The Hox protein family consists of homeodomain-containing transcription factors that are primary determinants of cell fate during animal development. Specific Hox function appears to rely on protein-protein interactions; however, the partners involved in these interactions and their function are largely unknown. Disconnected Interacting Protein 1 (DIP1) was isolated in a yeast two-hybrid screen of a 0–12-h Drosophila embryo library designed to identify proteins that interact with Ultrabithorax (Ubx), a Drosophila Hox protein. The Ubx-DIP1 physical interaction was confirmed using phage display, immuno precipitation, pull-down assays, and gel retardation analysis. Ectopic expression of DIP1 in wing and haltere imaginal discs malforms the adult structures and enhances a decreased Ubx expression phenotype, establishing a genetic interaction. Ubx can generate a ternary complex by simultaneously binding its target DNA and DIP1. A large region of Ubx, including the repression domain, is required for interaction with DIP1. These more variable sequences may be key to the differential Hox function observed in vivo. The Ubx-DIP1 interaction prevents transcriptional activation by Ubx in a modified yeast one-hybrid assay, suggesting that DIP1 may modulate transcriptional regulation by Ubx. The DIP1 sequence contains two dsRNA-binding domains, and DIP1 binds double-stranded RNA with a 1000-fold higher affinity than either single-stranded RNA or double-stranded DNA. The strong interaction of Ubx with an RNA-binding protein suggests a wider range of proteins may influence Ubx function than previously appreciated.

During animal development, differential transcription regulation is key to subdivision and specification of a diverse array of tissues. Position along the anterior-posterior axis in developing metazoans is specified by the Hox transcription factor family (1–7). Alterations in expression patterns of Hox transcription factors can rearrange appendages, a dramatic example of their capacity to instigate developmental programs (1). However, Hox transcription factors bind short, partially degenerate DNA binding sites via the highly conserved homeodomain, suggesting that DNA recognition solely by the homeodomain is insufficient to achieve the precise regulatory control required for Hox function in vivo (2). Further, members of the Hox family can activate or repress transcription, often of the same gene, depending on the cellular context (3, 4). Despite this variability, genetic and molecular studies demonstrate that Hox proteins specifically regulate a large number of genes throughout the genetic hierarchy (1, 5). Thus, Hox proteins require a mechanism to assimilate external information to produce differential DNA binding and transcription regulation functions.

The function of many transcription factor families relies on a complex web of protein interactions to generate the requisite specificity, diversity, and reliability of transcriptional outcomes (6–8). Indeed, the transcriptional activity of Hox proteins is influenced by homomeric and heteromeric cooperative DNA binding (9–13) and by specific interactions with components of the transcription apparatus (14). Hox proteins bind cooperatively to DNA with Extradenticle (Exd) in arthropods or with Pbx in vertebrates via their YPWM motifs (10, 13). Residues flanking this YPWM motif stabilize the protein complex and also make contacts with the DNA (13, 15). Thus, interactions with heterologous proteins may provide the functional specificity requisite for Hox function in vivo. Despite the potential importance of these interactions, very little is known about the range of Hox protein partners or the role of these partners in development.

Ultrabithorax (Ubx) is a Hox protein that orchestrates development of the posterior thorax of Drosophila melanogaster. Ubx specifies parasegments 5 and 6 and contributes to the differentiation of more posterior segments (6, 16–20). Within these regions, Ubx influences midgut, central nervous system,

Received for publication, November 24, 2003, and in revised form, March 4, 2004
Published, JBC Papers in Press, March 23, 2004, DOI 10.1074/jbc.M312842200

Sarah E. Bondos‡, Daniel J. Catanese, Jr.‡§, Xin-Xing Tan‡¶, Alicia Bicknell, Likun Li**, and Kathleen S. Matthews‡‡
From the Department of Biochemistry and Cell Biology, Rice University, Houston, Texas 77005
Ubx Interacts with a dsRNA-binding Protein, DIP1

The overlapping cDNA and expressed sequence tags from the BerkeleyFlyBase were aligned and combined using DNA Strider 1.2. Homology searches of DIP1 were completed using the Prosite Data base (30) and the Blast program (31).

Phase Display Assay

The T7Select Phage Display System (Novagen) was used according to the manual. DIP1-c, the longest isoform, was cloned into T7Select 1–1b vector between the EcoRI and XhoI restriction sites and was fused to the region encoding the C terminus of the 10B capsid protein gene of T7 phage to display, on average, 1 DIP1-c protein on each product phage as a fusion protein. Phage were amplified, and a specific amount of phage lysate containing either 500 or 1,000 phages was applied to the surface of a Petri dish upon which cell extracts containing biotinylated Ubx Ib had been immobilized (see below). Controls utilized cell extracts with vector lacking the Ubx Ib coding region. The dish was washed five times with 100 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1% Tween 20. Phage that had been captured by Ubx Ib were eluted with 1% SDS. Two 20-min elutions at room temperature were collected and were diluted 20-fold with LB medium. The phage lysate was titrated with Escherichia coli BLT5615 both before and after biopanning. These data were used to calculate the percentage of phage retained.

Protein Expression and Purification

Ubx Ib, cloned into pET3c between the NdeI and BamHI restriction sites, was a gift from Philip A. Beachy (Johns Hopkins University). Ubx Ib was expressed in E. coli BL21(DE3)pLysS and purified as described previously (32).

To produce biotinylated Ubx Ib for phase display assays, Ubx Ib was cloned into the pDW383 vector (33) between the EcoRI and BamHI restriction sites. Biotinylated Ubx Ib was expressed in E. coli AR120. Cell growth and protein induction were performed as described (32). Cell pellets from a 200-ml growth were resuspended in 2 ml of 10% sucrose, 50 mM Tris-HCl, pH 7.4, with 0.5 mM PMSF and 0.8 mg/ml lysozyme and frozen at –80 °C. The frozen cell pellet was thawed on ice and diluted with an equal volume of 10% sucrose, 50 mM Tris-HCl, pH 7.4, 0.8 M NaCl, 20 mM EDTA, 4 mM DTT, and 10 μl of 100 mM PMSF. The cells were incubated on ice for 45 min. Subsequently, 4 μl of 10 mg/ml DNase I was added before a further 30-min incubation at room temperature. The slurry was centrifuged for 30 min at 11,000 × g, and the supernatant was applied to avidin-coated Petri dishes to immobilize biotinylated Ubx Ib. The plates were washed with 100 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1% Tween 20, to remove all bacterial proteins prior to the addition of phage.

