Development 111, 393–405 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

Molecular definition of the morphogenetic and regulatory functions and the *cis*-regulatory elements of the *Drosophila Abd-B* homeotic gene

ANNE M. BOULET, ALAN LLOYD and SHIGERU SAKONJU*

Howard Hughes Medical Institute, Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, Utah 84132, USA

* Author for correspondence

Summary

The *Abdominal-B* (*Abd-B*) gene, a member of the *Drosophila* bithorax complex, is required during development to specify the identity of parasegments 10–14. Based on genetic studies, Casanova, J., Sánchez-Herrero, E. and Morata, G. (1986) *Cell* 47, 627–636, proposed that the *Abd-B* gene consists of two distinct elements that provide a morphogenetic (m) function in PS 10–13 and a regulatory (r) function in PS 14, where it represses m function. Here we present molecular confirmation of this genetic model. Using specific antibodies, we show that the $55\times10^3 M_r$ and $30\times10^3 M_r$ *Abd-B* proteins, predicted by cDNA analysis, are indeed present in PS 10–13 and PS 14, respectively. We also examine *Abd-B* mRNA and protein expression patterns in embryos mutant for either the m or r function. These data allow us to unambiguously assign m function to the $55\times10^3 M_r$ protein and r function to the $30\times10^3 M_r$ protein. Furthermore, as postulated by the model, transcription of the mRNA encoding the m protein is derepressed in PS 14 in the absence of r function. We have also studied the effect of mutations mapping in the *infra-abdominal* (*iab*) region located downstream of the *Abd-B* gene. Genetic studies suggest that the *iab* region contains *cis*-acting regulatory elements controlling *Abd-B* expression in PS 10–12. We present molecular evidence for the presence of downstream *cis*-regulatory elements by analyzing *Abd-B* mRNA and protein patterns in *iab-6* and *iab-7* embryos. Our analysis reveals the presence of parasegment and cell-specific regulatory elements of the *Abd-B* gene within each *iab* region. The *Abd-B* gene may provide a model for the understanding of similarly complex homeotic genes in higher organisms.

Key words: bithorax complex, *Abdominal-B*, *cis*-regulatory elements, *infra-abdominal* mutations.

Introduction

The homeotic genes of *Drosophila melanogaster* act during development to specify the unique identities of each segmental unit (for review see Akam, 1987). The bithorax complex (BX-C) specifies the identities of the posterior thorax and the abdominal segments (Lewis, 1978). There are three lethal complementation groups within the BX-C, designated *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*) (Sánchez-Herrero *et al.* 1985*a,b*; Tiong *et al.* 1985; Casanova *et al.* 1987). The order of these genes on the chromosome reflects the order of segments along the anterior–posterior axis that are affected by mutations in each gene (Karch *et al.* 1985; Duncan, 1987). The *Ubx* gene is required for the proper identity of the third thoracic and first abdominal segments (T3 and A1). The next adjacent gene, *abd-A*, is required in the second through the seventh abdominal segments (A2–A7). The *Abd-B* gene is active in the fifth through the ninth abdominal segments, corresponding to parasegments 10–14 (a parasegment consists of the posterior compartment of one segment and the anterior compartment of the adjacent segment, Martinez-Arias and Lawrence, 1985). *Abd-B* directs proper development of certain posterior larval and adult structures including genitalia in the adult fly.

The majority of *Abd-B* mutations fall into one of three classes (Casanova *et al.* 1986). Class I mutations affect the development of PS 10–13. Class II mutations cause transformations of PS 14. Mutations of Class III are strictly lethal and affect PS 10–14. Class I and Class II mutants complement each other, but both fail to complement Class III mutants. It has been proposed that Class I and Class II lesions inactivate two distinct elements of the *Abd-B* gene and that Class III mutations inactivate both of these elements simultaneously. The activity affected by Class I mutations is called the morphogenetic or m element because it is required to produce the morphological diversity of the region from PS 10 through 13. The activity absent in Class II mutants is called the regulatory or r element.

This element is required for terminalia development and appears to suppress the activity of the *Abd-B* m element as well as other homeotic genes in PS 14. Class I, II and III mutants will be referred to as m^-r^+, m^+r^- and m^-r^- mutants, respectively.

It has recently been shown that the *Abd-B* gene contains four overlapping transcription units, utilizing at least three separate promoters (DeLorenzi *et al.* 1988; Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988; Celniker *et al.* 1989; Zavortink and Sakonju, 1989). These mRNAs will henceforth be referred to as class A, B, C (Zavortink and Sakonju, 1989) and gamma transcripts (Kuziora and McGinnis, 1988). Data derived from *in situ* localization of *Abd-B* transcripts in wild-type embryos suggest that the m element corresponds to the class A transcript, and that r element functions may be carried out by either the class B or C mRNA species, or both acting together (DeLorenzi *et al.* 1988; Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988; Celniker *et al.* 1989; Zavortink and Sakonju, 1989). The gamma transcript is expressed in PS 15 (Kuziora and McGinnis, 1988), outside the domain of known *Abd-B* mutant effects, and will not be considered further in this report. cDNA sequence analysis suggests that the class A transcript encodes a 55×10^3 M_r protein while the class B and class C mRNAs encode a 30×10^3 M_r protein that lacks the amino-terminal portion of the 55×10^3 M_r protein (Celniker *et al.* 1989; Zavortink and Sakonju, 1989). Celniker *et al.* (1989) have provided evidence that two *Abd-B* proteins are translated in embryos. We and others have proposed that the 55×10^3 M_r protein provides the m function while the 30×10^3 M_r protein is responsible for the r function (DeLorenzi *et al.* 1988; Celniker *et al.* 1989; Zavortink and Sakonju, 1989).

A number of mutations that affect only a portion of the domain of the *Abd-B* morphogenetic (m) function have been identified (Lewis, 1978; Karch *et al.* 1985). These alleles, designated *infra-abdominal*, alter segmental identities in the region from PS 10 through 13. *infra-abdominal-5* (*iab-5*) mutations affect only PS 10, *iab-6* mutations affect PS 10 and 11, and *iab-7* mutations cause defects in PS 10, 11 and 12 (Karch *et al.* 1985; Duncan, 1987). In contrast, the m^-r^+ mutants mentioned above affect the entire region from PS 10 through PS 13. *Abd-B* mutants are unable to complement the *iab-5*, *6* and *7* mutants in *trans*. Thus the wild-type *iab* elements must be present in *cis* to obtain proper *Abd-B* activity. Molecular mapping of the DNA lesions associated with various *iab* alleles showed that breakpoints of a particular category of *iab* mutant are clustered (Karch *et al.* 1985; Duncan, 1987), and *iab* alleles affecting *Abd-B* are located close to the *Abd-B* transcription unit. These, and additional observations, have led to the hypothesis that *iab* mutations cause a disruption of *cis*-acting regulatory sequences controlling *Abd-B* expression (Karch *et al.* 1985; Casanova *et al.* 1987; Peifer *et al.* 1987).

Our current understanding of the mRNA and protein products of the *Abd-B* gene provides a means to test, at the molecular level, the genetic model of Casanova *et al.* (1986) and the proposal that the *iab* regions represent *cis* regulatory elements for *Abd-B* expression. *In situ* hybridization and antibody staining of whole-mount *Drosophila* embryos were initially used to characterize the wild-type patterns of *Abd-B* mRNA and protein expression. We then examined the expression of the m (class A) and r (class B/C) transcripts in genetically m^-r^+, m^+r^- and m^-r^- mutant embryos. Our results provide the first conclusive evidence that the expression patterns of the two *Abd-B* proteins conform to predictions for the morphogenetic and regulatory elements of Casanova *et al.* (1986). In addition, a study of the mRNA and protein patterns in *iab-6*, and *iab-7* mutants provides evidence that the associated DNA lesions affect the regulation of *Abd-B* transcription in a parasegment and cell-specific fashion.

Materials and methods

Drosophila *strains*

Abd-B and *iab* mutant strains were provided by E. B. Lewis, I. Duncan, and G. Morata and are described in Karch *et al.* (1985), Casanova *et al.* (1986), Duncan (1987) and Celniker and Lewis (1987). *38000.11A* is associated with an inversion with a breakpoint at genomic position +115 within the BX-C and has been classified as an *iab-6* mutant (E. B. Lewis, personal communication). Uab^{1revB9} is an *abd-A*$^-$ *Abd-B* (m^+r^-) mutant isolated by M. Reagan and I. Duncan (personal communication) and is derived from Uab^1 (Lewis, 1978). The *abd-A*$^-$ mutation was introduced to eliminate the dominant gain-of-function phenotype associated with the Uab^1 breakpoint at map position −14 (Karch *et al.* 1985); the original m^+r^- phenotype is unaltered in Uab^{1revB9}.

To allow identification of homozygous mutant embryos, most mutant chromosomes were rebalanced over a TM3 chromosome carrying a *ftz–lacZ* P element insert (provided by S. Smolik-Utlaut). All embryos carrying one or two copies of the balancer will show β-gal expression in a pattern of seven stripes, corresponding to the normal *ftz* pattern. By double labeling embryos with an anti-β-gal antibody and the anti-*Abd-B* antibody, homozygous mutant embryos can be identified by the absence of the striped β-gal pattern. *Tab* or Uab^{1revB9} chromosomes cannot be balanced over TM3, *ftz–lacZ*. *Tab* heterozygous adults are sterile in the absence of a chromosome carrying a duplication of the bithorax complex (Celniker and Lewis, 1987). The Uab^{1revB9} chromosome contains markers which are incompatible with the TM3 balancer.

Transcript localization

Whole mount *in situ* hybridization with digoxigenin-labeled probes was carried out according to Tautz and Pfeifle (1989). Probes were prepared using the random primer labeling method or using asymmetric PCR amplification. The PCR method was designed to produce predominantly the desired strand.

