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Immunochemical dissection of the Ultrabithorax homeoprotein family in *Drosophila melanogaster*

(bithorax complex/homeotic genes/homeodomain/monoclonal antibodies)

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ABSTRACT **The homeotic gene Ultrabithorax (*Ubx*) specifies metameric identities in multiple tissues of the thorax and abdomen in *Drosophila melanogaster*. Alternatively spliced Ultrabithorax mRNAs encode five protein isoforms that differ in internal sequences immediately adjacent to a homeodomain DNA-binding motif. Each of these proteins is phosphorylated *in vivo* at multiple serine and threonine residues. An extensive panel of monoclonal antibodies was raised against the Ultrabithorax proteins, including antibodies specific for individual isoforms and antibodies that discriminated between different phosphorylation states. Characterization of these antibodies provided insights into shared and isoform-specific features of Ultrabithorax protein structure that may be functionally important. Immunohistochemical staining experiments demonstrated that each isoform is expressed in a different stage- and tissue-specific pattern and suggested that Ultrabithorax protein phosphorylation is also developmentally regulated. These results support the hypothesis that alternative splicing and phosphorylation modulate developmentally specific functions of the *Ubx* gene.**

The homeotic genes of *Drosophila melanogaster* specify the developmental fates of cells in different metameres (reviewed in refs. 1 and 2). Each of the proteins encoded by these genes contains a homeodomain, a 60-amino acid helix–turn–helix motif that mediates DNA-binding interactions and is also found in other proteins that regulate development (reviewed in ref. 3). Various lines of evidence indicate that these proteins act as regulators of transcription (4–6). The homeotic gene Ultrabithorax (*Ubx*) specifies the identities of posterior thoracic and anterior abdominal metameres (reviewed in ref. 1). Alternative splicing of the *Ubx* transcripts generates at least five different mRNAs (7, 8). The members of the resulting Ultrabithorax (UBX) protein family share common amino- and carboxyl-terminal regions of 247 and 99 amino acids, respectively, but differ in the sequence connecting the common regions according to the combination of three small optional elements: a 9-amino acid element encoded by the sequence between alternative donor splice sites at the end of the common 5′ exon (the "b element") and two 17-amino acid elements (I and II), each encoded by a separate microexon (see Fig. 1*A*). The *Ubx* homeodomain is located in the carboxyl-terminal common region and is separated from the differential region by 4 amino acid residues. Ribonuclease protection experiments have demonstrated temporal regulation of *Ubx* alternative splicing (7, 8), and similar experiments have also suggested tissue-specific regulation (8), raising the possibility that the differential elements may modulate developmentally specific UBX functions. In addition, each of the UBX isoforms is phosphorylated *in vivo* at multiple serine and threonine residues (9).

We have taken an immunochemical approach to study the regulation and functional significance of UBX protein isoform expression and modification. We report the generation of an extensive library of monoclonal antibodies against the UBX proteins, including antibodies that recognize structural features of individual UBX isoforms and antibodies that discriminate between different phosphorylation states of these proteins. Characterization of these antibodies provides insights into the structure of the UBX proteins, and immunohistochemical staining experiments reveal stage- and tissue-specific regulation of UBX isoform expression and phosphorylation. These results support the hypothesis that alternative splicing and phosphorylation modulate developmentally specific UBX functions.