DIP1-c was cloned between the NdeI and XhoI restriction sites in the pET28a vector (Novagen) to express an N-terminally His6-tagged DIP1-c in E. coli BL21(DE3)pLysS. Overnight Luria broth culture (8 ml) was used to inoculate each of 12 LB cultures (1 liter each) at 37 °C. At midlog phase, DIP1-c expression was induced with 1 mM isopropyl-β-d-thiogalactopyranoside, and the cells were harvested 2 h later. Cells collected from 2-liter growth and stored at –20 °C were thawed on ice and lysed in lysis buffer (50 mM NaH2PO4, pH 8.0, 300 mM NaCl, 1 mM imidazole, 5% glucose, 10 mM DTT). After lysis, DNase I and RNase A (each 20 mg/ml) were added. The lysate was centrifuged at 13,000 × g for 15 min, and the supernatant was filtered before loading onto a 5-ml HITRAP heparin column (Amersham Biosciences) at 4 °C on an Akta fast protein liquid chromatography apparatus (Amersham Biosciences). The heparin column was pre-equilibrated with Buffer A (50 mM NaH2PO4, pH 8.0, 100 mM NaCl, 5% glucose, 10 mM DTT, passed through a 0.2-μm filter, and degassed) and washed with Buffer A after loading the protein. DIP1-c was eluted with an 8-column gradient of Buffer A to Buffer A with 1 mM NaCl, and fractions were analyzed by SDS-PAGE. DIP1-c fractions were pooled and loaded onto a ~2.5-ml Ni2+-nitrilotriacetic acid-agarose (Qiagen) column pre-equilibrated with lysis buffer at 4 °C. The column was washed with lysis buffer with 20 mM imidazole, and DIP1-c was eluted in lysis buffer with 100 mM imidazole. Fractions were analyzed by SDS-PAGE. DIP1-c fractions were dialyzed twice at 4 °C against 1-liter exchanges (1 h each) of 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 2.5 mM CaCl2, 5% glucose, 10 mM DTT. Biotinylated thrombin (Novagen) was added at a 1:100 dilution, mixed well by inverting the tube, and allowed to cleave His6-DIP1-c for 16 h at 4 °C. The protein mixture was passed through 100 μl of avidin (Novagen) and 500–1000 μl of Ni2+-nitrilotriacetic acid-agarose resin slurries and incubated at 4 °C for 30 min. Centrifugation at 2,200 × g for about 10 s removed the resin along with biotinylated thrombin and His6 tag released from DIP1-c. The resulting DIP1-c was

EXPERIMENTAL PROCEDURES

Yeast Two-Hybrid Screen

The “interaction trap” method developed by the Brent Laboratory for yeast two-hybrid screens (26) has been utilized in these experiments. Matchmaker LexA two-hybrid system (Clontech) was used, and a 0–12 h D. melanogaster embryonic cDNA library (27) was a gift from Roger Brent (The Molecular Sciences Institute, Berkeley, CA). The plasmid pLexA-UbxNa216 (21) contains coding sequences for amino acids 216–389 of the Ubx Ib isoform fused to the LexA DNA binding domain, a construct initially used to eliminate transcriptional activation by Ubx previously (32). The C terminus of the protein contains a glutamine/alanine-rich region that contributes to transcription repression (22, 23). Although aspects of the transcription regulatory domains have been identified, the mechanisms, including protein interactions, that mediate these functions remain unknown. To identify potential protein partners that may modulate the function of Ubx, we have performed a yeast two-hybrid screen.

A partner prominently identified in our two-hybrid cDNA library screen was Disconnected Interacting Protein 1 (DIP1), which contains a putative nuclear localization signal and two double-stranded RNA-binding domains (dsRBDS). Global overexpression of DIP1 causes embryonic lethality (24). However, specific expression of DIP1 in the eye-antennal imaginal disc generates duplications of antenna and mouth structures and loss of the arista, reflecting aberrant expression of homothorax and spalt major and duplication of the distalless expression domain (24). DIP1 also interacts with other transcription regulators, including Disco and Su(var)3–9 (24, 25). Thus DIP1, like Ubx, can alter transcription regulation to impact developmental programs, although the molecular mechanism is unknown.

Herein, we detail a physical interaction between Ubx and DIP1 using modified yeast two-hybrid methods, phage display, immunoprecipitation, GST pull-down assays, and gel retardation supershifts. In addition, a genetic interaction between ubx and DIP1 is established. Formation of a DIP1-UbxDNA ternary complex and the demonstration that DIP1 represses transcription activation by Ubx in a modified yeast one-hybrid assay suggest a physiological role for the interaction. Intriguingly, DIP1 is shown to bind dsRNA with extremely high affinity. The strong interaction of a Hox protein with an RNA-binding protein implies that influences on Hox activity may be wider than previously appreciated.

Sequence Analysis

The C terminus of the protein contains a glutamine/alanine-rich region that contributes to transcription repression (22, 23). Although aspects of the transcription regulatory domains have been identified, the mechanisms, including protein interactions, that mediate these functions remain unknown. To identify potential protein partners that may modulate the function of Ubx, we have performed a yeast two-hybrid screen.

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diarylized at 4 °C against two 1-liter exchanges (1 h each) of 50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl, 5% glycerol, 10 mM DTT and stored at 4 °C for up to 1 week because freezing abrogates protein function. Every other day, solid DTT was introduced to about 100 μM to prevent oxidation effects on protein function.

**Immunoprecipitation**

Equimolar aliquots of purified Ubx Ib, purified DIP1-c, 2H.107° anti-Ubx antibody, and 10H.7 anti-Ubx antibody were combined and incubated with gentle rocking at 4 °C overnight. Hybridoma cell lines producing these antibodies were a gift from A. Javier Lopez (The John Hopkins School of Medicine) (34). Control incubations included either Ubx Ib or DIP1-c alone with the anti-Ubx antibodies. Each mixture was centrifuged at 16,000 × g. The supernatant was removed, and the pellet was washed with 50 μl of phosphate-buffered saline buffer with Tween 20 and recenterfuged at 5 min to discard any remaining supernatant. The supernatant, wash, and pellet fractions were analyzed by SDS-PAGE and Western blotting.