The probe that recognizes all *Abd-B* transcripts corresponds to residue numbers 2933 to 4053 of the B3 cDNA described in Zavortink and Sakonju (1989). The class A probe was derived from residues 508 to 1007 of the class A cDNA (the B3 cDNA clone), a portion transcribed from the class A specific exon. The class B probe was a *Taq*I–*Nae*I fragment of the E19 cDNA, a region transcribed from the class B specific exon (located at about position +184 on the map shown in

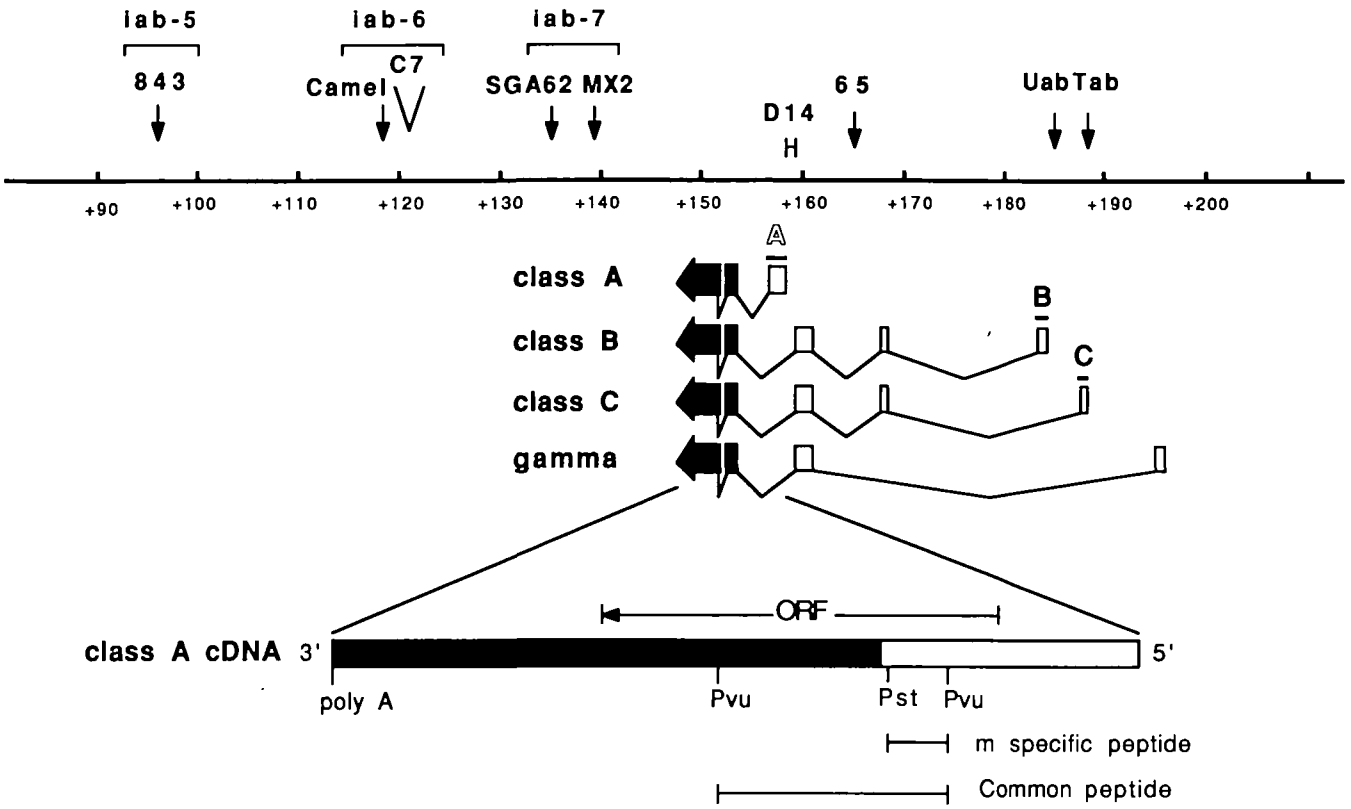

Fig. 1. Map of the *Abd-B* gene. The location of exons for each of the four classes of *Abd-B* transcripts are shown. The open bars denote sequences specific to class A, B, C, or gamma transcripts, while solid bars represent sequences common to all classes of transcript. The locations of class-specific probes used in *in situ* hybridization analysis is indicated by outlined capital letters. Breakpoints of *Abd-B* mutants used in this study are indicated above the line showing map coordinates in kilobases (Karch *et al.* 1985). The *C7* mutation is caused by an insertion at map position +121. The *D14* mutation is associated with a deletion of the class A mRNA transcription start site as described in Zavortink and Sakonju (1989). An enlarged version of the class A cDNA map is drawn at the bottom. The indicated regions were used to construct β-galactosidase fusion proteins for production of m-specific and common *Abd-B* antibodies in rats. Two *Pvu*II sites, at cDNA positions +1607 and +2485, and a *Pst*I site at +1790 are indicated (Zavortink and Sakonju, 1989). The extent of the ORF is indicated with the direction of translation from right to left. The mutations shown have been genetically classified as follows: *843*, *iab-5*; *Camel* and *C7*, *iab-6*; *SGA62* and *MX2*, *iab-7*; *D14*, m^-r^+; *65*, 'm^-r^-'; *Tab* and *Uab*^{1}, m^+r^- (Duncan, 1987; Casanova *et al.* 1986; discussion in text).

Fig. 1). The class C probe corresponds to an *Spe*I–*Xma*I fragment from the class C specific exon, located at position +188.

Production of antibodies

DNA fragments from a class A cDNA clone (B3 cDNA of Zavortink and Sakonju, 1989) were cloned into the appropriate form of the expression vector pWR590 (Guo *et al.* 1984) to produce in-frame fusions of *Abd-B* and β-galactosidase coding sequences. A *Pvu*II fragment extending from position +1607 to +2485 was used to produce an antibody that recognizes both predicted *Abd-B* proteins. A *Pvu*II-*Pst*I fragment (+1607 to +1790) was used to generate an antibody specific for the 55×10^3 M_r m protein encoded by the class A mRNA. Fusion proteins were isolated from *E. coli* and injected into rats at the Pocono Rabbit Farm (Canadensis, PA). Two rats were injected with each of the *Abd-B* fusion proteins. Antibodies were purified from crude serum according to standard procedures (Carroll and Laughon, 1987). First, anti-β-gal and anti-bacterial antibodies were removed by passage over column resin (CNBr-activated Sepharose, Pharmacia) coupled to proteins from an extract of *E. coli* carrying the pWR590 plasmid vector lacking an insert. The partially purified serum was then affinity purified using an anti-β-gal antibody column cross-linked to the larger *Abd-B*-β-gal fusion protein.

All four rat anti-*Abd-B* antibodies were able to detect *Abd-B* protein on Western blots and three were able to detect protein in embryos. However, only the common antibody from rat 2 stains embryos with a very low level of background staining.

Detection of Abd-B *proteins on Western blots*

Drosophila embryo extracts were prepared by homogenization of dechorionated 0–12 h embryos in SDS gel sample buffer (0.1 M DTT, 2% SDS, 0.08 M Tris–HCl, pH 6.9, 10% glycerol, 0.004% bromophenyl blue) using 5 μl buffer per mg embryos (dry weight). Extracts were boiled 5–10 min and centrifuged 2 min prior to loading on 8.5 to 10% SDS–polyacrylamide gels (Krause *et al.* 1988). *E. coli* extracts containing full-length *Abd-B* m or r protein were provided by S. Cumberledge. Proteins were dry blotted (Polyblot, American Bionetics) from gels to nitrocellulose or nylon membranes. Filters were incubated with primary antibodies at

a dilution of 1:500 or 1:1000 in non-fat milk solution (20 mM Tris–HCl, pH 7.5, 0.5 M NaCl, 5% non-fat dry milk). After washing, filters were incubated with goat anti-rat antibodies conjugated with alkaline phosphatase (Jackson Immuno Research Laboratories) at a dilution of 1:10000. The enzyme reaction was carried out using substrates for the standard color reaction (Bio-Rad) or using the chemiluminescent substrate AMPPD (Tropix Inc.) for increased sensitivity. Chemiluminescent products were visualized by exposure of X-ray film. We estimate that the chemiluminescent method is at least 5 times more sensitive than the standard color reaction.

Antibody staining of whole mount embryos

Detection of *Abd-B* proteins in *Drosophila* embryos was carried out according to the procedure described in Boulet and Scott (1988). Affinity-purified *Abd-B* antibodies were used at a dilution of 1:200 to 1:400. Anti-β-galactosidase antibodies (Boulet and Scott, 1988) were used at a dilution of 1:400. To establish the location of parasegment boundaries, embryos were stained with an anti-*invected* monoclonal antibody (referred to as the anti-*engrailed* antibody, provided by J. Kassis and D.-H. Huang, used at a dilution of 1:4). This monoclonal antibody recognizes the *engrailed* protein in the embryo. Fluorescein-conjugated goat anti-rabbit antibodies (Cappel) were used at 1:200. Rhodamine-conjugated and fluorescein-conjugated goat anti-rat antibodies (Jackson Immuno Research Laboratories) were diluted to 1:2000. Goat-anti-mouse antibodies conjugated with rhodamine (Boehringer Mannheim Biochemicals) were used at 1:400. All secondary antibodies were preabsorbed to fixed *Drosophila* embryos. A fluorescein-coupled anti-HRP antibody (provided by M. Bastiani) was used at a dilution of 1:10000.

Results

Class A, B, and C Abd-B *mRNAs are expressed in discrete spatial and temporal patterns*

Sánchez-Herrero and Crosby (1988), DeLorenzi *et al.* (1988) and Kuziora and McGinnis (1988) have reported the *in situ* localization of transcripts from the *Abd-B* locus in wild-type embryos. These studies were carried out prior to the identification of the four transcript classes (Zavortink and Sakonju, 1989) and did not distinguish between transcripts arising from the class B and class C promoters. We have utilized the technique of hybridization to RNA in whole mount *Drosophila* embryos (Tautz and Pfeifle, 1989) to individually localize class A, B and C transcripts. Fig. 1 depicts the *Abd-B* gene structure and the locations of probes that distinguish between the class A, B and C mRNA species. To provide a description of expression patterns of transcripts described in Zavortink and Sakonju (1989), a brief summary of our results using wild-type embryos is given below with an emphasis on features not described in the previous studies.

Embryos stained with a probe that recognizes sequences common to all *Abd-B* transcripts show that the domain of *Abd-B* expression encompasses PS 10–14 (data not shown; Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988). In cellular blastoderm stage embryos, the class A probe detects a ring of transcript in the primordium of PS 13 as determined by percent egg length (Fig. 2A; Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988). Class A transcripts are present at high levels in PS 13 and at lower levels in PS 11, 12 and 14 in a stage 12 embryo (Fig. 2B). A very weak hybridization signal is occasionally detected in PS 10. After germ band retraction, class A transcripts are detected in posterior A7 (anterior PS 13) and surrounding the developing posterior spiracles in A8 (Fig. 2C). In the ventral nervous system, class A transcripts are detected in PS 11 through PS 13 (Fig. 2D). The hybridization signal obtained with the class A probe in PS 14 (Fig. 2B, C, D) is much weaker than that seen with the probe recognizing all *Abd-B* transcripts. The PS 14 signal could be due to a low level of transcription from the class A promoter or to detection of precursor mRNAs transcribed from the class B or C promoters.