MATERIALS AND METHODS

Expression and Purification of UBX Proteins. The five *Ubx* open reading frames (ORFs) for which cDNAs are known (7, 8) were expressed in *Escherichia coli* under the control of the λP_L promoter in the vector pAS-1 (10). To generate expression constructs for these isoforms, the 900-base-pair (bp) *Stu* I–*Xho* I fragment spanning the optional elements in a pAS-1 construct carrying *Ubx* ORF Ib (pAS-Ib) (11) was replaced with the corresponding fragment derived from cDNA clones for Ia, IIb, IIa, and IVa. The *Ubx* Ia fragment was derived from plasmid pϕ3602, IIa was from EC13, and IVa was from EC1 (7). The *Ubx* IIb fragment was derived from cDNA E6-16T#5 (8). Expression of the pAS-UBX constructs was induced in *E. coli* AR120 (λ cI^+; *nalA*::Tn*10*) with nalidixic acid (12). Cells were lysed in 20 mM Tris·HCl, pH 7.4/10 mM EDTA/2 mM dithiothreitol (DTT)/5% (vol/vol) glycerol/0.4 M NaCl/0.4 mg of lysozyme per ml/0.15% (wt/vol) Brij-58 containing 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 2 μg each of aprotinin, leupeptin, and pepstatin per ml. Residual DNA was precipitated from the cleared lysate with 1% (wt/vol) polyethyleneimine. The supernatant was adjusted to 35% saturation with ammonium sulfate, and the precipitate was recovered by filtration and then extracted with 15% saturated ammonium sulfate in CM buffer [20 mM Mes, pH 6.5/200 mM NaCl/0.1 mM EDTA/0.5 mM DTT/5% (vol/vol) glycerol/0.5 mM PMSF]. The remaining precipitate was dissolved in CM buffer and chromatographed on carboxymethyl-Sepharose. Individual UBX ORFs were expressed in *Drosophila* Schneider cell line 2 (S2 cells) under the control of the hsp70 promoter as described (9).

Synthetic Peptides. Peptides were synthesized manually as carboxyl-terminal amides on 2,4-dimethoxybenzhydrylamine resin using 9-fluorenylmethoxycarbonyl chemistry

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Abbreviations: UBX, protein(s) encoded by the Ultrabithorax (*Ubx*) gene; ORF, open reading frame; CNS, central nervous system.

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(13, 14). Peptides were cleaved from the resin with trifluoroacetic acid (TFA) containing 4.5% phenol and 0.5% ethanedithiol (13) and were purified by reversed-phase chromatography on octadecylsilica using a gradient of 0–40% acetonitrile in 0.1% TFA. Cysteine residues were deprotected by treatment with equimolar mercuric acetate in TFA followed by excess 2-mercaptoethanol in 0.75 M Tris·HCl at pH 8.0. The peptide suspensions were acidified with glacial acetic acid, clarified by centrifugation, then desalted on a column of Sephadex G-10 in 20% acetic acid, and lyophilized. Peptides (15 μmol) were conjugated to mouse serum albumin (0.3 μmol) as carrier using *N*-succinimidyl-3-(2-pyridyldithio)-propionate (15) when the amino-terminal residue was a cysteine or by diazotization using bis(diazo)benzidine (16) when the amino-terminal residue was a tyrosine. Crosslinking with glutaraldehyde (17) was used in all other cases.

Immunizations and Generation of Hybridomas. Female BALB/c mice 4–6 weeks old were immunized by subcutaneous injection with 12.5 μg of antigen in 200 μl of complete Freund's adjuvant. The mice were given booster immunizations three times at 21-day intervals by intraperitoneal injection with the same amount of antigen in incomplete Freund's adjuvant. Individual spleens were taken for fusion with SP2/0 myeloma cells as described (18). Hybridoma supernatants were screened by enzyme-linked immunosorbent assay (ELISA) and by immunoblotting against each of the UBX proteins. For ELISA, the UBX proteins were applied to the microassay plates as purified native preparations (25 ng per well), and the assays were performed following the Bio-Rad alkaline phosphatase protocol. Immunoblotting assays were performed on SDS/polyacrylamide gel electrophoretograms of crude bacterial lysates or S2 cell extracts using alkaline phosphatase-conjugated goat anti-mouse IgG as secondary antibody (19). Hybridomas were cloned by limiting dilution.

RESULTS

Isoform-Specific Antibodies. The following strategies were used to generate monoclonal antibodies against shared and isoform-specific epitopes in the UBX proteins: immunization with each of five partially purified native UBX proteins (Fig. 1*B*) and immunization with synthetic peptides corresponding to each of the optional elements and to sequences encoded by the exon junctions that distinguish individual UBX isoforms (Fig. 1*C*). Table 1 summarizes the characteristics of 68 independent antibodies that discriminated among the UBX isoforms, 31 of which derive from 819 UBX-positive hybridomas obtained by immunization with purified UBX proteins, with the remainder raised from the synthetic peptides.

