	-	+	-	42F	-	+	-	<b>64B</b>	+	+	+	84F	+	-	-
5C	-	-	+	23D	+	-	-	43C	+	-	+	64C	-	+	+	85A	-	-	+
5D	+	-	-	23F	-	+	-	43D-E	+	-	-	64E	+	-	-	85B	-	-	+
<b>6C/D</b>	+	+	+	24A	-	-	+	43F	+	-	-	65D	+	+	-	85C	+	-	-
<b>7A/B</b>	+	+	+	<b>24C</b>	+	+	+	44B	+	-	-	65F	-	+	-	85D	-	-	+
7D	-	+	-	24D	-	-	+	44C/D	+	+	-	66A	+	+	-	<b>85F</b>	+	+	+
7F	+	-	-	24E	+	-	-	44E	+	-	+	66C	+	+	-	<b>86B</b>	+	+	+
8D	+	-	+	<b>25A</b>	+	+	+	44F	-	-	+	66D	-	+	+	86C	+	-	+
<b>8D/E</b>	+	+	+	<b>25B</b>	+	+	+	45F	+	-	+	66E	+	-	-	86D	+	-	-
8F	-	+	+	25C	+	-	+	46A	-	+	-	66F	+	-	-	86E	+	-	+
9A/B	+	-	-	25D?	+	+	-	46B	-	+	-	67A	+	-	-	<b>87A</b>	+	+	+
9B	+	+	-	26A	+	+	-	46C	+	-	+	67C	+	-	-	87B	+	-	+
9C	-	+	-	26B	+	+	-	46D	-	+	-	<b>67D</b>	+	+	+	87C	+	-	+
9D/E	+	+	-	26D	+	-	-	46F	+	+	-	67E	+	-	+	87F	+	-	+
9E	-	+	-	26E	+	-	-	47A	-	-	+	68B	-	+	-	88A	-	+	-
9F	-	+	-	27A	+	-	-	47D	-	-	+	68C	+	-	-	88B	-	+	+
10A	+	+	-	27D	+	-	-	47F	+	+	-	68D	+	-	+	88D	+	+	-
10B	-	+	-	27E	+	+	-	<b>48B</b>	+	+	+	68E	-	-	+	88F	+	+	-
10C	+	-	-	28B	-	+	-	48C	-	+	-	68F	+	+	-	89A	+	+	-
10F	+	+	-	28D	-	+	+	<b>48F</b>	+	+	+	69A	+	+	-	<b>89B</b>	+	+	+
11A	+	-	-	28E	-	+	-	49A	+	-	-	69F	+	-	+	89C	+	-	+
<b>11B</b>	+	+	+	29D	+	-	+	49B	+	-	-	70A	+	+	-	Ubx	+	-	+
11E	-	-	+	29F	+	-	-	49C	+	+	-	70B	+	+	-	Abd-A	-	++	+
11F	-	-	+	<b>30A</b>	+	+	+	49D	+	-	-	<b>70F</b>	+	+	+	Abd-B	-	+	+
12A	-	-	+	<b>30B</b>	+	+	+	49E	-	-	+	71E	+	-	-	89E	+	-	-
12B	-	-	+	30D	+	+	-	49F	+	+	-	71F	-	+	-	89F	+	-	+
12D	-	-	+	30F	-	-	+	50A	+	-	-	72	-	-	+	90B	+	+	-
12F	-	-	+	31B	-	+	-	50B	-	+	-	73A	-	+	-	90E	+	-	+
13A	+	-	-	31D	-	+	-	50C	+	-	+	73B	-	+	-	90F	+	+	-
13C	+	+	-	31E	-	+	-	50E-F	+	-	-	73C	-	+	-	91A	+	+	-
13D	-	+	+	31F	-	+	-	51B	-	+	-	73D	+	+	-	91C	+	-	-
13E	+	-	-	32B	-	+	-	51C	-	+	+	73E	+	+	-	91D	+	-	-
13F	-	+	-	32C	-	+	-	51E	+	-	-	74E	+	-	-	92B	-	+	+
14C	-	+	-	32E	-	+	-	51F	+	+	-	<b>74F</b>	+	+	+	92D	-	-	+
14D	+	-	+	33A	-	+	-	52A	+	+	-	75A	-	+	-	<b>92E</b>	+	+	+
15B	+	-	-	33B	-	+	+	52C	+	-	+	75B	+	+	-	92F	-	+	+
15C	+	-	-	33E	-	+	+	52D	+	-	+	75C	-	-	+	93B	-	+	+
16B	-	-	+	34A	+	-	-	<b>52E</b>	+	+	+	<b>75D</b>	+	+	+	93C	+	+	-
16A	-	+	-	34B	+	-	-	<b>52F</b>	+	+	+	75E	-	+	-	93D	+	-	-
16C	-	+	+	34C	-	+	+	53F	-	+	-	75F	-	+	-	<b>93E</b>	+	+	+
16D	-	+	-	34D	-	+	+	54C	+	+	-	76B	+	-	-	93F	+	+	-
16F	-	+	-	34E	-	+	+	54D	+	+	-	76C	+	-	+	94A	-	+	-
17A	+	-	+	35A	-	-	+	54E	-	+	-	<b>76D</b>	+	+	+	94B-C	+	-	-
17F	-	+	-	35B	-	-	+	55D	-	+	+	77A	+	-	-	94D	-	+	+
18A	-	+	-	35D	+	+	-	55E	-	+	+	77B	+	-	-	94E	-	-	+
18C/D	-	+	-	35E	-	-	+	55F	-	-	+	77E	+	-	+	95B	-	-	+
18F	-	+	-	<b>35F</b>	+	+	+	56B	-	+	-	78	-	-	+	95D	+	-	+
20	-	-	+	<b>36A</b>	+	+	+	56C	-	-	+	78C	+	-	-	95E	-	-	+
				36D	-	+	+	56E	-	+	+	79B	+	-	-	96A	-	-	+
				37A	-	-	+	57A	-	-	+	79C	+	-	-	96B	-	+	-
				37C	-	+	-	57B	-	-	+	79F	+	+	-	96C	+	-	-
				37F	-	+	+	57F	+	+	-					96D	+	-	-
				38B	-	+	-	58A	+	-	-					96E	-	-	+
				38F	-	+	-	58D	-	+	-					96F	+	-	+
				39A	-	-	+	58E	+	+	-					97D	+	-	-
								<b>58F</b>	+	+	+					97F	+	-	-
								59B	+	-	+					98A	+	-	+
								59D	+	-	-					98C	+	-	-
								60A	+	-	+					98D	+	-	-
								60C	+	+	-					98F	+	-	-
								<b>60D</b>	+	+	+					99B	+	-	+
								<b>60E</b>	+	+	+					99D	+	-	-
																99F	+	-	-
																100A	+	-	+
																100C	+	-	-
																100D	+	-	-