The pattern of transcription from the class B promoter has not been previously described. Using a probe specific for the class B transcripts, a strong hybridization signal is detected in PS 14 and anterior PS 15 of the extended germ band (Fig. 2E). In later embryos, a strong hybridization signal is seen in the terminal region of the ventral nervous system (Fig. 2G) and a much weaker signal is detectable in the epidermis in segment A9 (PS 14; Fig. 2F). Because the epidermal signal in later embryos is very weak, it is possible that this signal reflects hybridization to unspliced precursors of class C mRNAs. Thus, class B transcripts appear to be expressed exclusively or predominantly in the ventral nervous system of later stage embryos.

The class C probe detects a ring of transcripts in cellular blastoderm embryos at a position just posterior to the class A signal (Fig. 2H). Class C transcripts are present at high levels in the epidermis of PS 14 and anterior PS 15 in extended germ band embryos (Fig. 2I) and, after germ band shortening, in a narrow band of cells in posterior A8 and anterior A9 (PS 14; Fig. 2J). The level of epidermal staining seen with probe C is much higher than that seen with probe B after germ band retraction (compare Fig. 2K and 2G, arrowheads). Hybridization in PS 14 of the ventral nervous system is also detected with the class C probe (Fig. 2K). The different distribution of class B and C transcripts suggest that they play different functional roles during development.

The Abd-B *gene encodes two protein products with unique patterns of expression in embryos*

To provide direct evidence for the presence of the postulated *Abd-B* m and r proteins *in vivo* and to examine the distribution of these proteins in developing embryos, antibodies directed against *Abd-B*-β-galactosidase fusion proteins were prepared (see Materials and methods). A *Pvu*II fragment (Fig. 1) was used to produce antibodies recognizing both protein species. A *Pvu*II–*Pst*I fragment (Fig. 1) was used to generate antibodies specific for the predicted *Abd-B* m protein. Purified fusion proteins synthesized in *E. coli* were injected into rats, and the antibodies from two rats injected with each antigen were affinity-purified using

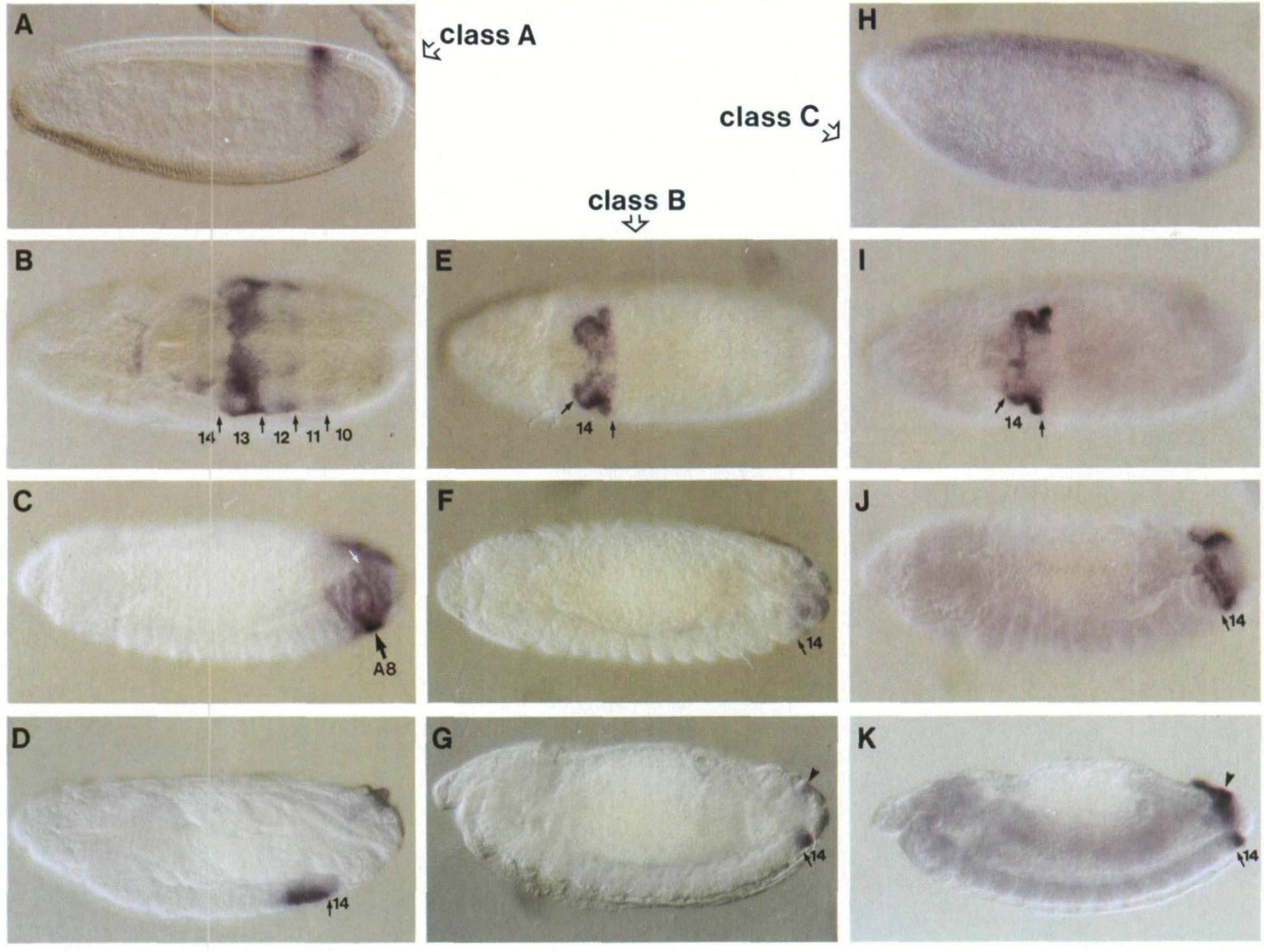

Fig. 2. Patterns of RNA expression determined by whole mount *in situ* hybridization using class A, class B or class C specific probes. Approximate positions of relevant parasegmental boundaries are marked with arrows in B, D, E, F, G, I, J and K. (A) Cellular blastoderm embryo hybridized with the class A specific probe. (B) Dorsal view of the class A transcript pattern in an embryo undergoing germ band retraction. (C) Embryo after germ band shortening hybridized with the class A probe. Large arrow marks the position of A8. The white arrow indicates anterior A9, in which hybridization is weaker than seen with the common probe (data not shown). (D) Lateral view of the pattern of class A transcription in the VNS. Staining is very weak in PS 14 as compared to that seen in PS 13 and to that detected in PS 14 with the common probe (data not shown). (E) Stage 12 embryo stained with the class B probe. (F) Germ band retracted embryo hybridized with the probe specific for class B transcripts. (G) Lateral view of the class B transcription pattern in the VNS. Staining is present only in PS 14. (H) Cellular blastoderm stage embryo hybridized with the class C probe. (I) Embryo during germ band shortening stained with the class C probe. (J) Class C mRNA expression in an embryo after germ band retraction. (K) VNS staining obtained with the class C probe. Hybridization is restricted to PS 14. Note the high level of epidermal staining (arrowhead) relative to that seen with the class B probe in (G).

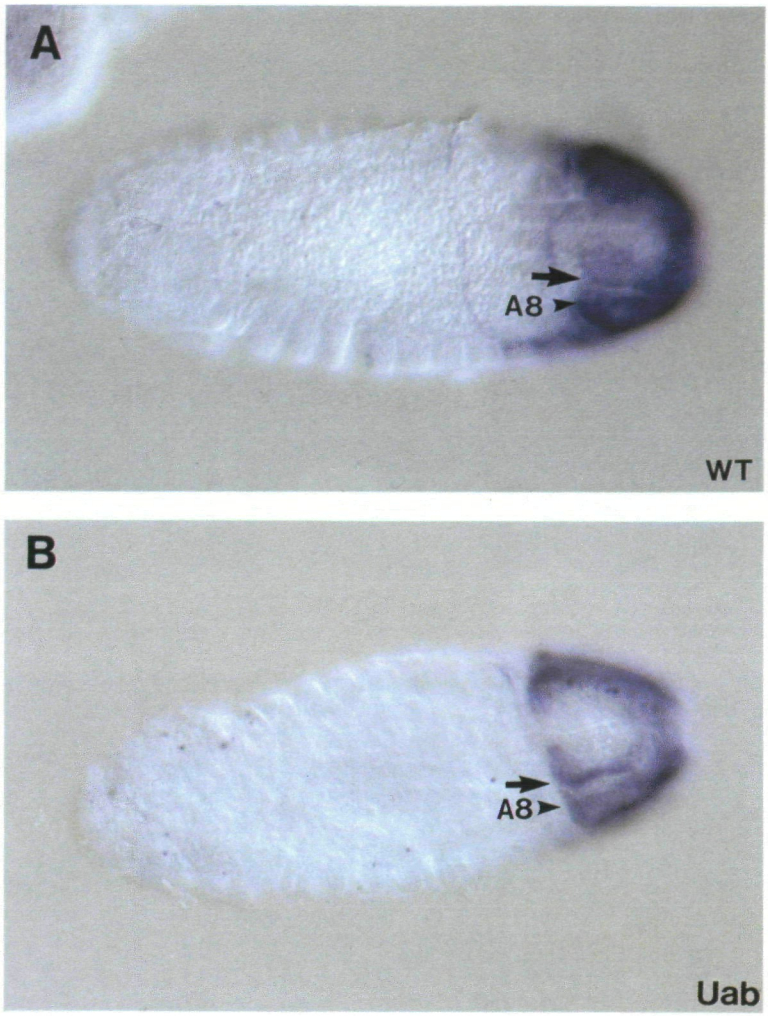

Fig. 6. Class A transcript pattern in late stage 12 wild-type and Uab^{1revB9} mutant embryos. Genotypes are indicated on each panel. Arrowheads point out segment A8. (A) Wild-type embryo hybridized with the class A specific probe. (B) Class A transcript pattern in a Uab^{1revB9} embryo. Uab^{1revB9} embryos show a higher level of class A mRNA expression just posterior to the segment border between A8 and A9 (anterior A9 or posterior PS 14; arrow) than does the wild-type embryo.