Three themes characterize this population of antibodies. (*i*) The strongest and most frequent of the antibodies raised from the UBX proteins discriminated among them by ELISA and immunoblots according to the presence of elements I and/or II (e.g., monoclonal antibodies Ia-3.11F and Ia-4H.6; Table 1 and Fig. 2*A*). These antibodies reacted specifically with the microexon peptides (either P-MI or P-MII but not P-B) conjugated to mouse serum albumin, indicating that residues in elements I and II make up at least part of the epitopes recognized in the intact protein (data not shown).

(*ii*) The b element appears to influence the accessibility of these epitopes. Thus, most of these antibodies reacted more strongly with isoforms that contained the b element, even when the immunogen lacked that element (e.g., Ia-3.11F; Table 1 and Fig. 2*A*). Furthermore, the next most frequent antibody type generated by UBX proteins reacted specifically with isoforms Ib and/or IIb, and these antibodies were obtained by immunization not only with the corresponding proteins but also with isoform Ia (Table 1). In contrast, only one antibody specific for isoform Ia, one for IIa, and two for Ia and IIa were identified, and all of these were quite weak in ELISA and immunoblotting assays. Antibodies raised against synthetic UBX peptides also showed a preference for isoforms that contain the b element, even when it was not part of the immunogen (Table 1). Antibodies induced by P-MI reacted only with isoform Ib (e.g., MP3-3BD.2; Fig. 2*A*), and most antibodies induced by P-MII reacted preferentially, but not exclusively, with isoforms that also contain the b element (e.g., MP4-5C.2; Fig. 2*A*). Immunization with the junction peptides JP-Ia and JP-IIa yielded polyclonal sera that reacted weakly not only with Ia and IIa, respectively, but also with the respective b forms. In contrast, JP-Ib and JP-IIb elicited polyclonal sera that reacted exclusively with isoform Ib or IIb, respectively.

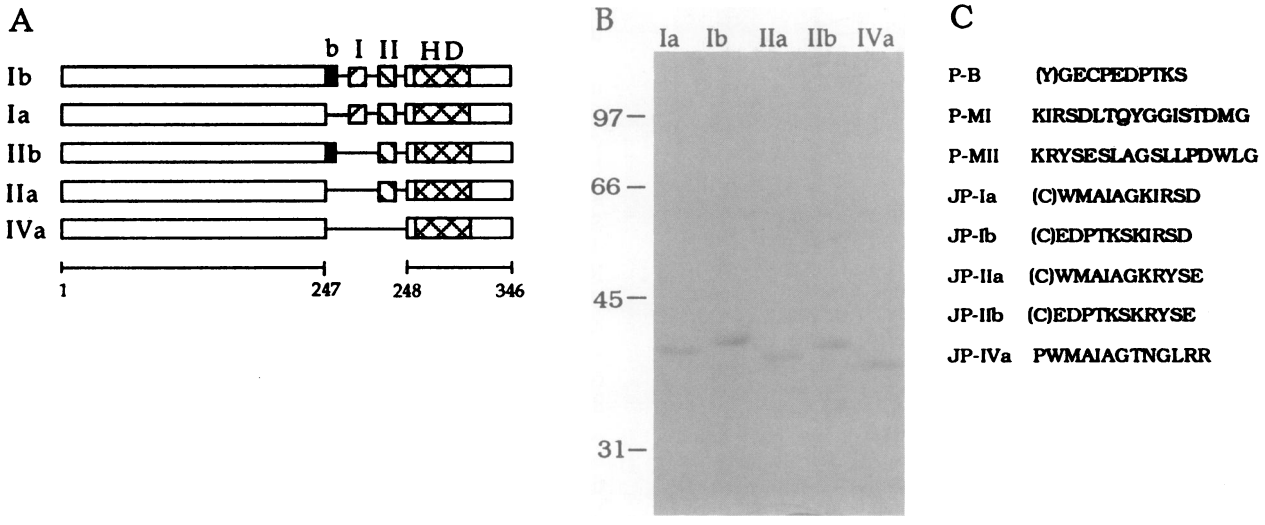

FIG. 1. (*A*) Primary structure of the UBX protein isoforms. Regions encoded by separate exons are represented as rectangles connected by horizontal lines. Amino acid coordinates for the common regions are indicated beneath isoform IVa. HD identifies the homeodomain; b, I, and II identify the optional elements. (*B*) Partial purification of UBX proteins. The five UBX proteins were expressed and isolated as described in the text. One-half microgram of each protein was electrophoresed through a 10% SDS/polyacrylamide gel and stained with Coomassie blue. The isoform is indicated above each lane; the positions of molecular mass markers (in kDa) are shown at the left. (*C*) Primary structure of synthetic UBX peptides. P-B, b element; P-MI, element I; P-MII, element II; JP-Ia through JP-IVa, peptides spanning the exon junctions unique to each isoform. Residues enclosed by parentheses were added to facilitate coupling to carrier protein.