**GST Pull-down Assay**

The coding region of full-length Ubx Ib was subcloned into the pGEX-6P-2 vector (Amersham Biosciences, Inc.) via the EcoRI and XhoI restriction sites to produce pGEX-Ubx. The Ubx precursor was transfected into 10 mM Tris-HCl, pH 7.4, 0.8 mg/ml lysosome, with 10 μl of 100 mM PMSF. Cell pellets were stored at −20 °C. Cell pellets thawed on ice were diluted with an equal volume of 10% (w/v) sucrose, 50 mM KCl, 10 mM NaCl, 0.1% Triton X-100, 10 mM MgCl₂, each NTP at 4 mM, 10 mM MgCl₂, 10% poly(I)-poly(C) at 4.95 × 10⁶ M, or poly(C) at 4.95 × 10⁻⁹ M. After three washes in buffer (10 min of incubation/wash), membranes were air-dried and exposed to a Fuji phosphorimaging plate for 16 h. Data were analyzed using MacBas version 2.0 software.

**RNA Template Preparation**

Several RNAs, used for gel retardation experiments, were produced by in vitro transcription.

**Human Immunodeficiency Virus (HIV) TAR RNA—**The DNA template used contained the sequence of the TAR RNA (5'-GGGCAACUGAGGC-GGACCAUCU-3') and the T7 RNA polymerase (RNAP) promoter (5'-TAAATACGACTCATACTAG-3'). This template (1 μl), re-suspended in water, was annealed with the T7 primer oligonucleotide (1.2 μM).

**Adenovirus VA1 RNA—**The template was amplified by PCR from pVA1, a gift from Gerton Akusjarvi (Upssala University, Sweden). The primers, 5'-ATTATACGACTCATACTAGGCGAGTGTTCTGTTGGT-3' and 5'-AAAAAGGGGCTCCCTCCCGTGTCCTGTC-3', were used to generate a 182-base pair product that contains the VA1 sequence under the T7 RNAP control. The PCR product was purified using the Qiagen PCR Clean-up kit, and the DNA template was resuspended in water.

**In Vitro Transcription—**Each transcription reaction consisted of 30 mM Tris-HCl, pH 8.1, 2 mM spermidine, 0.01% Triton X-100, 25 mM MgCl₂, each NTP at 4 mM, 10 mM DTT, 1 μM DNA template (HIV TAR or adenovirus VA1), and 50 μg/ml T7 RNAP (a gift from Yousef Shamoo, Rice University). The reactions were incubated at 37 °C for 3 h. To stop the reactions, an equal amount of 2× formamide solution (90% (v/v) formamide, 1× TBE (39), 25 mM EDTA, 0.02% bromphenol blue, 0.01% xylene cyanol) was added, and the samples were boiled for 1 min. The reactions were loaded on a 20% acrylamide (19:1) gel complexed with the T7 RNAP polymerase (RNAP) promoter (5'-TAAATACGACTCATACTAG-3'). This template (1 μl), re-suspended in water, was annealed with the T7 primer oligonucleotide (1.2 μM).

**Drosophila Genetics**

The Drosophila line containing the UAS-DIP1-c insertion was created by Alan Pelisson (CNRS, France) and obtained from Ana Campos (McMaster University, Hamilton, Canada). The D1F1-LBD(Dp1Y) I line was provided by Bloomington Stock Center (stock number 5999). The P(GawB)BxMS1016 Gal4 flies were acquired from Sean Carroll (University of Wisconsin, Madison, WI), and flies containing the Uba₈₋₆₄ genomic insertion were received from Bloomington Stock Center (stock number 457). The S880 wild-type fly line was obtained from Michael Stern (Rice University, Houston, Texas). DIP1-c was precipitated with ethanol and resuspended in water treated with diethyl pyrocarbonate.

**Dephosphorylation—**Transcribed RNA was dephosphorylated for 1 h at 37 °C using 10 units of alkaline phosphatase (Sigma) in 50 mM Tris-HCl, pH 7.9, 100 mM NaCl, 10 mM MgCl₂, 1 mM DTT. The dephosphorylated products were precipitated with ethanol and resuspended in water.

**Radialol蛏ing—**RNA was radiola belized with 7₂⁻³²P ATP (MP Bio- medicals, Inc.) using polynucleotide kinase as described by Promega. Radiolabeled RNA was purified in 20 mM Tris-HCl, pH 7.5, or TE buffer, pH 8.0 (39), with a NickTm column (Amersham Biosciences). The column was washed in 400 μl of buffer, and the RNA eluted in an additional 400 μl of buffer. The RNA was aliquoted and stored at −20 °C.

**RNA Gel Retardation—**DIP1-cRNA binding was measured in 20 mM Tris-HCl, pH 7.5, 100 mM KC1, 100 μg/ml BSA, 5 mM DTT, 10% glycerol. The final concentration of RNA in each reaction was ~4 × 10⁻¹¹ M.
Ubx Interacts with a dsRNA-binding Protein, DIP1

Identification of DIP1 as a Partner for Ubx Ib—A yeast two-hybrid screen was used to search for proteins that interact with the *Drosophila* Hox protein Ubx Ib. The Ubx Ib isoform was selected to include all possible amino acid sequences (Fig. 1). Because Ubx Ib contains an activation domain, the LexA-Ubx fusion is constitutively active, precluding detection of partners (21). A mutant in which the N-terminal 216 amino acids are deleted to abrogate transcription, UbxN216, was therefore generated for the initial yeast two-hybrid assays (21). UbxN216 was fused to the LexA DNA binding domain, and a 0–12 h *Drosophila melanogaster* cDNA library was fused to the B42 activation domain. Isolation and sequencing of cDNA from colonies that exhibited β-galactosidase production using wild-type Gal4, was used as a control to ensure DIP1-c effects on Ubx were specific. The transformants were plated on SD minimal medium plates lacking uracil, histidine, and tryptophan and containing galactose as the carbon source. The plates were incubated at 30 °C until colonies became visible. X-Gal (40 mg/ml) was added to the plates to monitor transactivation of the lacZ reporter gene by Ubx Ib.

RESULTS

DNA Labeling and DNA Gel Retardation

Complementary DNA oligonucleotides (top strand, 5′-GTCTGAT-CAGTGAAGCCTTTAAAGGCCGTATG-3′) were annealed, and the dsDNA was labeled for 1.5 h in reactions containing 0.3 mM DNA, 10 units of polynucleotide kinase, and 1 mM kinase buffer (Promega), 2 mM spermidine, and 100 μCi of [γ-32P]ATP (MP Biomedicals, Inc.) in a final volume of 20 μl. The labeling reaction was terminated by the addition of 2 μl of 0.5 mM EDTA, and the labeled DNA was loaded onto a NICK column (Amersham Biosciences) pre-equilibrated in TE buffer, pH 8.0 (39). The column was washed in 400 μl of TE, and the DNA was eluted in an additional 400 μl of TE buffer. DNA was aliquoted and stored at −20 °C.