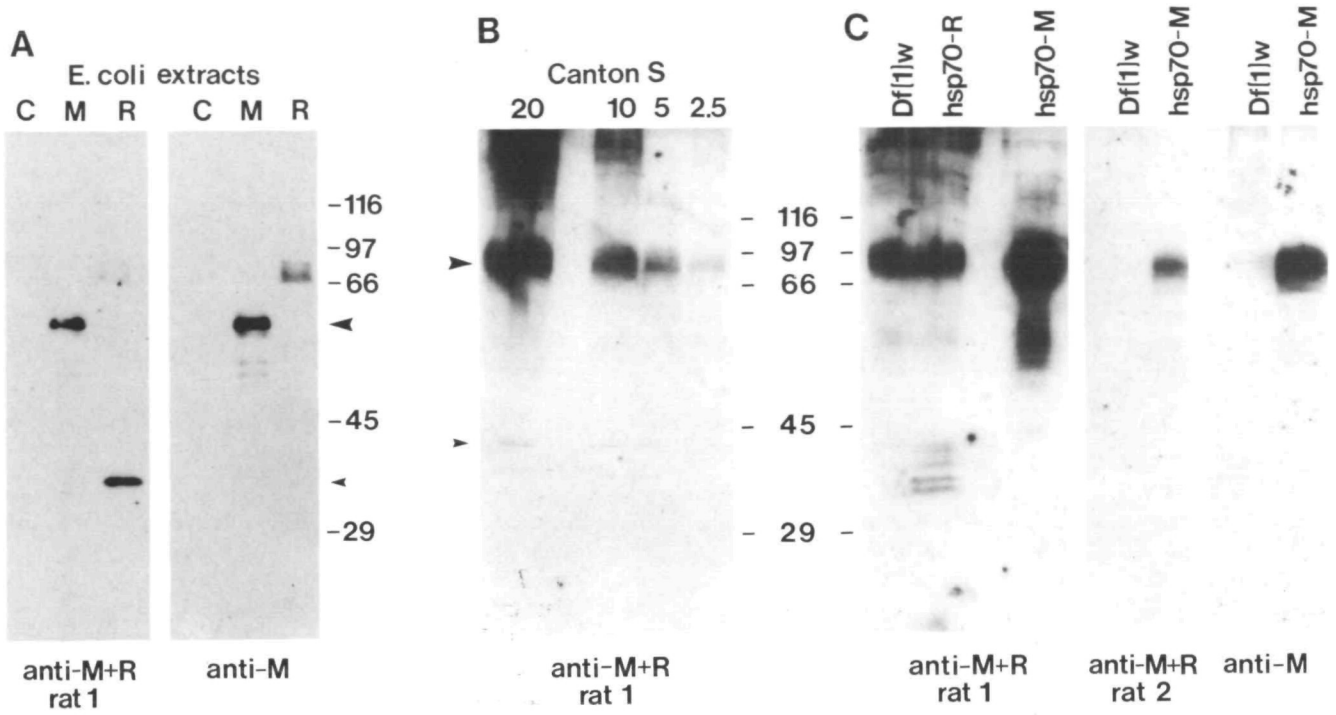

Fig. 3. Western blot analysis using *Abd-B* antibodies. The *Abd-B* antibodies directed against a polypeptide domain common to the two *Abd-B* protein species (common antibodies, anti-M+R, rat 1 and rat 2) and an antibody directed against a region specific to the m protein (m-specific antibody, anti-M) were used to probe Western blots of extracts from *E. coli* expressing full-length m and r proteins and extracts of *Drosophila* embryos. (A) Lanes C contain extracts from *E. coli* carrying the expression vector lacking an *Abd-B* insert. Extracts containing m or r protein produced in bacteria were run in lanes M and lanes R, respectively. The panel on the left shows the proteins recognized by the common antibody (anti-M+R, rat 1). The panel on the right is a blot probed with the m-specific antibody (anti-M). Both antibodies recognize the m protein, but only the common antibody detects the r protein. The band seen in the R lanes that migrates as a higher relative molecular mass than the m protein is likely to be an *E. coli* protein that cross-reacts with both of our antibodies. This protein is present at lower levels in the C and M lanes; 5-fold more of the extract from *E. coli* expressing the r protein was loaded on the gel than of the control or m extract. (B) Western blot of Canton S embryo extracts probed with the common antibody derived from rat 1 (anti-M+R, rat 1). 2.5, 5, 10, and 20 μl aliquots of extracts prepared as described in Materials and methods were loaded on the gel. (C) Western blot of extracts from heat shocked embryos probed with common antibodies from rats 1 and 2 and with an m-specific antibody. Control (untransformed *Df(1)w*), *hsp70*-M and *hsp70*-R embryos were heat shocked at 37°C for one hour. In the first panel, probed with anti-M+R, rat 1, 16 μl of extract from untransformed *Df(1)w*, *hsp70*-M embryos and *hsp70*-R embryos was loaded on the gel. In the second and third panels, probed with anti-M+R, rat 2 and anti-M, respectively, 25 μl of *Df(1)w* and *hsp70*-M extracts were loaded. The anti-M+R, rat 2 and anti-M antibodies detect only a very low level of m protein in untransformed embryos while a strong signal is obtained with anti-M+R, rat 1. The multiple bands migrating just below the position of the endogenous r protein probably result from degradation of the heat-shock-induced product.

standard procedures (Materials and methods). The common and m-specific antibodies were tested on Western blots of *E. coli* extracts expressing full-length *Abd-B* m ($55\times10^3 M_r$) or full-length *Abd-B* r ($30\times10^3 M_r$) protein (S. Cumberledge, unpublished). As expected, the common antibody recognizes both m and r proteins, and the m-specific antibody recognizes only the m protein (Fig. 3A).

The *Abd-B* common and m-specific antibodies were used to probe Western blots of wild-type embryo extracts. The common antibody reacts with a cluster of polypeptides of approximately $80–90\times10^3 M_r$ and a single protein of about $40\times10^3 M_r$ (Fig. 3B). A subset of the polypeptides in the higher relative molecular mass cluster is also detectable in embryonic nuclear extracts (data not shown). Transformant lines expressing the m protein (*hsp70*-M) or the r protein (*hsp70*-R) under control of the *hsp70* promoter have been generated (A. Boulet, unpublished). To confirm that the protein species detected with our *Abd-B* antibody correspond to the endogenous m and r proteins, Western blots of extracts from heat-shocked *hsp70*-M embryos were probed with the common and m-specific antibodies. Both types of antibodies detect a cluster of proteins migrating at the same position as the cluster seen in untransformed embryos (Fig. 3C). Likewise, the common antibody recognizes a protein in extracts of *hsp70*-R embryos comigrating with the smaller protein detected in untransformed embryos (Fig. 3C). The presumptive m and r proteins are present at much higher levels in heat-shocked *hsp70*-M and *hsp70*-R transformed embryos, respectively, than in heat-

shocked control embryos (Fig. 3C). These observations demonstrate that the two clusters of embryonic proteins detected in the Western blots correspond to the predicted m and r proteins. The results also suggest extensive modifications of these proteins *in vivo*.

Since previous *Abd-B* protein localization studies used antibodies recognizing both *Abd-B* proteins, specific expression patterns for the two proteins could not be established (Celniker *et al.* 1989; DeLorenzi and Bienz, 1990). We have used our antibodies to determine the distribution of *Abd-B* m and r proteins in wild-type embryos. In stage 12 embryos, the m-specific *Abd-B* antibody detects a high level of protein in PS 13, but generates little or no signal in PS 14 (Fig. 4D). In embryos of approximately the same developmental stage, the common antibody detects *Abd-B* protein in PS 13 and PS 14 at approximately equal levels (Fig. 4A). The expected low level of m protein staining in PS 11 and 12 is partially obscured by the high background staining obtained with the m-specific antibody. In later stage embryos, the m-specific antibody detects a relatively high level of protein in PS 11 through PS 13, but little or no protein in PS 14 (Fig. 4E,F). In contrast the common antibody detects a high level of *Abd-B* protein in PS 14 (Fig. 4B,C) Thus, our data show that the *Abd-B* protein detected in PS 11–13 represents the $55\times10^3 M_r$ m protein encoded by class A transcripts, while the protein detected in PS 14 comprises mainly, or exclusively, the $30\times10^3 M_r$ r protein translated from class B/C mRNAs.

Analysis of Abd-B *mRNA and protein distributions in Class I, II and III* Abd-B *mutants*

The genetic model of Casanova *et al.* (1986) postulated two distinct Abd-B functions, m and r, that are inactivated individually by Class I (m^-r^+) or II (m^+r^-) mutations, respectively, or simultaneously by Class III (m^-r^-) mutations. To assign the two genetic functions to *Abd-B* mRNA and proteins, whole mount *in situ* hybridization analysis and antibody staining were used to examine m^-r^+, m^+r^- and m^-r^- embryos.

First, we examined embryos carrying an m^-r^- mutation for *Abd-B* protein expression. When embryos from a heterozygous *iab-7*^{D16} (Karch *et al.* 1985) stock were stained with the *Abd-B* antibody, protein was not detectable in approximately one quarter of the embryos. The embryos lacking *Abd-B* protein also show signs of aberrant development in the posterior regions (data not shown). Thus the complete absence of *Abd-B* proteins correlates with the loss of both m and r genetic functions.

iab-7^{D14}, an m^-r^+ mutation, is associated with a 411 bp deletion extending from −66 bp to +345 bp relative to the class A transcription initiation site (Fig. 1; Zavortink and Sakonju, 1989). This provides strong evidence that the class A transcript, and therefore the $55\times10^3 M_r$ protein encoded by this transcript, corresponds to the m element of Casanova *et al.* (1986). *In situ* hybridization and antibody staining were used to determine whether mutant *iab-7*^{D14} embryos indeed lack the m, or class A, mRNA and whether this correlates with a lack of protein in PS 10–13. In addition, the expression of the class B and C mRNAs, putatively encoding the r function, should remain unaltered in this mutant. To allow unambiguous identification of homozygous *iab-7*^{D14} embryos, the mutant chromosome was balanced with a chromosome marked with a *ftz–lacZ* P element insert (see Materials and methods). The homozygous embryos can also be recognized by failure of the posterior spiracles to form after germ band retraction (Fig. 5C). Our results show that *Abd-B* mRNA (Fig. 5A) and protein are absent from PS 10–13 but present at apparently wild-type levels in PS 14 of homozygous *iab-7*^{D14} embryos (Fig. 5B,C,D; compare B to wild-type embryo in H). Thus the m function defined by Casanova *et al.* (1986) is provided by the class A transcript and its encoded $55\times10^3 M_r$ protein.