Table 1. Isoform-specific anti-UBX monoclonal antibodies

Specificity	Immunogen*	No.	Example
Ia, Ib, IIa, IIb	Ia	7	Ia-4H.6
	P-MII	12	MP4-5C.2
Ia, Ib, IIa	Ia	1	Ia-1BD.5
Ib, IIb	Ib	2	Ib-10H.11
	Ia	1	Ia-6G.9
	P-MII	2	MP4-4F6.1
	P-B	4	M2-7A.7
Ia, IIa	Ia	2	Ia-2.9F
Ia, Ib	Ia	2	Ia-3.11F
IIa, IIb > Ia, Ib†	Ia	1	Ia-4A.1
Ib	Ib	4	Ib-2D.2
	Ia	2	Ia-3H.10
	P-MI	13	MP3-3BD.2
	JP-Ib	1	MP7-B.3
Ia	Ia	1	Ia-9C.6
	JP-Ia	1	MP8-6C.12
IIa	IIa	1	IIa-4.9c‡
	JP-IIa	1	MP9-4E.4
IIb	IIb	2	IIb-6D.9
	JP-IIb	3	MP7-A.4
IVa†	Ia§	5	Ia-1BC.6

*See the legend to Fig. 1 for nomenclature.
†In ELISA only; nonselective in immunoblots.
‡In ELISA only; does not react in immunoblots.
§Also obtained with the other UBX isoforms.

(*iii*) Anti-IVa monoclonal antibodies that reacted preferentially with isoform IVa in ELISA reacted with all UBX isoforms on immunoblots (e.g., Ia-1BC.6; Table 1, Fig. 2*A*) and were generated by immunization with any isoform (Table 1). These results indicate that the relevant epitopes are present in all isoforms in their denatured states but are recognized more readily in isoform IVa in the native state. Accordingly, monoclonal antibody Ia-1BC.6 immunoprecipitated isoform IVa preferentially from crude bacterial lysates and only reacted with the other isoforms during prolonged incubations (data not shown). Evidently, anti-IVa specificity results not from the only unique sequence in isoform IVa (i.e., the JP-IVa sequence, Fig. 1*C*) but rather from differences among the native structures of the UBX proteins—a conclusion consistent with our finding that JP-IVa did not elicit anti-UBX antibodies.

