Alternative Splicing Modulates Ubx Protein Function in *Drosophila melanogaster*

Hilary C. Reed, Tim Hoare, Stefan Thomsen, Thomas A. Weaver, Robert A. H. White, Michael Akam and Claudio R. Alonso

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FIGURE S1.—Isoform expression levels do not correlate with isoform-specific functions. Levels of Ubx expression for the different Ubx isoforms detected in Western Blot experiments using the anti-Ubx FP3.38 antibody. Embryos were recovered from a 0-4 hs collection and were left to develop for further 12 hours at 25°C (average age 12-16hs). Sampled embryos were the progeny of the cross 69B-Gal4 x UAS-Ubx (A) or 24BGal4 x UAS-Ubx (B). All collected embryos were heterozygous for the UAS-Ubx construct and for the Gal-4 driver construct. Embryos were dechorionated in 50% bleach and transferred to a methanol/heptane mix and washed in methanol repeated times. Before use embryos were re-hydrated in 1X PBS, 0.1% Triton X-100, counted, transferred to protein sample buffer, boiled for 10 minutes and stored at -20°C until use. Embryo extracts were resolved in a 10% PAGE SDS gel loading a volume equivalent to ten embryos per lane. After electrophoresis, samples were electro-transferred to a nitrocellulose membrane and subjected to a standard antibody staining protocol (Alkaline Phosphatase detection). Although expression levels for the different isoforms vary across the different transgenic lines there is no correlation between expression level and isoform in vivo function as assessed by the activation pattern of the reporter gene dpp-lacZ (summarised in C; see also Fig. 3) or the endogenous dpp gene.
**FIGURE S2.**—Isoform expression levels do not correlate with isoform-specific functions. **(A-I)** Expression levels of Ubx achieved by UAS lines for different Ubx isoforms as detected by fluorescence confocal microscopy. Stage 13 embryos carrying the 24B Gal4 driver, a UAS-GFP reporter, and a UAS-Ubx isoform construct were fixed, stained for Ubx (FP3.38) and GFP proteins, and analysed by confocal laser scanning microscopy. Embryos from Ubx Ia and Ubx IVa lines (i.e. from transgenic lines analysed in gel lanes “Ia” and “IVa2” in Supp Fig S1) were collected and stained in parallel. All pictures were captured using identical settings in both computer and microscope, therefore fluorescence intensities can be used as an estimate for expression levels. **(A-C)** 24B-Gal4, UAS-GFP, UAS-UbxIa embryos. **(D-F)** 24B-Gal4, UAS-GFP, UAS-UbxIVa embryos. **(G-I)** Wildtype embryos. **(J-K)** Quantification of Ubx levels achieved by UAS-UbxIa and UAS-UbxIVa lines when driven by the gal-4 driver 24B. Mean values of 24B-driven Ubx expression were normalised by the endogenous levels of Ubx. Embryo areas used for signal quantification are illustrated in **(J)**. Quantification results **(K)** expressed as a ratio between the 24B-driven signal over the endogenous Ubx level in one CNS hemisegment indicate the absence of significant differences in the expression levels between the transgenic lines for the different splicing isoforms (Quantification was performed using the histogram function of Adobe Photoshop). These experiments confirm that as suggested in western blot experiments, these two transgenic lines achieve comparable expression levels of Ubx in mesodermal tissues (labelled with GFP in green) while showing a markedly different ability to activate the molecular target dpp during embryogenesis (see Fig 3). This data further supports the conclusion that qualitative differences in the structure of the proteins compared in this study are causal to the distinct activation patterns of the dpp target gene. (All embryos in ¾ ventral view oriented with anterior to the left).
FIGURE S3.—Increased levels of ectopic UbxIb do not lead to the activation of dpp-lacZ in the somatic mesoderm. All embryos carry the dpp-lacZ reporter gene and were stained with an anti-β-galactosidase antibody. (A-B) Expression of dpp-lacZ at stage 13 (A) and stage 16 (B) when UbxIb is expressed throughout the mesoderm with the 24BGal-4 driver line. Little β-galactosidase expression is seen in the somatic mesoderm. (C-D). The expression of dpp-lacZ at stage 13 (C) and stage 16 (D) when UbxIb is expressed throughout the mesoderm with a combination of 24B-Gal4 and twist-Gal4 driver lines. The delivery of higher levels of UbxIb fails to activate dpp-lacZ in the somatic mesoderm. This experiment further confirms that there is no correlation between isoform expression level and in vivo function as revealed by the dpp activation patterns (see Fig. 3). (All embryos are in dorsal view, oriented with anterior to the left; SM: somatic mesoderm).
FIGURE S4.—In vitro DNA-binding assays with Ubx proteins. (A) [35S] labelled natural Ubx isoforms (see lanes Ubx Ia wt and Ubx IVa wt) and artificially engineered Ubx isoforms (lanes Ubx Ia ALA and Ubx IVa ALA) were expressed in a rabbit reticulocyte system and analysed by PAGE-SDS. Exd wild type was also expressed and analysed in the same manner (lane Exd wt). All preparations rendered products of appropriate size. Note that translation reactions without RNA program produced no visible products (lanes 'no program'). Quantification of the [35S] signal from relevant bands was normalised by the number of methionines in each protein product and used to estimate protein units (see Materials and Methods). Molecular weight standards are indicated to the left of the panel. (B) Sequence of the Hox-Exd composite element used in DNA binding experiments. Hydrogen bonds between bases and aminoacid residues in Ubx (red) or Exd (blue) are indicated by solid lines. Core sites for each protein are also indicated (boxes). This diagram was prepared from crystallographic data and other information provided in reference (Passner et al. 1999). (C) Electrophoretic mobility shift assay (EMSA) with the Hox-Exd probe testing the specificity of protein-DNA complexes detected in our experiments. Ubx and Exd proteins were incubated with a [32P] labelled Hox-Exd double stranded oligonucleotide and the products were resolved in a low-ionic strength polyacrylamide gel. In the absence of competitor, Ubx and Exd produce a clear protein-DNA ternary complex (lane 1) of low mobility (arrow). When homologous unlabelled oligo (specific competitor) was added, the complexes detected disappear as the molarity of the competitor increases (lanes 2-4). When identical amounts of an unlabelled non-specific competitor of similar length were added to the reaction (lanes 5-7), the signal in ternary complexes remained stable, confirming that the detected DNA binding activity was specific for the labelled probe. (D) PhosphorImager quantification of the experiment shown in (C). Maximum relative binding, detected in the absence of competitor has been defined as 100%. Competition level is plotted as fold of unlabelled competitor relative to labelled probe. For further details on binding quantification please see Materials and Methods.
**FIGURE S5.**—Ubx protein expression in wild type and *Ubx^{MX17}* mutants. (A-B) Ubx protein expression in wild type (A) and *Ubx^{MX17}* (B) extended germ band embryos (Stage 10). Both genotypes show the characteristic Ubx protein expression in the epidermis from PS6-PS12 with lower expression levels detected in PS5. (C-D) Ubx protein expression in wild type (C) and *Ubx^{MX17}* (D) Stage 16 embryos displaying Ubx signal in the epidermis and the CNS. As in earlier stages, both genotypes show identical patterns of expression. Note that Ubx expression can be detected with very high resolution; see, for instance, that both genotypes show signal in a subset of the ventral midline cells (arrows) which contribute to the population of glial cells in the developing CNS. Ubx proteins were detected using the FP3.38 monoclonal antibody (which detects all Ubx isoforms) in parallel for both genotypes. (All embryos with anterior to the left, A-B in lateral view, C-D in ventral view).