Expression of *Abd-B* mRNA and protein was also examined in three mutants showing defects in r function. *Tab* and *Uab*^{1revB9} have been classified as m^+r^- mutants based on phenotype and the ability to complement the m^-r^+ allele *iab-7*^{D14} (Casanova *et al.* 1986; Duncan, personal communication). These mutations affect development of the posterior spiracles and induce a partial transformation of PS 14 into PS 13 in homozygous mutant embryos causing formation of a rudimentary setal belt in A9 (Karch *et al.* 1985; Celniker and Lewis, 1987). *iab-7*^{65} has been classified as an atypical m^-r^- *Abd-B* allele in which the r function is absent but the m function is not completely destroyed (Casanova *et al.* 1986; Sánchez-Herrero and Crosby, 1988). In fact, *iab-7*^{65} is able to partially complement the m^-r^+ mutant *iab-7*^{D14} (A. Boulet, unpublished).

The patterns of *Abd-B* mRNA and protein expression in homozygous *Tab*, *Uab*^{1revB9} and *iab-7*^{65} embryos are consistent with these mutations having an effect on the amount of r element activity. In embryos undergoing germ band shortening, *Abd-B* mRNA (data not shown) and protein are present at greatly reduced levels in PS 14 relative to wild-type levels (Fig. 5E,F,G; compare to 5H). The reduction of *Abd-B* mRNA and protein in PS 14 but not in PS10–13 supports the assignment of the r function to the $30\times10^3 M_r$ protein encoded by class B and/or class C transcript.

Transcription of mRNA encoding the Abd-B *m protein is derepressed in the absence of the r function*

In three mutants with genetic defects in r function, *Abd-B* expression was reduced in PS 14 but not entirely eliminated. This result may support the proposal of Casanova *et al.* (1986) that m activity is derepressed in PS 14 in the absence of r function. In the cases of *Uab*^{1revB9} and *Tab*, the close proximity of the associated breakpoints to the class B transcription start site raises the possibility that these mutations do not completely disrupt class B transcription (Fig. 1; Zavortink and Sakonju, 1989). In *iab-7*^{65} embryos, however, the PS 14 mRNAs cannot be transcribed from the class B or C promoters since these transcription units have been disrupted by a breakpoint located about 6 kb upstream from the class A initiation site (Fig. 1; Zavortink and

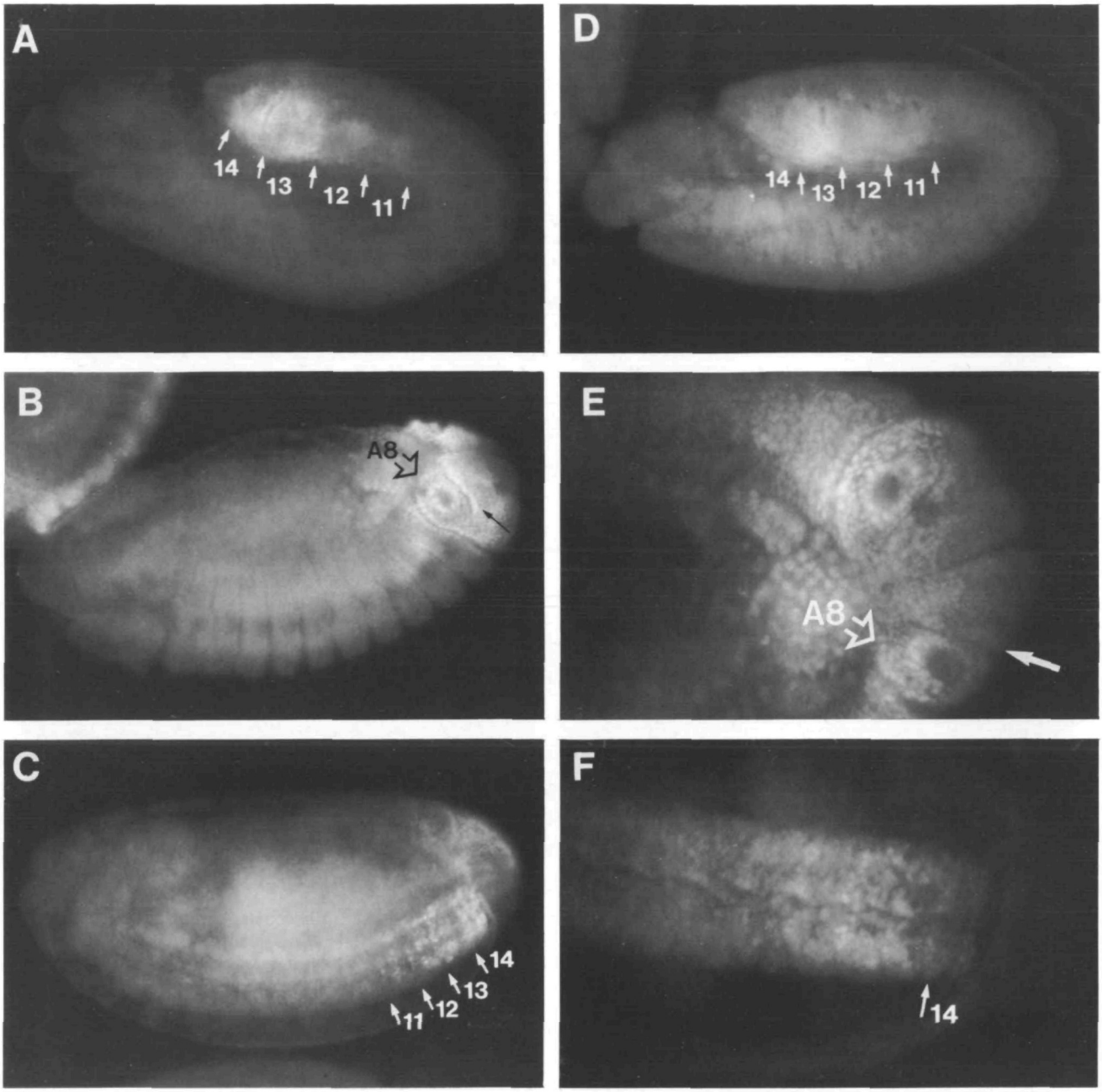

Fig. 4. Expression of *Abd-B* proteins in whole mount embryos detected by the common antibody (anti-M+R, rat 2) or the m-specific antibody. Approximate locations of borders of relevant parasegments are indicated by small arrows in A, C, D and F. (A) Embryo undergoing germ band retraction (stage 12) stained with the common *Abd-B* antibody (anti-M+R, rat 2). (B) Embryo after germ band retraction stained with the common antibody. The open arrow marks A8, and the small black arrow points out the high level of staining in anterior A9 or the posterior portion of PS 14. (C) *Abd-B* protein expression in the VNS detected with the common antibody. (D) Stage 12 embryo stained with the m-specific antibody. (E) Embryo at the same stage as that shown in B stained with the m-specific antibody and photographed at higher magnification. Open arrow marks A8. Note that the m-specific antibody does not detect the high level of *Abd-B* protein in anterior A9 or posterior PS 14 (solid arrow) seen with the common antibody in B. (F) High magnification view of protein expression in the VNS detected by the m-specific antibody. M protein expression is very low in PS 14.

Sakonju, 1989). To directly test the hypothesis of Casanova *et al.* (1986), class A specific probes were used to determine whether m transcripts are present at increased levels in PS 14 of Uab^{1revB9} embryos. Approximately one quarter of the embryos from a heterozygous Uab^{1revB9} stock showed a higher level of class A transcripts in posterior PS 14 (anterior A9) than seen in wild-type embryos (Fig. 6B; compare to wild-type embryo in A). This evidence supports the hypothesis that class A or m expression is derepressed in PS 14 due to a mutation that affects r expression in PS 14.

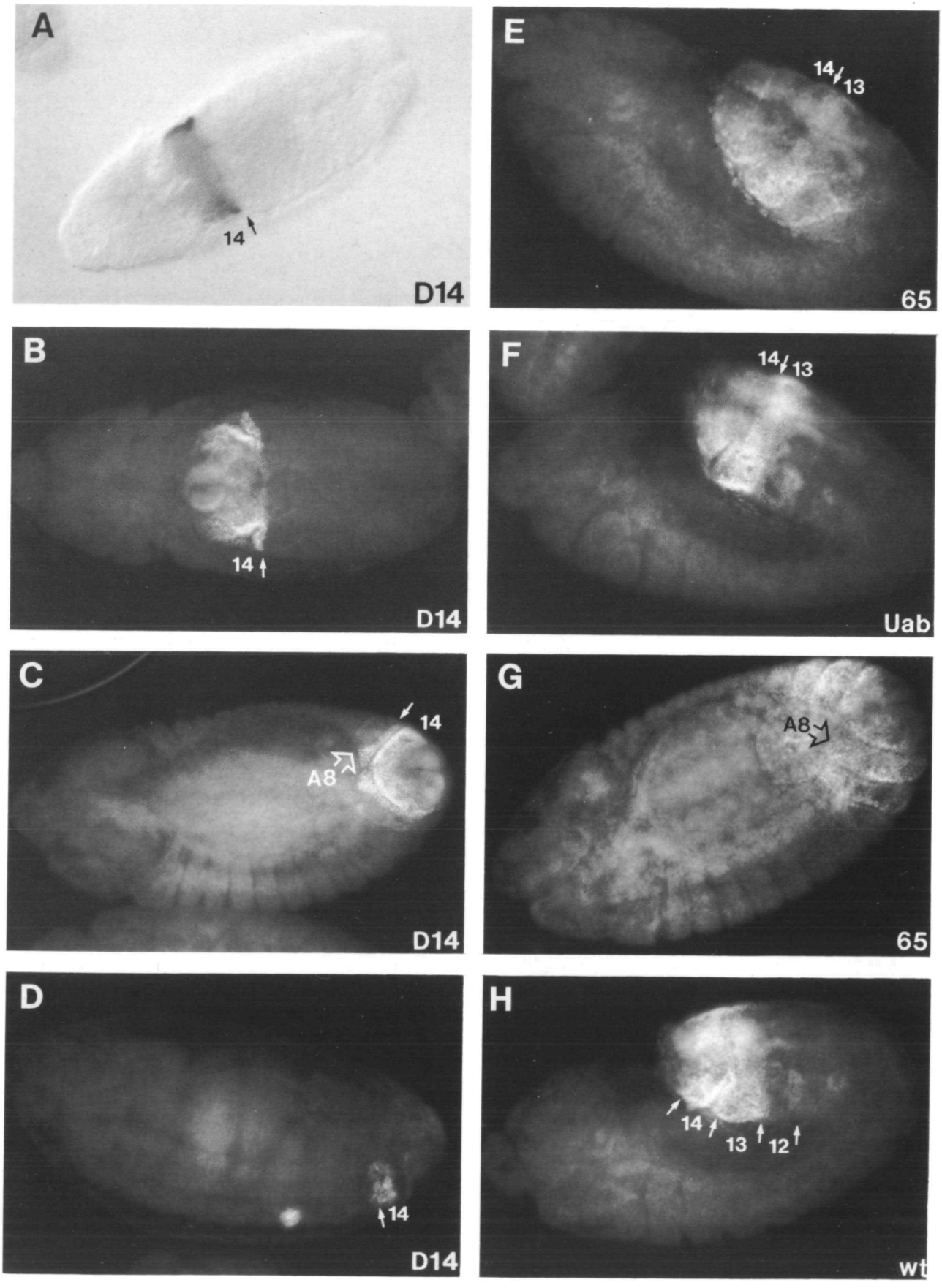
A
14
D14
E
14/13
65
B
14
D14
F
14/13
Uab
C
14
A8
D14
G
A8
65
D
14
D14
H
14
13
12
wt