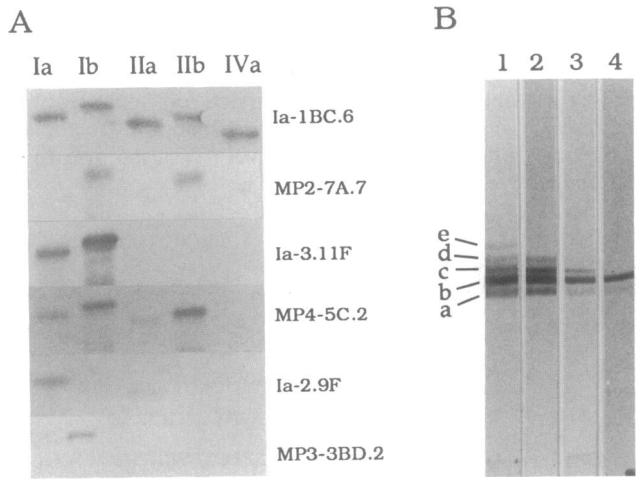

FIG. 2. (*A*) Immunoblots of UBX proteins with isoform-specific monoclonal antibodies. Crude extracts of *E. coli* cells expressing individual UBX isoforms (identified at the top of the lanes) were subjected to Western immunoblotting using the monoclonal antibodies identified at the right (see text and Table 1). Only the UBX region of each blot is shown; the smears below the main bands are UBX degradation products. (*B*) The three classes of phosphorylation-sensitive anti-UBX monoclonal antibodies. UBX isoform Ia was expressed under the control of the hsp70 promoter in stably transformed S2 cells. Crude extracts from these cells were analyzed by Western immunoblotting. Band b is the nonphosphorylated isoform Ia and bands c, d, and e are phosphorylated Ia variants (see text). Band a appears to be a pseudoisoform IVa generated by aberrant splicing of Ia mRNA (A.J.L., unpublished data; see ref. 9 for similarly aberrant splicing of Ib and IIb mRNAs.) Lane 1, monoclonal antibody not sensitive to phosphorylation (Ia-5B.12); lane 2, S3 antibody (Ia-5F.2); lane 3, S2 antibody (Ib-3F.6); lane 4, S1 antibody (Ia-4C.12).

Antibodies Sensitive to UBX Protein Phosphorylation. UBX proteins expressed in *Drosophila* embryos and in S2 cells from cDNA expression constructs are phosphorylated at multiple serine and threonine residues in the amino-terminal common region (9). Increasing phosphorylation correlates with decreasing mobility of the UBX proteins on SDS/polyacrylamide gels. This is shown in lane 1 of Fig. 2*B*, where band b consists of the nonphosphorylated Ia isoform and bands c, d, and e consist of progressively more highly phosphorylated Ia protein (ref. 9; see Fig. 2 legend for an explanation of band a). Similar states of phosphorylation have been observed for the other UBX isoforms (9).

Thirteen monoclonal antibodies that recognized epitopes common to all UBX isoforms reacted only with subsets of the phosphorylation states of these proteins. Three classes of these phosphorylation-sensitive antibodies were defined according to the results shown in Fig. 2*B*. The S1 class (lane 4) reacted only with unmodified protein (band b), the S2 class (lane 3) reacted with the unmodified protein and with UBX protein at the lowest level of phosphorylation (band c), whereas the S3 class (lane 2) reacted with all but the most highly phosphorylated forms in band e. These results suggest that phosphorylation of the UBX proteins masks the epitopes recognized by these antibodies, either because an amino acid residue that is part of or close to the epitope is phosphorylated or because phosphorylation causes a conformational change in the region containing the epitope.

Distribution of Epitopes Recognized by Anti-UBX Monoclonal Antibodies. Fig. 3*A* describes the strategy used to map the approximate location of antigenic determinants recognized by a random selection of monoclonal antibodies generated by immunization with UBX Ia. Of 62 antibodies tested, 49 reacted on immunoblots, and 44 of these could be classified unambiguously as described in Fig. 3*A*. The resulting map is shown in Fig. 3*B*. The distribution of determinants is strikingly nonuniform: the majority are clustered between amino acid residues 37 and 183 (regions B, C, and D) and a smaller group appears in the 49 carboxyl-terminal amino acids (region H). Regions C and D are characterized by a high content of glycine and polar and charged residues (7) and are highly susceptible to proteolysis with trypsin, glutamyl endopeptidase, or elastase in the native protein (data not shown). Region H includes the last 14 amino acids of the homeodomain and the 35 carboxyl-terminal amino acids, which are rich in polar and charged residues and are also highly sensitive to proteolysis in the native protein. In contrast, regions A, E, and most of F, in which no determinants were detected, contain few or no charged residues and are resistant to proteolysis. These observations suggest that the region between residues 106 and 198 forms an exposed and relatively unstructured domain between two more compactly folded domains formed by the A, B, and E–G regions.

Antigenic determinants recognized by the phosphorylation-sensitive antibodies were detected only in regions C and D (Fig. 3*B*). All but 1 of 10 antibodies that recognized determinants in region D were sensitive to phosphorylation, whereas only 4 of 16 antibodies that recognized determinants in region C were sensitive to phosphorylation. The two class S1 antibodies recognized determinants within region D as did