DNA gel retardation was performed as previously described (41, 42) with minor variations. Protein and DNA were diluted in DNA binding buffer: 50 mM Tris-HCl, pH 7.5, 100 or 200 mM KCl, 10% glycerol, 5 mM DTT, 100 μg/ml RSA. Ubx Ib activity was measured as previously described (41, 42). All Ubx Ib preparations were −90–100% active. DIP1-c supershift assays contained 3 × 10⁻¹² μM DNA (100–300 cpm/μl). DIP1-c concentration ranged from 4 × 10⁻⁸ to 2.5 × 10⁻⁷ M. In a duplicate set of lanes, Ubx Ib was present at 6.3 × 10⁻⁸ M.

Yeast One-hybrid Analysis of DIP1-c Effect on Transactivation by Ubx Ib

The Matchmaker LexA Two-hybrid System (Clontech) was used in these experiments with modification of the pGβ vector. The coding region of full-length, wild-type Ubx Ib was subcloned into the pLexA vector via the EcoRI and BamHI restriction enzyme sites to produce pLexA-Ubx. The plasmid pGβ was modified by removing the coding region of the B42 activation domain with digestion of XhoI and EcoRV to produce vector pGβA242AD. The DIP1-c coding region was subcloned into the vector pGβA242AD using EcoRI and XhoI, and the resulting plasmid, pGβA242AD-DIP1, was transformed into the yeast strain EGY48 carrying pLexA-Ubx and the reporter plasmid p6op-lacZ, with pJGAB42AD as a negative control. The vector pCL1, which allows for constitutive activation of the lacZ reporter gene using wild-type Gal4, was used as a control to ensure DIP1-c effects on Ubx were specific. The transformants were plated on SD minimal medium plates lacking uracil, histidine, and tryptophan and containing galactose as the carbon source. The plates were incubated at 30 °C until colonies became visible. X-Gal (40 mg/ml) was added to the plates to monitor transactivation of the lacZ reporter gene by Ubx Ib.

FIG. 1. Schematic of the protein sequences for Ubx Ib and DIP1-c. A, a schematic of the Ubx Ib sequence, showing the position of various functional domains: homeodomain (HD), the B, mI, and mII microexons subject to alternative splicing, the YPWM motif (Y) (site of Exd interaction), a polyglycine region (*Poly G*), the activation domain (AD) that has a predicted α-helix and activation enhancing region (*AER*) (21), and a glutamine- and alanine-rich region (QA motif) that participates in transcriptional repression (22, 23). Also depicted is the region of Ubx Ib that interacts with DIP1, as determined by yeast two-hybrid experiments. B, a schematic of the DIP1-c sequence, showing regions that exhibit homology to other proteins, including the PEST and nuclear localization (*NLS*) sequences. Two predicted dsRNA-binding domains (*dsRBD*), a sequence homologous to the DEAD box (D), and four clustered PXXP motifs (P). Also depicted is the region of DIP1-c that interacts with Ubx Ib (*solid line*), as determined by deletion mutants, as well as a region N-terminal (*dashed line*) that enhances the Ubx Ib-DIP1-c interaction.
the N terminus. Interaction in the yeast two-hybrid system indicates these full-length proteins have higher affinity for each other than for endogenous yeast proteins.

**Verification of the Ubx Ib-DIP1-c Interaction**—The interaction of Ubx Ib and DIP1-c was first confirmed by switching their positions in the yeast two-hybrid assay, such that Ubx Ib was fused to the B42 activation domain and DIP1-c was fused to the LexA DNA binding domain. This experiment detects whether either protein interacts with LexA or B42 to generate a false positive. Because the fusion of full-length wild-type Ubx Ib with the B42 activation domain resulted in a toxic protein, a special “colony lift” procedure was developed for this assay. Yeast colonies were first grown on a glucose-containing plate, repressing expression of the toxic Ubx-B42 fusion protein, and then the colonies were transferred to a plate containing galactose to induce fusion protein expression. The interaction of full-length Ubx Ib and DIP1-c was confirmed in these “reverse” yeast two-hybrid experiments.

The Ubx Ib-DIP1-c interaction was further verified using phage display methods. Two advantages of phage display are that wild-type, full-length Ubx Ib can be utilized, and there are no yeast proteins present that might bridge Ubx Ib and DIP1-c. Full-length biotinylated Ubx Ib was bound to a Petri dish coated by avidin, and the Petri dish was incubated with phage expressing DIP1-c on the surface. After five washes, 77% ± 4% (four experiments) of the phage was retained on the plate, indicating a strong Ubx Ib-DIP1-c interaction. The positive control for this system employed interaction of Ubx Ib and Exd. The Ubx Ib-Exd partnership (two experiments) resulted in 74 and 76% phage retention, values comparable with Ubx Ib-DIP1-c. Negative controls yielded <10% phage retention. The experiment was also repeated with identical results using purified Ubx Ib without a biotin tag (data not shown). Therefore, biotin does not cause structural rearrangement in Ubx Ib that enhances interaction with DIP1-c. These assays demonstrate that full-length Ubx Ib can interact effectively with full-length DIP1-c in the absence of yeast or bacterial proteins.

Finally, the full-length proteins were shown to interact by immunoprecipitation and GST pull-down assays. DIP1-c was cloned into the plasmid pET28a, which contains sequences for adding a His6 tag to the N terminus of the protein. This modification allows rapid purification and detection of the protein. All buffers contained 5% glucose to minimize precipitation of DIP1-c as established by an aggregation assay (32). DIP1-c runs at an anomalously high molecular weight on SDS-PAGE, a trait previously reported for other proteins containing dsRNA-binding domains (38, 43). Ubx Ib was immobilized on a Petri dish and GST-Ubx was precipitated from a bacterial lysate. GST-Ubx was precipitated with the 10H.7 antibody to Ubx, and the last five lanes were probed with the α-tetra-His antibody (Qiagen), an antibody to the His6 tag on DIP1-c. S, supernatant; P, pellet; W, wash. B, either His6-DIP1-c alone or GST-Ubx lysate and His6-DIP1-c were combined with glutathione-Sepharose 4B beads (Amersham Biosciences). Following separation, the beads were washed (W) and then eluted (E) with reduced glutathione. The Western blot was probed with the α-tetra-His antibody (Ab). S, supernatant; W, three washes; E, eluate. C, the GST pull-down assay was repeated with DIP1-c treated with thrombin to remove the His6 tag. The Western blot was probed with rabbit polyclonal antibodies to DIP1.