Fig. 5. *Abd-B* mRNA and protein expression in m^-r^+ and m^+r^- mutant embryos. *Abd-B* mRNA was localized in the embryo in A by hybridization with the common probe. All other embryos were stained with the common antibody, anti-M+R, rat 2. Each panel is labeled in the lower right corner with the mutant genotype. Arrows in A, B, C, D, E, F and H indicate approximate positions of relevant parasegment borders. (A) *Abd-B* mRNA expression in a stage 12 *iab-7*^{D14} homozygous embryo. (B) *Abd-B* protein expression in a stage 12 *iab-7*^{D14} homozygous embryo. (C) *iab-7*^{D14} embryo after the germ band has retracted. Note the absence of any evidence of posterior spiracle formation in A8 (open arrow). (D) *Abd-B* protein expression in the ventral nervous system of an *iab-7*^{D14} embryo. (E) Pattern of *Abd-B* protein detected in a stage 12 *iab-7*^{65} homozygous embryo. (F) *Abd-B* protein expression in a *Uab*^{1revB9} homozygous embryo undergoing germ band shortening. Note the reduced level of protein in PS 14 of the embryos shown in E and F relative to that seen in the wild-type embryo in panel H. (G) *Abd-B* protein pattern in an *iab-7*^{65} embryo after the germ band has retracted. Morphogenetic movements required in A8 for formation of the posterior spiracles do not appear to occur (open arrow). (H) *Abd-B* protein expression in a wild-type embryo during germ band retraction.

Distribution of Abd-B *protein products in* iab *mutant embryos*

The *iab* mutations were originally thought to disrupt individual 'abdominal' genes (Lewis, 1978). More recently, it has been proposed that iab-5, -6 and -7 alleles represent regulatory mutations of the *Abd-B* gene (Peifer *et al.* 1987; Casanova *et al.* 1987); for example, *iab-7* lesions would destroy or remove sequences required for *Abd-B* expression in PS 10–12 and *iab-6* and *iab-5* lesions would affect PS 10 and 11 or PS 10 expression, respectively. To test whether expression of *Abd-B* protein and mRNA in *iab* mutant embryos fits the latter model, several *iab* mutant lines were examined by *in situ* hybridization and antibody staining. Again, mutant chromosomes were balanced with a marked chromosome to allow identification of homozygous mutant embryos.

Abd-B mRNA and protein expression were examined in the *iab-7* mutant, *Abd-B*^{MX2}. This mutation is associated with a breakpoint located approximately 10 kb downstream of the *Abd-B* transcription units (Fig. 1; Karch *et al.* 1985). Whole mount *in situ* hybridization shows that *Abd-B* mRNA expression in *Abd-B*^{MX2} homozygous embryos is restricted to PS 13–15 (Fig. 7A). *Abd-B* antibody staining demonstrates that *Abd-B* protein expression is also limited to PS 13–15 (Fig. 7B). *Abd-B* protein is not seen in PS 11 and 12 of the extended germ band in *Abd-B*^{MX2} homozygotes. *Abd-B* protein is also clearly absent from PS 11 and 12 in the ventral nervous system where it is easily detected in wild-type embryos (Fig. 7C). These observations indicate that the regulatory elements affected in *iab-7* mutations are required for proper expression of *Abd-B* in PS 10–12 of stage 12 embryos and the ventral nervous system (VNS) and epidermis of embryos after germ band retraction.

The *Abd-B* mutant *SGA62* has been difficult to classify due to dominant gain-of-function effects caused by the chromosome rearrangement (Duncan, 1987). Karch *et al.* (1985) refer to *SGA62* as an *iab-6* allele. However, the *SGA62* breakpoint maps between the breakpoints of two alleles, *Abd-B*^{MX1} and *Abd-B*^{MX2} (Karch *et al.* 1985), reclassified by Duncan (1987) as *iab-7*. The mRNA and protein patterns observed in *SGA62* mutant embryos are essentially identical to those seen in *Abd-B*^{MX2}: *Abd-B* protein and mRNA is not detected in PS 11 and 12 of homozygous embryos (Fig. 7D; data not shown). Ectopic *Abd-B* protein expression has not been detected in *SGA62* embryos.

The mutations *Camel* (Karch *et al.* 1985) and *38000.11A* have been classified as *iab-6* alleles by Duncan (1987; personal communication). Embryos homozygous for these mutations show very similar patterns of *Abd-B* mRNA and protein expression (Fig. 7E,F,G and data not shown). The *Abd-B* gene is expressed in PS 12–15: mRNA and protein are not detectable in PS 11 at stages when expression is easily seen in wild-type embryos.

Embryos homozygous for the *iab-5*^{C7} mutation (Karch *et al.* 1985) were examined by *in situ* hybridization and antibody staining. *iab-5*^{C7} homozygous embryos show *Abd-B* protein expression in PS 12–14 of the ventral nervous system; PS 11 expression is not detectable (Fig. 7H). Based on the absence of mRNA and protein in PS 11, as well as the location of the *iab-5*^{C7} insertion with respect to other *iab* mutant lesions (Karch *et al.* 1985), we conclude that *iab-5*^{C7} is an *iab-6* rather than an *iab-5* allele.

We have not attempted to examine mRNA and protein expression in true *iab-5* mutants. *Abd-B* transcripts can be detected in PS 10 in only some wild-type stage 12 embryos. Therefore, the appearance of this staining is not consistent enough to reach a firm conclusion on the presence or absence of *Abd-B* expression in PS 10 of *iab-5* mutant embryos.

Discussion

Spatial and temporal expression of transcripts from three Abd-B *promoters*

We have examined the transcription patterns of mRNAs transcribed from three promoters of the *Abd-B* gene using the technique of whole mount *in situ* hybridization to *Drosophila* embryos. Previous studies of *Abd-B* transcript localization (Sánchez-Herrero and Crosby, 1988; DeLorenzi *et al.* 1988; Kuziora and McGinnis, 1988) were carried out before the three *Abd-B* transcription start sites had been identified. Our results, obtained with probes specific for each of the three classes of *Abd-B* mRNA, show that each class is expressed in a unique spatial and temporal pattern. Class A mRNAs are expressed in PS 10–13 while class B and C mRNAs are confined to PS 14 and anterior PS 15. The epidermal staining patterns of class B and class C transcripts are not identical. For example, the class C mRNA is expressed at much higher levels after germ

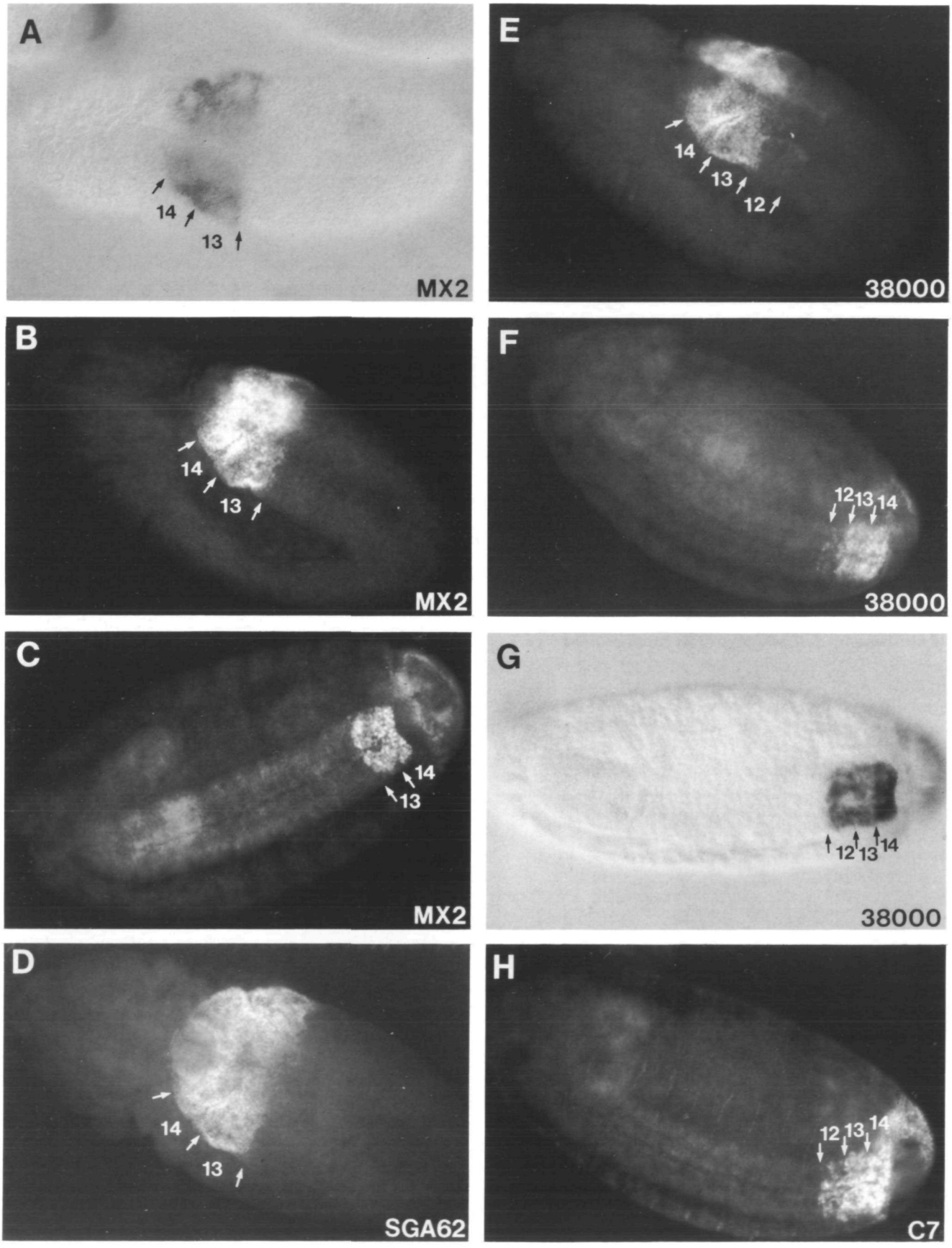
A
14
13
MX2
E
14
13
12
38000
B
14
13
MX2
F
12
13
14
38000
C
14
13
MX2
G
12
13
14
38000
D
14
13
SGA62
H
12
13
14
C7