\*Chromosomes.

Bold indicates the common binding sites.

## Conclusions

In conclusion, the present work on BX-C gene and encoded protein expression along the AP axis of the fat body has allowed us to address important questions about the function of the resident genes of the complex. First, the data show that the BX-C genes are differentially expressed along the AP axis of the fat body, with patterns parallel to those observed in the epidermis. This observation provides strong support to the hypothesis that this mesodermal tissue also has a segmental character and that the BX-C may be involved in the specification of at least three domains. Second, the expression of the BX-C proteins in the nuclei of the fat body has allowed us to obtain evidence of activity of the BX-C. The observation of visible puffs at this locus suggests that there are important changes in chromatin conformation related to the observed differential activity of the genes within the complex. The differential puffing of this region will also permit examination of the presence or absence of regulatory proteins on the inactive or active BX-C locus. Third, it has been possible, for the first time, to determine the chromosomal binding sites of the BX-C encoded proteins in vivo under normal physiological conditions.

Taken together, these data provide an important basis for identifying specific downstream targets of the BX-C encoded proteins, and for identifying new chromosomal proteins that regulate the BX-C locus. Recent technological advances have increased the sensitivity of microarray analyses allowing investigators to perform experiments on small numbers of cells. Hence, it should now be possible to compare the transcriptional profile of different domains of the larval fat body using this technique. A comparison of microarray data with the results described in this report should help identify candidate genes that are likely to be direct, as opposed to indirect, targets of each of the BX-C genes. Finally, on a practical note, it should be pointed out that in addition to the fat body and salivary gland, other tissues in *Drosophila* have polytene chromosomes that might be suitable for the types of approaches described in this report.

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