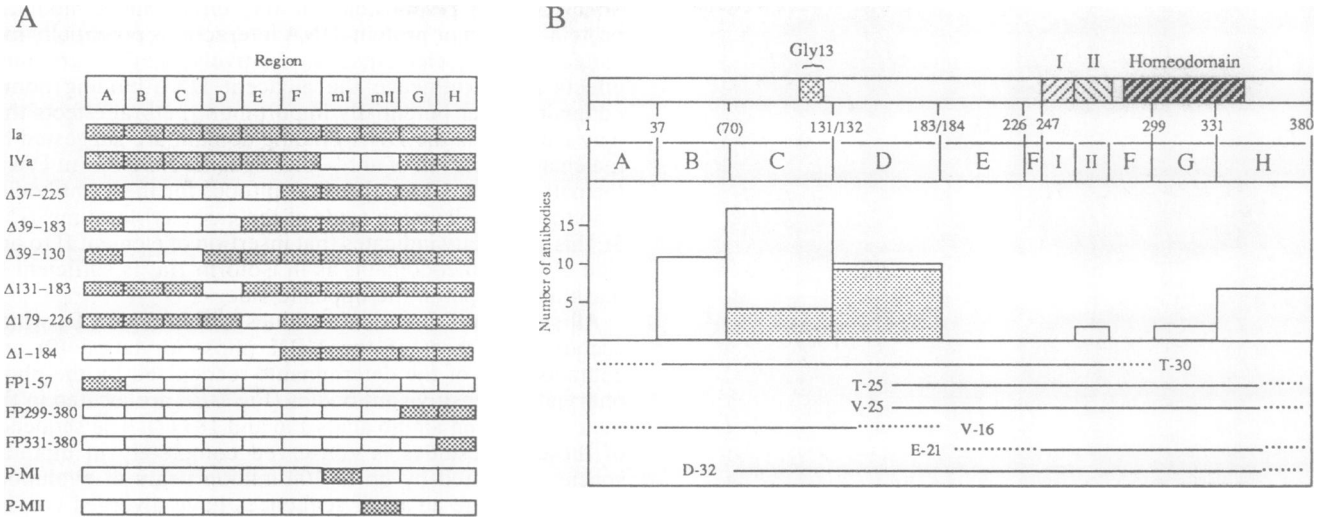

FIG. 3. Location of antigenic determinants recognized by monoclonal antibodies raised against UBX Ia. (*A*) Mapping strategy. Immunoblotting assays were used to determine the ability of monoclonal antibodies to react with normal UBX isoforms, in-frame deletion derivatives, β-galactosidase–UBX fusion proteins, and proteolytic fragments of the UBX proteins. Reactivity with synthetic peptides PM-I and PM-II conjugated to mouse serum albumin was determined by ELISA. These antigens (except the proteolytic fragments; shown in *B*) are identified at the left. Deletion derivatives are designated by the codons removed (e.g., Δ37-225). Fusion proteins are designated by the UBX codons included in the fusion (e.g., FP1-57). The pattern of reactivity with this partially overlapping set of antigens was used to assign the key antigenic determinants recognized by each antibody to 1 of 10 regions of the protein (A-H, mI, and mII) indicated at the top. The pattern of reactivity for antibodies that recognize determinants within the indicated regions is indicated by the shaded (reactive) and unshaded (unreactive) rectangles. In-frame deletions of the *Ubx* coding sequence were constructed in pP_{Ac}UBX-IVa as described (9) and transferred into the expression construct pAS-Ia by substitution of the *Stu* I/*Xho* I fragment within the *Ubx* cDNA. Fusion proteins were constructed in the pUR vector series (20). (*B*) Approximate location of antigenic determinants on the UBX Ia primary structure. Landmarks on the UBX Ia protein are indicated at the top as in *A*. Gly13 is a stretch of 13 consecutive glycine residues. Amino acid coordinates and the regions of the protein defined as in *A* are indicated below the protein diagram. The histogram shows the number of independently isolated monoclonal antibodies that recognize antigenic determinants within the indicated region, deduced as described in *A*. Open bars, all antibodies; stippled bars, phosphorylation-sensitive antibodies. Below the histogram are shown the approximate extents of several proteolytic fragments of UBX Ia as deduced from their size and pattern of reaction with the antibodies. T-30, T-25, E-21, and V-16 are 30-, 25-, 21-, and 16-kDa proteolytic fragments, respectively, generated by limited digestion of native isoform Ia with trypsin (T), elastase (E), or glutamyl endopeptidase (V). D-32 is a prominent 32-kDa degradation product generated during expression in *E. coli*. The amino- and carboxyl-terminal ends of these fragments have not been determined precisely (dotted lines).

seven of the eight S2 antibodies. One S2 antibody and all three S3 antibodies recognized determinants in region C. These results suggest that the phosphorylation events that mask the epitopes recognized by these antibodies occur in regions C and D. This is consistent with the results of phosphopeptide mapping experiments, which indicate that the UBX phosphorylation sites are located within regions C and D (9).