Fig. 7. *Abd-B* RNA and protein expression in *iab-6* and *iab-7* mutant embryos. Approximate locations of relevant parasegment boundaries are marked with arrows. Genotypes of embryos are given in the lower right corner of each panel. (A) $Abd\text{-}B^{MX2}$ homozygous embryo during germ band retraction hybridized with a probe that recognizes all *Abd-B* transcripts. (B) Stage 12 $Abd\text{-}B^{MX2}$ homozygous embryo stained with the common *Abd-B* antibody. (C) *Abd-B* protein expression in the ventral nervous system of an $Abd\text{-}B^{MX2}$ homozygous embryo. (D) Stage 12 *SGA62* homozygote stained with the common *Abd-B* antibody. *Abd-B* RNA and protein is not detectable in PS 10–12 of $Abd\text{-}B^{MX2}$ and *SGA62* homozygous embryos; these mutants can be classified as *iab-7*. (E) *Abd-B* protein expression in *38000.11A* homozygous embryo undergoing germ band retraction. (F) *Abd-B* protein expression in the ventral nervous system of a *38000.11A* homozygous embryo. (G) *38000.11A* embryo hybridized with the probe recognizing all *Abd-B* transcripts. Note the presence of protein in PS 12 and the absence of a detectable signal in PS 11. *38000.11A* is classified as an *iab-6* mutant. (H) *Abd-B* antibody staining in the ventral nervous system of an $iab\text{-}5^{C7}$ homozygote.

band retraction than the class B mRNA. The sum of the individual class A, B and C patterns is very similar to the pattern seen with a probe that recognizes all *Abd-B* transcripts.

Assignment of morphogenetic and regulatory functions to the two Abd-B *proteins*

Analysis of the sequences of *Abd-B* cDNAs and the patterns of transcript expression have led to the hypothesis that the $55\times10^3 M_r$ and $30\times10^3 M_r$ *Abd-B* proteins carry out morphogenetic (m) and regulatory (r) functions, respectively (Sánchez-Herrero and Crosby, 1988; DeLorenzi *et al.* 1988; Kuziora and McGinnis, 1988; Celniker *et al.* 1989; Zavortink and Sakonju, 1989). We have tested the following aspects of this hypothesis: (1) the existence of the predicted m and r proteins in the embryo, (2) the localization of the m and r proteins to specific parasegments and (3) the correspondence between morphogenetic (m) and regulatory (r) mutant effects and expression of the *Abd-B* proteins.

To determine whether the two predicted *Abd-B* proteins are indeed expressed in the embryo, antibodies were generated against a $55\times10^3 M_r$ protein-specific domain and against a domain common to both the $55\times10^3 M_r$ and $30\times10^3 M_r$ proteins. These antibodies recognize proteins of the predicted relative molecular masses in extracts of *E. coli* carrying *Abd-B* m ($55\times10^3 M_r$) and r ($30\times10^3 M_r$) constructs. Western blot analysis shows that two *Abd-B* protein products of different relative molecular masses are synthesized in wild-type *Drosophila* embryos. The larger protein has an apparent relative molecular mass much greater than that predicted from the DNA sequence. Since m and r proteins synthesized in *E. coli* migrate close to the expected relative molecular masses, this discrepancy is probably not due to aberrant migration in SDS polyacrylamide gels seen with a number of other homeodomain-containing proteins (Ollo and Maniatis, 1987; Jack *et al.* 1988; Krause *et al.* 1988). Rather, the larger apparent relative molecular masses of *Abd-B* proteins on our Western blots may be due to post-translational modifications occurring in embryos. The appearance of proteins of $80\text{–}90\times10^3 M_r$ and about $40\times10^3 M_r$ in heat shocked *hsp70*-M embryos suggests that the presumptive modifications can occur in most, if not all, cells of the embryo. Celniker *et al.* (1989) reported detection of two protein products, of $55\times10^3 M_r$ and $30\times10^3 M_r$, in nuclear extracts of wild-type embryos using a monoclonal *Abd-B* antibody. The $55\times10^3 M_r$ protein species detected on their Western blots may represent a partially degraded form of the m protein. Alternatively, the difference in the embryonic stages at which the extracts were prepared in the two studies could account for the discrepancy in the apparent relative molecular masses.

The pattern of staining in *Drosophila* embryos obtained with the antibody specific to the $55\times10^3 M_r$ protein clearly shows that this protein is present in PS 13, but absent from, or present at very low levels in, PS 14. This result supports the assignment of the morphogenetic function, confined to PS 10–13, to the $55\times10^3 M_r$ protein. We hereafter will refer to this protein as the m protein. The precise anterior border of m protein expression is difficult to determine because the antibody specific to the m protein gives a high background of nuclear staining in embryos. Since *opa* repeats, encoding Gln residues, are present throughout the m-specific domain, the high background may be due to cross-reactivity with *opa* repeats in other proteins. In contrast to the staining pattern obtained with the m protein-specific antibody, the antibody generated against a domain common to the $55\times10^3 M_r$ and $30\times10^3 M_r$ proteins detects protein in PS 11 through PS 14. The staining patterns obtained with the antibodies recognizing the common *Abd-B* domain agree with those described by Celniker *et al.* (1989) and DeLorenzi and Bienz (1990). Taken together, the patterns seen with our two antibodies indicate that the $30\times10^3 M_r$ protein is expressed in PS 14. Thus the $30\times10^3 M_r$ protein must be responsible for the regulatory function, and will be referred to as the r protein. Although such an assignment has been suggested previously (Celniker *et al.* 1989; DeLorenzi and Bienz, 1990), it is only by demonstrating that the m protein is absent from PS 14, or present at very low levels, that the protein detected in PS 14 can be definitively identified as the $30\times10^3 M_r$ or r protein.

The results from antibody staining of m^-r^+, m^+r^- and m^-r^- *Abd-B* mutants support the hypothesis that the *Abd-B* gene consists of two distinct genetic elements (Casanova *et al.* 1986) which can be assigned to the m ($55\times10^3 M_r$) and r ($30\times10^3 M_r$) proteins. The lack of detectable *Abd-B* protein in m^-r^- mutant embryos strongly suggests that the phenotype of these mutants represents the null phenotype for the *Abd-B* gene. $iab\text{-}7^{D14}$ (m^-r^+) homozygous mutant embryos clearly lack class A mRNA and m protein in PS 10–13. In these embryos, PS 14 expression of class B and C

mRNAs and the r protein are unaffected. Conversely, in m^+r^- mutants, the expression of *Abd-B* mRNAs and protein is greatly diminished in PS 14, while the expression of class A mRNA and the m protein is not significantly affected in PS 10–13. These results provide a molecular genetic correlation of different *Abd-B* mutant classes with the products of the *Abd-B* gene and substantiate the model postulated by Casanova *et al.* (1986).

We have also shown that the presence of *Abd-B* mRNA and protein in PS 14 of embryos mutant for r function is partly or perhaps entirely due to derepression of the class A transcript that encodes m protein. The increase in class A transcription in PS 14 can be interpreted in several ways. First, the class A promoter may be repressed by r protein in PS 14 of wild-type embryos; in the absence of r protein, the class A promoter would be derepressed. Alternatively, r^- mutations may have removed or disrupted a cis-regulatory element that normally represses transcription of class A mRNA in PS 14. A third possibility is that DNA rearrangements associated with existing r^- mutations juxtaposed sequences that activate the class A promoter or a cryptic promoter in PS 14. An effect of the juxtaposed DNA sequence in $iab\text{-}7^{65}$ can be observed in the increase of the m protein expression in PS 12. Aberrant expression of the m protein may explain why $iab\text{-}7^{65}$ behaves more like an m^-r^-, or null, *Abd-B* allele than an m^+r^- mutant.

The r function of *Abd-B* cannot as yet be assigned to any one of the class B, C or gamma transcript classes; in fact, each may contribute to r element activity. Our results indicate that an r^- phenotype can be caused by a variety of molecular defects. For example, the lack of all three classes of transcripts, B, C and gamma, or the absence of only one class may produce a phenotype classified as r^-. It is also possible that derepression of the m protein in PS 14 does not occur in all r^- mutants. More careful studies of *Abd-B* mutant phenotypes may reveal differences between r^- mutants that could be attributed to disruption of one or more of the class B, C and gamma transcription units, and may help to clarify the relationship between the class B, C and gamma transcripts and the genetic function of the r element.