DIP1-c interaction has been confirmed by three different yeast two-hybrid, two-phase display, and immunoprecipitation and GST pull-down experiments.

**Ectopic Expression of DIP1 in Wings and Halteres**—Expression of uxb in the wing or haltere imaginal discs promotes haltere formation from the default developmental programs (44). Because Hox proteins play a causal role in appendage specification in *Drosophila* and DIP1 is expressed in wing and haltere imaginal discs (24), we determined the effects of DIP1 overexpression on development of these appendages using the Gal4-UAS system (35). P[GawB]Bx*HS1096* Gal4 drives transcription in the wing and haltere imaginal discs, predominantly on the dorsal side (45), and the UAS-DIP1-c responder expresses well in *Drosophila* (24). Ectopic expression of DIP1-c in wing imaginal discs resulted in a dramatic reduction in wing size and a thicker, shriveled appearance (Fig. 3, A and B), a phenotype that does not occur with either element in isolation. Because individual cells were visible, cell density could be measured. The change in wing size was due to a change in cell number, not cell size. The wing pictured in Fig. 3B is one of the larger ones observed, but the smaller wings were concave and could not lie flat for microscopy without folding or tearing. On rare occasions (<2%), bifurcated wings were observed. Bifurcations were always on the smallest, most concave wings and therefore may occur in flies with the highest levels of ectopic DIP1-c expression. In addition, both wing veins and the line of bristles along the margin were lost, although some heavy bristles are clustered proximal to the notum and at the most distal edge. Ectopic expression of DIP1-c in haltere imaginal discs resulted in a range of deformed halters (Fig. 3, D and E) compared with the wild-type haltere (Fig. 3C). Most halters contained a misshapen capitellum, and ~10% were also bifurcated. Like the wing overexpression data, ectopic expression
phenotypes demonstrate that DIP1-c is a functional protein in vivo that influences growth and patterning.

Genetic Interaction of Ubx Ib and DIP1-c—Ectopic expression of ubx in the wing imaginal disc also generates small shriveled wings with excess bristles (45). The similarity of the DIP1 and ubx ectopic expression phenotypes in the wing suggests a potential for a genetic interaction between the two genes. To explore this possibility, we utilized the Ubx<sup>bx-34e</sup> gypsy insertion line, which causes a reduction of ubx gene expression in the haltere imaginal disc (44). Homozygotes have an enlarged haltere, with long, dark costal bristles (Fig. 4A), whereas halteres in the heterozygotes appear wild-type (Fig. 4B). Initial efforts examined whether alterations in DIP1 expression could modulate the Ubx<sup>bx-34e</sup> heterozygote phenotype. Indeed, flies containing a single copy of Ubx<sup>bx-34e</sup> and a duplication of the region of the X chromosome containing the DIP1 gene onto the Y chromosome have a punctate line of short, dark bristles on the capitellum with a denser cluster of bristles at the distal tip (Fig. 4C). To ensure that this effect was caused by the DIP1 gene, DIP1-c was ectopically expressed in the wing and haltere imaginal discs of Ubx<sup>bx-34e</sup> heterozygous flies using the MS1096-Gal4 driver. Ubx is naturally expressed across the entire haltere imaginal disc with highest levels in the pouch (46). Therefore, Ubx and ectopic DIP1-c domains will overlap. These flies had long bristles across the entire surface of the haltere, an enhancement of the Ubx<sup>bx-34e</sup> heterozygous phenotype that is not visible in wild-type flies or flies with only the Ubx<sup>bx-34e</sup> and MS1096-Gal4 elements (Fig. 4, D-L). For halters mounted in the same orientation, negative controls heterozygous for just MS1096-Gal4 and Ubx<sup>bx-34e</sup> had twice as many bristles as wild type, whereas flies heterozygous for MS1096-Gal4, UAS-DIP1-c, and Ubx<sup>bx-34e</sup> had four times as many bristles as wild type. Thus, ectopic expression of DIP1-c, either by duplication or by specific overexpression, enhances the Ubx<sup>bx-34e</sup> effect. This genetic interaction between the ubx and DIP1 genes provides in vivo evidence for a physiological role of the demonstrated physical association between the two proteins.

Identification of Functional Motifs within the DIP1 Sequence—Although DIP1 is not homologous to another known protein throughout its entire length, the sequence does contain regions similar to known motifs that indicate potential biological function (Fig. 1). Three short motifs, a nuclear localization signal (residues 142–159) (47), a DEAD box (residues 260–263), potentially involved in Mg<sup>2+</sup> binding (48), and a PEST motif (residues 107–138) (49), were found in DIP1 (Fig. 1B), along with multiple potential phosphorylation, glycosylation, and myristoylation sites (data not shown). These short motifs, if actually employed in vivo, may serve to regulate the function of DIP1. Importantly, two dsRBDS (residues 171–237 and 335–403) (50) were also identified in DIP1. dsRBDS are 65–68-amino acid motifs that are divided into two categories: type A domains, which are conserved throughout the motif in sequence and secondary structure of α-β-β-α (where α denotes α-helix and β is β-sheet), and type B domains, which are only conserved in the C-terminal helix (37, 50). Interestingly, the first dsRBD of DIP1 is a type A domain whereas the second dsRBD is a type B domain (data not shown) (24). Yeast two-hybrid experiments indicate that DIP1-c self-associates (data not shown), a characteristic common to proteins containing dsRBDS (38, 51). Although these domains are generally only loosely conserved, the highest sequence similarity was to two domains found within RED1, an RNA editase (52). DIP1-c is 29% (87/292) similar to human RED1, in which there is 41% (28/68) identity and 50% (34/68) similarity between the first dsRBD of each protein.

Nucleic Acid Binding by DIP1-c—The ability of purified DIP1-c to bind RNA was initially tested using Northwestern blotting (Fig. 5). DIP1-c was able to bind poly(I)-poly(C) at subnanomolar concentrations, indicating a strong affinity for dsRNA. In contrast, a 22-fold higher concentration of poly(C) was required to observe weak, but significant, binding in the same range of protein concentrations. Also, DIP1-c binds a 30-base pair dsDNA duplex with low affinity (Fig. 6 and Table 1). Strong interaction with dsRNA and weak interaction with
single-stranded RNA and dsDNA is characteristic of proteins containing double-stranded RNA-binding domains (37, 38, 50). Therefore, the double-stranded RNA-binding domains identified through sequence similarity are functionally active.