Those antibodies that reacted preferentially with native isoform IVa recognized determinants located in the common carboxyl-terminal region H (Fig. 3*B*; e.g., Ia-1BC.6), consistent with the ability of these antibodies to react with all isoforms in their denatured states.

Developmental Regulation of UBX Isoform Expression and Phosphorylation. Immunohistochemical staining experiments using the isoform-specific antibodies demonstrated that UBX isoforms Ia and Ib are expressed primarily in the epidermis, mesoderm, and peripheral nervous system during embryonic development (Fig. 4 *c* and *d*), whereas isoforms IIa and IIb are expressed primarily in the central nervous system (CNS; Fig. 4 *e* and *f*) and isoform IVa is expressed exclusively in the CNS (Fig. 4 *g* and *h*).

Immunohistochemical staining experiments using the phosphorylation-sensitive antibodies suggested that UBX proteins in the embryonic CNS are underphosphorylated relative to those in the epidermis and mesoderm. With one exception (which stained UBX strongly in all tissues), phosphorylation-sensitive antibodies stained parasegments 5–13 of the CNS strongly but failed to stain or stained only very weakly the epidermis and mesoderm, especially during early developmental stages prior to germ band retraction (Fig. 4 *i* and *j*). Treatment of the embryos with phosphatases prior to incubation with phosphorylation-sensitive antibodies enhanced the intensity of staining of the epidermis and mesoderm relative to the CNS, although not to the levels observed with the common antibodies (data not shown).

A detailed description of the complex temporal and spatial expression patterns of the UBX isoforms will be given elsewhere.

DISCUSSION

Functional differences between the UBX isoforms have been demonstrated in *Drosophila* S2 tissue culture cells using reporter gene cotransfection assays (4) and in transgenic embryos by analyzing metameric identity transformations produced by ectopic expression of UBX proteins (22). Although the functions of the UBX isoforms appear to overlap substantially with respect to epidermal development, isoform Ia, but not IVa, can redirect developmental fates in the embryonic peripheral nervous system when expressed ectopically (22). In addition, the phenotype produced by the Ubx^{195} mutant allele, which contains a stop codon in microexon II, suggests that isoform IVa provides *Ubx* function in the late CNS but not in other tissues or stages (21). These observations are consistent with the developmental patterns of UBX isoform expression observed using the antibodies generated in this study. Together, these results support the hypothesis that regulated alternative splicing modulates the developmental functions of the *Ubx* gene products. The observation of developmentally specific immunohistochemical staining with phosphorylation-sensitive antibodies (Fig. 4 *i* and *j*) suggests that phosphorylation may also modulate tissue- and stage-specific functions of the UBX proteins. Like *Ubx*, the homeotic genes Antennapedia (*Antp*) (23, 24), labial