The iab *region downstream of the* Abd-B *gene contains parasegment-specific* cis*-regulatory elements*

The BX-C has been shown to consist of three lethal complementation groups (Sánchez-Herrero *et al.* 1985*a,b*; Tiong *et al.* 1985). These correspond to the three homeodomain protein-encoding genes, *Ubx*, *abd-A* and *Abd-B*. Non-lethal bithorax complex mutations, such as *bx*, *abx*, *pbx*, *bxd* and *iab-2* through *iab-7* do not appear to disrupt additional BX-C genes. Mutations in these domains affect spatially distinct subsets of *Ubx*, *abd-A* or *Abd-B* functions, and the protein-coding transcription units must be intact to achieve wild-type *bx*, *abx*, *pbx*, *bxd* and *iab* activity. For these reasons, it has been postulated that these regions contain *cis*-elements that regulate the expression of the protein-coding BX-C genes. Based on mutant expression patterns in the *Ubx* domain, Peifer *et al.* (1987) have postulated that the *bx*, *abx*, *pbx* and *bxd* (or *iab*) regions consist of enhancers that activate the *Ubx* (or *abd-A* and *Abd-B*) promoter in cells at a specific position within each parasegment. The cell-specific enhancers are postulated to reside within parasegment-specific DNA domains. The parasegment-specific domains are thought to be activated at a particular site along the body axis and in all more posterior regions. The associated enhancers would then promote gene expression in a specific set of cells in each parasegment in which the DNA domain is activated. For the *Abd-B* gene, the enhancers located in the *iab-5*, *iab-6* and *iab-7* regions would be activated in PS 10–13, PS 11–13, and PS 12 and 13, respectively. Our data from the analysis of *iab-6* and *iab-7* mutants demonstrate the presence of parasegment-specific regulatory elements in the *iab* region of the *Abd-B* gene. Sánchez-Herrero and Akam (1989) have obtained similar results to those reported here for the *iab* mutant $Abd\text{-}B^{MX2}$. The associated DNA lesions clearly disrupt or effectively remove parasegment-specific regulatory elements that are required for expression of the *Abd-B* gene in PS 10, 11 and 12. A similar parasegment-specific loss of expression has been observed for the *Ubx* gene in the mutant abx^2 (White and Wilcox, 1985). However, our data does not provide any indication that a single enhancer controls expression in a subset of analogous cells in adjacent parasegments. According to the Peifer *et al.* (1987) model, equivalent cells expressing *Abd-B* under the control of the *iab-6* enhancer in PS 11, 12 and 13 should lack *Abd-B* products in *iab-6* mutant embryos. Instead we observe that *Abd-B* expression in PS 12 and 13 of *iab-6* homozygotes appears to be unaltered while expression in PS 11 is entirely eliminated. Therefore, our results suggest that cell-specific enhancers are either reiterated in successive *iab* regions or that the *iab* regions contain only parasegment-specific regulatory elements which activate a common set of cell-specific enhancers.

The phenotypes of the *iab* mutants in adult flies corresponds fairly well with the patterns of *Abd-B* protein expression in homozygous *iab* embryos. Therefore, it seems that regulatory regions required for transcription in embryos in each parasegment lie close to, or are coincident with, the sequences required for the *Abd-B* expression in larvae and/or pupae that directs proper development of adult structures.

A combination of genetic studies and molecular analysis has allowed elucidation of the relationship between the genetic functions and complex transcriptional structure of the *Abd-B* gene. The *XlHbox1* gene of *Xenopus* also utilizes separate promoters to produce short and long proteins containing the same homeodomain (Cho *et al.* 1988). The Hox3.3 genes of mouse and human are closely related to the *Xenopus XlHbox1* gene (Schughart *et al.* 1989). Our knowledge of the *Abd-B* gene may aid in the study of these and other complex genes of higher organisms.

We thank E. B. Lewis, I. Duncan and G. Morata for *Abd-B*

and *iab* mutant fly strains, S. Smolik-Utlaut for the *ftz-lacZ* balancer line and J. Mahaffey and T. Kaufman for expression vectors. We thank J. Kassis, D.-H. Huang and M. Bastiani for providing antibodies. Advice on antibody purification and Western blotting from B. Appel and L. Boyd is greatly appreciated. We also thank D. Gard for providing hypersensitized Kodak Technical Pan film, S. Cumberledge for *E. coli* extracts, and C. Thummel, S. Cumberledge, M. Lamka and B. Appel for comments on the manuscript. Finally, we thank M. Zavortink for construction of the *Abd-B* fusions and initial fusion protein purifications.

References

Akam, M. E. (1987). The molecular basis for metameric pattern in the *Drosophila* embryo. *Development* **101**, 1–22.

Boulet, A. M. and Scott, M. P. (1988). Control elements of the P2 promoter of the *Antennapedia* gene. *Genes and Dev.* **2**, 1600–1614.

Campos-Ortega, J. A. and Hartenstein, V. (1985). *The Embryonic Development of* Drosophila melanogaster. Berlin: Springer-Verlag.

Carroll, S. and Laughon, A. (1987). Production and purification of polyclonal antibodies to the foreign segment of β-galactosidase fusion proteins. In *DNA Cloning*, vol. 3 (ed. D. M. Glover). Oxford, England: IRL Press Limited.

Casanova, J., Sánchez-Herrero, E., Busturia, A. and Morata, G. (1987). Double and triple mutant combination of the bithorax complex of *Drosophila*. *EMBO J.* **6**, 3103–3109.

Casanova, J., Sánchez-Herrero, E. and Morata, G. (1986). Identification and characterization of a parasegment specific regulatory element of the *Abdominal-B* gene of *Drosophila*. *Cell* **47**, 627–636.

Celniker, S. E., Keelan, D. J. and Lewis, E. B. (1989). The molecular genetics of the bithorax complex of *Drosophila*: characterization of the products of the *Abdominal-B* domain. *Genes and Dev.* **3**, 1424–1436.

Celniker, S. E. and Lewis, E. B. (1987). *Transabdominal*, a dominant mutant of the bithorax complex, produces a sexually dimorphic segmental transformation in *Drosophila*. *Genes and Dev.* **1**, 111–123.

Cho, K. W. Y., Goetz, J., Wright, C. V. E., Fritz, A., Hardwicke, J. and deRobertis, E. M. (1988). Differential utilization of the same reading frame in a *Xenopus* homeobox gene encodes two related proteins sharing the same DNA-binding specificity. *EMBO J.* **7**, 2139–2149.

DeLorenzi, M., Ali, N., Saari, G., Henry, C., Wilcox, M. and Bienz, M. (1988). Evidence that the *Abdominal-B* element function is conferred by a *trans*-regulatory homeoprotein. *EMBO J.* **7**, 3223–3231.

DeLorenzi, M. and Bienz, M. (1990). Expression of *Abdominal-B* homeoproteins in *Drosophila* embryos. *Development* **108**, 323–329.

Duncan, I. (1987). The bithorax complex. *A. Rev. Genet.* **21**, 285–319.

Guo, L.-H., Stepien, P. P., Tso, J. Y., Brousseau, R., Narang, S., Thomas, D. Y. and Wu, R. (1984). Synthesis of human insulin gene, VIII. Construction of expression vectors for fused proinsulin production in *Escherichia coli*. *Gene* **29**, 251–254.

Jack, T., Regulski, M. and McGinnis, W. (1988). Pair–rule segmentation genes regulate the expression of the homeotic gene *Deformed*. *Genes and Dev.* **2**, 635–651.

Karch, F., Weiffenbach, B., Peifer, M., Bender, W., Duncan, I., Celniker, S., Crosby, M. and Lewis, E. B. (1985). The abdominal region of the bithorax complex. *Cell* **43**, 81–96.

Krause, H. M., Klemenz, R. and Gehring, W. J. (1988). Expression modification and localization of the *fushi-tarazu* protein in *Drosophila* embryos. *Genes and Dev.* **2**, 1021–1036.

Kuziora, M. A. and McGinnis, W. (1988). Different transcripts of the *Drosophila* Abd-B gene correlates with distinct genetic subfunctions. *EMBO J.* **7**, 3233–3244.

Lewis, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565–570.

Martinez-Arias, A. and Lawrence, P. (1985). Parasegments and compartments in the *Drosophila* embryo. *Nature* **313**,

Ollo, R. and Maniatis, T. (1987). *Drosophila* Kruppel gene product produced in baculovirus expression system is a nuclear phosphoprotein that binds to DNA. *Proc. natn. Acad. Sci. U.S.A.* **84**, 5700–5704.

Peifer, M., Karch, F. and Bender, W. (1987). The bithorax complex: control of segment identity. *Genes and Dev.* **1**, 891–898.

Sánchez-Herrero, E. and Akam, M. (1989). Spatially ordered transcription of regulatory DNA in the bithorax complex of *Drosophila*. *Development* **107**, 321–329.

Sánchez-Herrero, E., Casanova, J., Kerridge, S. and Morata, G. (1985*a*). Anatomy and function of the bithorax complex of *Drosophila*. *Cold Spring Harb. Symp. quant. Biol.* **50**, 165–172.

Sánchez-Herrero, E. and Crosby, M. A. (1988). The *Abdominal-B* gene of *Drosophila melanogaster*: Overlapping transcripts exhibit two different spatial distributions. *EMBO J.* **7**, 2163–2173.

Sánchez-Herrero, E., Vernos, I., Marco, R. and Morata, G. (1985*b*). Genetic organization of *Drosophila* bithorax complex. *Nature* **313**, 108–113.

Schughart, K., Pravtchera, D., Newman, M. S., Hunihan, L. W., Jiang, Z. and Ruddle, F. H. (1989). Isolation and regional localization of the murine homeobox-containing gene Hox3.3 to mouse chromosome region 15E. *Genomics* **5**, 76–83.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive *in situ* hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma* **98**, 81–85.

Tiong, S. Y. K., Bone, L. M. and Whittle, J. R. S. (1985). Recessive lethal mutations within the bithorax complex in *Drosophila*. *Molec. gen. Genet.* **200**, 335–342.

White, R. A. H. and Wilcox, M. (1985). Regulation of the distribution of *Ultrabithorax* proteins in *Drosophila*. *Nature* **318**, 563–567.

Zavortink, M. and Sakonju, S. (1989). The morphogenetic and regulatory functions of *Drosophila Abdominal-B* gene are encoded in overlapping RNAs transcribed from separate promoters. *Genes and Dev.* **3**, 1969–1981.

(*Accepted 29 October 1990*)

Note added in proof:

Recently, S. Celniker, S. Sharma, D. Keelan and E. B. Lewis (EMBO J. in press) have also examined *Abd-B* expression in *iab* mutants. We thank Ed Lewis for communicating their results prior to publication.