The affinity of DIP1-c for commonly used dsRNAs was compared with other dsRBD proteins to determine whether the DIP1-c domains are sufficiently active for in vivo function. Two dsRNA ligands were transcribed in vitro and tested as ligands in gel retardation assays (Fig. 6). dsRNA binding proteins are reported to bind dsRNA ligands with high affinity ($K_a \approx 10^{-9}$ M), regardless of sequence or physiological relevance (53–56). In these assays, DIP1-c bound with high affinity to HIV TAR RNA (50 nM $K_{app} \approx 1$ nM) but bound with much higher affinity to the adenovirus VA1 RNA ($K_{app} \approx 50$ pM). The binding affinities of DIP1-c for dsRNA targets are comparable with or greater than those reported for other dsRNA-binding proteins (Table I).

Alternative Splicing of DIP1—Alternative splicing of the DIP1 gene was indicated by the presence of four major bands on a Northern blot of mRNA isolated from 0–16 h embryos from the S880 wild-type $D. melanogaster$ strain. The existence of multiple DIP1 RNA isoforms was confirmed by reverse transcriptase PCR (Fig. 7). Sequences of these DNAs indicated differences that are derived from alternative splicing. Several DIP1 isoforms have previously been deposited at GenBank.2 We have identified a novel isoform, DIP1-d, in which the putative nuclear localization signal is not present (Fig. 8). A version of DIP1 potentially confined to the cytoplasm suggests the protein may have multiple functions (24).

Identification of the Protein-Protein Interaction Regions of $Ubx$ Ib and DIP1-c—Comparison of the $Ubx$ Ib-DIP1-c interaction domains with known functional domains provides a means to predict activities that may be altered or precluded by the $Ubx$ Ib-DIP1-c interaction. Deletion mutants of each protein were used in the yeast two-hybrid system to identify the sequences of each protein required for the $Ubx$ Ib-DIP1-c interaction. A series of deletion mutants were made in which sequences were successively removed from the N- and C-terminal ends of DIP1-c (Fig. 9). Residues 1–266 are not required for interaction, since the interaction was identified originally for an N-terminal truncated form of DIP1-c. Decreased interaction with $Ubx$ Ib can be detected when residues 267–307 are removed, as indicated by pale blue colonies. From the C terminus,

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2 The nucleotide sequences for the DIP1 isoforms have previously been deposited in the GenBank™ data base with the following accession numbers: AF175713 (DIP1-a), AF182154 (DIP1-b), AF218310 and AF175711 (DIP1-c), AJ217028 (DIP1-d), AJ250866 (KLETT-a), and AJ250867 (KLETT-b).
RNA. Poly(I)-poly(C) dsRNA at 2.24 M was used for the experiment on the left; Poly(C) single-stranded RNA at 4.95 × 10⁻³ M was utilized for the blot shown on the right. The first column on both membranes was filtered with water, the second column with solutions containing the indicated amounts of DIP1-c, and the final column with solutions containing identical amounts of BSA. The RNA binding in the DIP1-c column is due solely to the presence of the DIP1-c protein.

residues 366–410 can be removed with no adverse effect, but removing residues 327–365 abolishes the Ubx Ib-DIP1-c interaction. Thus, the DIP1-c sequence required for interaction with Ubx Ib resides in amino acids 327–365, and residues in the 267–307 region can enhance this interaction (Fig. 1B). These data are in agreement with GST pull-down experiments for key DIP1-c deletions (Fig. 9). This large region may be required to stabilize the tertiary structure supporting the interacting residues.

For Ubx Ib, the interaction domain must reside in the C-terminal 173 amino acids, since this protein was the original “bait” in the yeast two-hybrid experiments. All deletions made to UbxNX216 on the C-terminal end or on the N-terminal end abolish the Ubx Ib-DIP1-c interaction (Fig. 1A). Therefore, either multiple regions of Ubx Ib are required for this interaction, or a specific conformation of the protein supported by these regions is necessary to bind DIP1-c. The homeodomain alone is insufficient for this interaction. The DIP1-c interaction domain of Ubx Ib includes part of the activation domain, the Exd interaction motif, the three microexons, the homeodomain, and the repression domain. These Ubx Ib functions may be modulated by DIP1-c binding. Likewise, Ubx Ib binding may affect the affinity of the DIP1-c-RNA interaction due to the overlap of the region of Ubx Ib interaction and the second dsRBD. Preliminary results suggest that DIP1-c can bind RNA in the presence of Ubx Ib (data not shown); however, specific RNA sequences or structures may influence the effect of Ubx Ib on DIP1-c-RNA binding.

Ubx Ib and DIP1-c Form a Ternary Complex on Ubx Target DNA—For DIP1-c to influence transcription regulation by Ubx Ib in vivo, it must be able to participate in a ternary DIP1-c-Ubx Ib-DNA complex. To determine whether Ubx Ib can simultaneously bind both DIP1-c and its target DNA, a gel retardation assay was employed. As shown in Fig. 10 (lanes 12 and 13), Ubx Ib binds DNA with a single consensus binding site of 5’-TAATGG-3’ (57) to generate a single shifted band. Under the same conditions, only a small degree of DIP1-c-DNA binding was observed, consistent with the general ability of dsRBDs to weakly interact with dsDNA (37, 38, 50). However, when DIP1-c and Ubx Ib are combined, a novel DNA complex is observed. This “supershift” is only present when both proteins are added to the reaction. Western blot analysis detected both proteins in the supershifted band (data not shown). The supershift occurs at protein concentrations at which DIP1-c does not bind DNA, implying that DIP1-c binds directly to Ubx Ib to form the ternary complex. This complex was also observed in a buffer containing 200 mM KCl, which would be expected to disrupt nonspecific interactions. Although the homeodomain is encompassed by the region of Ubx Ib required for DIP1-c interaction, DIP1-c does not interfere with DNA binding by Ubx Ib and must leave the homeodomain binding surface exposed. This observation is consistent with the inability of the isolated homeodomain to bind DIP1-c. The binding of DIP1-c to Ubx Ib-DNA does not introduce a significant alteration in the affinity of Ubx Ib for DNA (data not shown), suggesting that DIP1-c does not modulate single site DNA binding by Ubx Ib. Observation of this supershift demonstrates Ubx Ib-DIP1-c interaction can occur at nanomolar protein concentrations.