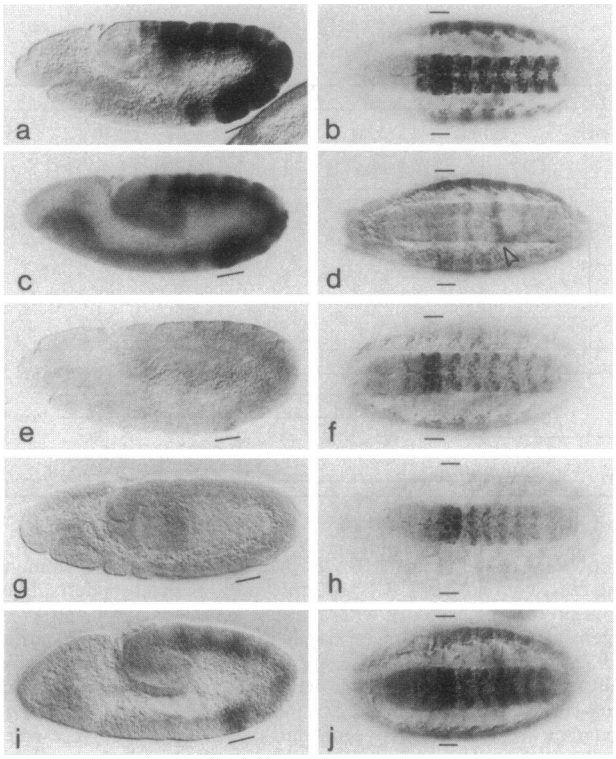

FIG. 4. Expression of UBX isoforms and phosphorylation states during embryogenesis. Embryos were stained with anti-UBX monoclonal antibodies and horseradish peroxidase-conjugated goat anti-mouse IgG (Vector). UBX protein localization appears black in a light gray background. All embryos are oriented with their anterior ends to the left. Parasegment 6 is indicated by a black bar. (*a*, *c*, *e*, *g*, and *i*) Lateral views of embryos at stage 10 (extended germ band). (*b*, *d*, *f*, *h*, and *j*) Ventral views of embryos at stage 16. (*a* and *b*) Ia-5B.12 (all UBX isoforms and phosphorylation states). At stage 10, UBX proteins are expressed strongly in nuclei of mesoderm (continuous internal band in *a*) and ectoderm (parasegmental bands in *a*). At stage 16, UBX proteins are expressed strongly in nuclei of the CNS (central bands in *b*) and in nuclei of the epidermis, mesoderm, and peripheral nervous system (lateral staining in *b*). (*c* and *d*) Ia-3.11F (Ia and Ib). The epidermis, mesoderm, and peripheral nervous system nuclei are stained at all stages but CNS nuclei are not stained. The arrowhead indicates visceral mesoderm staining seen through the overlying, unstained CNS. (*e* and *f*) MP4-4A.1 (primarily IIa and IIb; does not recognize IVa). Primarily CNS nuclei are stained. (*g* and *h*) Ia-1BC.6 stain of Ubx^{195} homozygote. Because the 1BC.6 epitope (Table 1) is carboxyl-terminal (Fig. 3) and the Ubx^{195} allele carries a stop codon in microexon II (21), only isoform IVa is visualized. Staining is seen exclusively in nuclei of the CNS during late embryogenesis. (*i* and *j*) Ia-6C.11 (phosphorylation-sensitive, S2). CNS nuclei are strongly stained, whereas epidermis and mesoderm are weakly stained.

(25), and proboscipedia (D. L. Cribbs, M. A. Pultz, D. Johnson, and T. C. Kaufman, personal communication) encode multiple protein isoforms as a result of alternative RNA splicing. Temporal and spatial modulation of alternative splicing have been described for *Antp* (23, 24), and the proteins encoded by this gene are also phosphorylated (S. Hayashi and M. P. Scott, personal communication). Thus, alternative splicing and protein phosphorylation may provide general mechanisms for the modulation of homeotic gene function.

The optional elements might modulate UBX function by direct participation in protein–protein or protein–DNA contacts or through effects on protein structure. Our results suggest that the b element increases the accessibility of antigenic determinants in elements I and II. The conformational changes responsible for this effect might modulate protein–protein or protein–DNA interactions potentially mediated by these elements. Alternatively, similar structural effects might influence the adjacent DNA-binding homeodomain. Other potentially important structural effects that may encompass the DNA-binding domain are suggested by the characterization of antibodies specific for isoform IVa in the native state. Given that the epitopes for these antibodies map to within 49 amino acids of the carboxyl terminus (Fig. 3), this specificity indicates that insertion of element II to one side of the homeodomain, as in isoform IIa, is sufficient to mask an epitope on the opposite side.

All serine and threonine residues within region D, which accounts for most of the UBX phosphorylation (9) and contains most of the determinants recognized by the phosphorylation-sensitive antibodies (Fig. 3*B*), are located in the interval between amino acids 156 and 183 (7). The sequence of these 27 residues is conserved completely in dipteran species separated by up to 100 million years of evolution, even though the adjacent sequences have diverged considerably (26). This pattern of sequence conservation suggests that phosphorylation in region D plays an important role in the function of the UBX proteins. The high antigenicity and susceptibility to proteolysis of this region indicate that it is highly accessible and could form contacts for protein–protein interactions that might be modulated by phosphorylation. The availability of antibodies that discriminate among the different isoforms and their phosphorylation states should facilitate more detailed analyses of the developmental regulation and functional significance of these UBX proteins.

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