DIP1-c Specifically Represses Ubx Ib Activation—The ability to form a ternary complex, as well as the overlap between the DIP1-c interaction domain and Ubx Ib activation and repression domains, suggests that DIP1-c may alter the mode (activation or repression) or the strength of transcription regulation. To test this hypothesis, a modified yeast one-hybrid assay was employed to investigate the function of the Ubx Ib-DIP1-c interaction (Table II). Full-length, wild-type Ubx Ib fused to the LexA DNA-binding domain can constitutively activate transcription in yeast, yielding blue colonies in the presence of X-gal (15). Transcriptional activation by Ubx Ib in this assay is independent of Ubx Ib-DNA binding but relies on LexA affinity for its operator. When full-length, wild-type DIP1-c (no B42 fusion) was expressed in these yeast, the colonies remained white on screening with X-gal, indicating that ß-galactosidase was not expressed. A control experiment was performed with pCL1, a vector which allows constitutive activation of the reporter gene by expressing wild-type yeast Gal4. When DIP1-c was co-expressed with Gal4, the colonies still turned blue in the presence of X-gal. These data suggest that DIP1-c is not a general inhibitor of transcription or translation but instead must specifically interact with Ubx Ib to affect its transcriptional activation. The observed inhibition of transactivation of Ubx Ib by DIP1-c is not caused by blocking DNA binding by Ubx Ib, because a ternary complex is observed in vitro, and Ubx Ib is not required to bind DNA in the fusion with LexA used in the yeast one-hybrid assay. Further, this inhibition is not caused by blocking DNA binding by LexA, or the Ubx Ib-DIP1-c interaction would not be observed in the yeast two-hybrid assays. Thus, an interesting possibility is that DIP1-c may regulate Ubx Ib function in vivo by modulating the balance between transcription activation and repression by Ubx.

DISCUSSION

The Hox family plays a critical role in segmental specification and cell fate determination in Drosophila development (58, 59). Given the global consequences of Hox function, activity must be tightly regulated in a temporal-, spatial-, and gene-specific manner. However, the high homology of the homeodomain combined with relatively promiscuous DNA binding in vitro is incompatible with the requirement for specific, yet plastic, function in vivo. A range of protein-protein interactions appears to be key to solving this Hox specificity puzzle (1, 6, 60–62). In order to understand how Hox proteins achieve their exquisite discriminatory function in vivo, these interactions
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Fig. 6. DIP1-c binds dsRNA with high affinity. The ability of DIP1-c to bind dsDNA, TAR RNA, and Adenovirus VA1 RNA was investigated using gel retardation. A, a gel retardation assay for dsDNA; B, an assay for TAR RNA; C, an assay for VA1 RNA. The final RNA or DNA concentration in each reaction for A and B was ~4 \times 10^{-11} \text{m} and ~1 \times 10^{-12} \text{m} for C. B, bound species; F, free DNA or RNA. Lane 1 of each gel contained no protein. For A and B, DIP1-c was present at concentrations of 5 \times 10^{-10} to 2 \times 10^{-6} \text{m} in lanes 2–20, whereas in C, DIP1-c was added at concentrations of 1.6 \times 10^{-11} to 6.3 \times 10^{-8} \text{m} in lanes 2–20. To the right of each gel is the binding curve that is representative of the data. For A (four gels) and B (three gels), the resulting curves are based on the disappearance of the free DNA or RNA species, whereas for C (four gels), the curves show both disappearance of free RNA (open circles) and the appearance of DIP1-c RNA complexes (closed circles). Table I summarizes the calculated binding constants for the three curves.

Table I  
Comparison of nucleic acid binding of DIP1-c with other dsRNA-binding proteins

<table>
<thead>
<tr>
<th>Ligand</th>
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<th>PKR</th>
<th>DIP1-c</th>
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<tr>
<td>Poly(I)-poly(C) dsRNA</td>
<td>1 nm</td>
<td>5 nm</td>
<td>~100 nm</td>
</tr>
<tr>
<td>HIV TAR RNA</td>
<td>ND</td>
<td>50 nm</td>
<td>~50 nm</td>
</tr>
<tr>
<td>Adenovirus VA1 RNA</td>
<td>+</td>
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<td>dsDNA</td>
<td>–</td>
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must be discovered, and their effects on function must be understood.

Potent transcriptional activation domains within Hox proteins and consequent protein toxicity to yeast cells have impeded identification of Hox protein partners by traditional yeast two-hybrid methods. By modifying this approach, confirmed the interaction with phage display, immunoprecipitation, GST pull-down assays, and gel retardation supershifts, and by identifying a genetic interaction, we have identified DIP1 as a Ubx Ib-interacting protein. The DIP1 expression domains in the central nervous system, imaginal discs, and embryo overlap with Ubx expression, and both proteins are localized to the nucleus (6, 18–20, 24). Importantly, the Ubx Ib-DIP1-c interaction requires Ubx amino acid sequences outside of the highly conserved homeodomain. Protein interactions in these much more variable regions could provide a source of functional discrimination within the Hox family.

The DIP1 protein contains two regions homologous to dsRNA-binding domains, a sequence for nuclear localization, PEST motifs, and four clustered PXXP sequences. Although, PXXP motifs are commonly used for mediating protein-protein interactions (63, 64), Ubx Ib does not contain a corresponding Src homology 3 domain. DIP1-c activity could thus be regulated (or regulate) through binding to other proteins via these PXXP motifs in vivo.

Northwestern blotting and gel retardation experiments demonstrate that DIP1-c, the longest isoform, binds with very high affinity to dsRNA and with ~1000-fold lower affinity to single-stranded RNA and dsDNA, consistent with active dsRBDs (37, 38, 50). The existence of alternative splicing products for DIP1 (GenBank) (24), as also observed in our experiments, suggests regulated expression of different forms in spatial and/or temporal patterns in Drosophila. In particular, we identified an isoform in which the putative nuclear localization signal is removed, potentially creating a cytoplasmic version of DIP1. The Campos laboratory identified additional isoforms and presented evidence supporting the existence of a cytoplasmic DIP1 isoform in nurse cells (24).

Previously, DIP1-b overexpression in the eye-antennal imaginal disc was shown to induce transformation phenotypes of adult head structures (24). Herein, we demonstrate the DIP1-c ectopic expression also alters the development of wings and halteres. Thus, DIP1-c can function in ubx-expressing cells. A genetic interaction was identified between underexpression of Ubx in the haltere and overproduction of DIP1, both by a duplication of the DIP1 gene and by the Gal4-UAS system. The enhancement of the Ubx mutant phenotype produced by increased DIP1 expression may be caused by interactions in vivo that hinder the normal function of Ubx in these cells.

Indeed, DIP1-c specifically blocks transcription activation by Ubx Ib in yeast cells. Differential transcriptional regulation by
Hox proteins, presumably mediated by protein interactions, are required for specific Hox function (1, 6, 21–23, 62, 65). Although a portion of the activation domain of Ubx Ib (amino acids 159–242) is required for DIP1-c interaction, neither deletion of amino acids 1–21 nor mutations between 223–233 abrogate the interaction, suggesting that these residues are not required for the DIP1-c partnership. However, interaction with general transcription factors may be sterically occluded by DIP1-c interaction, or DIP1-c binding may lock Ubx Ib into a conformation that inhibits transcriptional activation.

Gel retardation assays demonstrate ternary complex formation between Ubx Ib, DIP1-c, and DNA that would provide a mechanism by which DIP1-c could modulate transcription regulation in an Ubx Ib-specific manner. The small impact of DIP1-c on Ubx Ib-DNA affinity suggests that this regulation would derive from influence on other Ubx Ib functions, consistent...
with the loss of transcriptional activation by the Ubx Ib-DIP1-c complex in yeast. DIP1 expression coincides with the onset of zygotic transcriptional activity in the embryo, further suggesting a role for DIP1 in transcriptional regulation (24). Interestingly, the region of Ubx Ib that is required to interact with DIP1-c contains the C-terminal glutamine/alanine-rich region, which mediates transcription repression in arthropods (22, 23).

In vivo, Ubx represses both distalless and spalt in the absence of Extradenticle, a Hox co-factor required for Ubx-mediated repression of several genes (66). It will be of interest to determine if this repression is influenced by DIP1.

Conversely, Ubx Ib interaction may modulate DIP1 function. The Ubx Ib interaction domain on DIP-c includes part of the C-terminal dsRNA binding domain and hence may impact interactions with RNA. Several homeodomain proteins bind RNA in vivo. The fold of the RNA-binding domain of ribosomal protein L11 was shown to be similar in structure to the homeodomain (67). Further, Bicoid, a homeodomain protein that establishes the anterior-posterior gradient in the Drosophila embryo, binds the Bicoid response element in the 3'-untranslated region of caudal mRNA via its homeodomain, thus repressing translation (68, 69). Other transcription factors and DNA-binding proteins have been shown to bind RNA as well (70). The Hox protein Antennapedia interacts with Split Ends, a protein with RNA recognition motifs (71). The interaction of DIP1 in vitro with a zinc finger transcription factor required for eye development, Disco, and the histone methyltransferase, Su(var)3–9, supports the hypothesis that DIP1 acts as a bridge between regulation of DNA transcription and RNA processing/editing and suggests that this “bridge” is not exclusive to the Hox family of proteins (24, 25, 72–74). Indeed, overexpression of DIP1 in the eye-antennal disc alters the

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**Fig. 9.** Yeast two-hybrid and GST pull-down assays to determine DIP1-c sequences required for Ubx Ib binding. The constructs indicated were fused to the B42 activation domain in pB42AD and utilized in yeast two-hybrid assays with UbxN216 fused to the LexA DNA binding domain. Blue, yeast colonies exhibiting high levels of β-galactosidase activity, reflective of partner formation with Ubx Ib; Pale Blue, colonies with reduced enzyme activity and therefore reduced Ubx Ib-DIP1-c complex formation; White (no enzyme activity), Ubx Ib-DIP1-c binding has been abolished. To confirm these results, full-length DIP1-c and key deletions were expressed in vitro and tested for interaction with GST-Ubx Ib. ++, strong interaction in the GST pull-down assay; +, a weak interaction; −, no interaction. The protein motifs with DIP1-c identified through homology searches are also depicted.

**Fig. 10.** Ternary complex between Ubx Ib, DIP1-c, and Ubx target DNA. A gel retardation assay was employed to investigate the interaction between Ubx Ib and DIP1-c in the presence of Ubx target DNA. The 30-base pair DNA contains one Ubx consensus binding site, 5'-TAATGG-3' (57). The final DNA concentration in all reactions was 10⁻¹² M. No protein was present in lane 1. In lanes 2–6, DIP1-c was present at 4 × 10⁻¹⁰ to 4 × 10⁻⁹ M. Lanes 7–13 contained Ubx Ib at 6.3 × 10⁻¹⁰ M. Lanes 7–11 contained the same DIP1-c dilutions as lanes 2–6. The molar ratios of DIP1-c/Ubx Ib in lanes 7–11 were 0.6, 1.0, 1.6, 2.5, and 4.0, respectively. D, faint bands generated by DIP1-c/DNA in lanes 2–6; C, a DIP1-c/Ubx Ib DNA complex formed in lanes 7–11. U, Ubx Ib/DNA band in lanes 12 and 13; W, wells; F, free DNA.
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expression of homothorax and spalt major and generates an ectopic distalless expression domain, ultimately causing homoeotic transformations of head structures (24). Global overexpression of DIP1 is embryonic lethal (24), also consistent with a general function in transcription regulation and/or RNA metabolism.

The growing data demonstrating interaction of DNA- and RNA-binding proteins suggest that Hox proteins may participate in more diverse functions than previously indicated. Identification of other Hox proteins capable of DIP1 interaction will be of considerable interest, since this interaction provides potential for significant functional differences within this highly homologous class of proteins. Such studies will refine our understanding of the mechanisms by which Hox proteins regulate the complex cell specification process.

Acknowledgments—We thank the following: Roger Brent for the D. melanogaster embryonic cDNA library, Philip Beachy for the Ubx vector, Yousif Shamoo for the T7 RNA-polymerase construct and for technical assistance, Javier Lopez for Ubx monoclonal antibodies, Michael Stern for the LexA DNA binding domain. Blue, the yeast colonies exhibited high /H9004 B42AD (no B42 fusion), and full-length Ubx Ib was fused to the constitutive activation of the reporter gene using wild-type Gal4. Thus, DIP1-c is not a general inhibitor of transcription or translation.

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4. White, No Blue Yes
7. White, No Blue Yes
9. White, No Blue Yes
Hox Transcription Factor Ultrabithorax Ib Physically and Genetically Interacts with Disconnected Interacting Protein 1, a Double-stranded RNA-binding Protein
Sarah E. Bondos, Daniel J. Catanese, Jr., Xin-Xing Tan, Alicia Bicknell, Likun Li and Kathleen S. Matthews

doi: 10.1074/jbc.M312842200 originally published online March 23, 2004

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